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Effect of gender, gonadectomy and oestradiol-17β on growth in lambs under grazing conditions

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SUMMARY

To identify separate effects of gender, castration and exogenous oestrogen on growth, castrated lambs of both sexes and entire male lambs ($n = 8$) were implanted subcutaneously with three sizes of oestradiol-17β implants, or not implanted, and grazed on ryegrass and white clover pasture for 180 days. A group of non-implanted entire female lambs ($n = 8$) was run together with the others. Non-implanted entire male lambs grew faster, had heavier heads, less internal, non-carcass fat and more protein and less fat and water in the carcass than non-implanted entire females. In addition, they had higher 12th vertebral spine, thicker tibia, and heavier and larger humerus than entire female lambs. Castration of male lambs reduced live-weight gain, weight of head and content of protein in the carcass whereas it increased carcass fat content. In addition, it caused lengthening of cannon bones and reduced height of 12th vertebral spine and length of tibia. In females, gonadectomy increased height of 12th vertebral spine and diameter to length ratio of the radius. Oestradiol treatment increased live-weight gain, reduced total internal and carcass fat, and increased water and protein content of the carcass in gonadectomized animals of either sex, and increased weight of carcass and head in spayed ewe lambs. Oestradiol treatment inhibited longitudinal growth of cannon bones and stimulated that of vertebral column and ribs, but had little effect on the dimensions of limb bones apart from increasing their diameter. Oestradiol treatment had no effect on muscle length but increased muscle girth and weight, except for *m. splenius* in ram lambs where muscle weight was reduced. Effects of oestradiol on skeletal measurements in most cases were linearly related to dose of oestradiol. It was concluded that the variable effects of sex steroids on the skeleton were related to the differential pattern of skeletal maturation. In early maturing bones acceleration of the growth process by an exogenous sex steroid caused elongation to cease prematurely, whereas in late-maturing bones the acceleration effect on elongation did not result in premature cessation. This observation may explain the often contradictory reports in the literature on the effects of sex steroids on linear growth of bone.

INTRODUCTION

Oestrogens have been used widely as growth promoters in farm animals (Galbraith & Topps 1981; Hancock *et al.* 1991), yet they appear to inhibit skeletal growth in laboratory animals and man (Silberberg & Silberberg 1972; Short 1980). For instance oestrogen treatment stimulated linear growth of vertebral column and cannon bones (Wilkinson *et al.* 1955) and growth at the distal end of the femur (Shroder & Hansard 1959) in sheep, whereas it inhibited linear growth of limb bones, vertebrae and ribs in rats (Zondek 1936). These contradictory effects of oestrogens have been attributed to variation between species, in ranges of doses, ages of animals, duration of treatment or chemical identity of the hormone molecule used (Silberberg & Silberberg 1972; Spencer 1985). Dose effects were explained by the hypothesis that small doses of these hormones stimulate linear growth of bone whereas large doses inhibit it (Suzuki 1958; Short 1980). Experiments which have studied effects of lack of sex hormones by use of castration have also shown contradictory findings. Castration of male, and to a lesser extent of female, ruminants lengthened limb bones, particularly distal limb bones, resulting in animals which were taller than entires (Brannang 1971*a*, *b*). On the other

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hand, castration shortened the vertebral column resulting in animals shorter in body length than entires (Tandler & Keller 1910–1911; Brannang 1971*a*, *b*).

Skeletons of animals mature in a differential manner (Hammond 1932; Wallace 1948; Davies 1979; Davies *et al*. 1984) with the skull and cannon bones maturing earlier in life and the vertebral column later. The extent to which activity of epiphyseal plates can be affected by oestrogens at any particular stage of growth may vary for different bones in the skeleton. The present study examined the effects of endogenous sex hormones and an exogenous oestrogen, oestradiol, on skeletal growth of sheep in relation to the differential pattern of skeletal maturation during their first 8 months of postnatal growth.

MATERIALS AND METHODS

Experimental

One hundred and four Dorset Down X Coopworth lambs comprising 64 males and 40 females aged 8 to 10 weeks (mean live weight = 20·4 kg) were used in the experiment. Thirty-two of both sexes were gonadectomized at 8–10 weeks and were randomly allocated, within gender, to 13 groups ($n = 8$) (Table 1). Four groups (one of each sex class) were non-implanted controls (0) and the other nine groups (three each of wether, ram and spayed ewes) were treated with low (L, 2 implants), medium (M, 1 implant) and high (H, 1 implant) dose oestrogen implants (Table 1) one week after the last gonadectomy operation. Animals were grazed throughout the experimental period of 180 days on pasture consisting predominantly of perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.). The implants were formed of silicone rubber containing oestradiol-17β moulded in three sizes; small (L) (surface area, SA, 75 mm²), medium (M) (SA 603 mm²) and large (H) (SA 1574 mm²) which contained 3, 22 and 52 mg oestradiol, respectively. Animals with the 2 L dose implants were re-implanted with another 2 implants 41 days later to overcome the possibility of exhaustion of hormone from the implants. Blood samples (10 ml) were collected on the day before implantation and at 1 and 175 days after implantation into heparinized glass tubes, centrifuged and the plasma stored at −20 °C. Plasma oestradiol concentration was measured by radioimmunoassay (Peterson *et al*. 1975). All procedures involving the use of animals were approved by the Lincoln University Animal Ethics Committee.

Table 1. *Allocation of lambs to sex, gonadectomy and oestrogen treatment groups (0 = control, L = low dose (6 mg), M = medium dose (22 mg) and H = high dose (52 mg) oestradiol implants)*

	Oestrogen dose level				
Sex group	0	L	M	H	Total
Wethers	8	8	8	8	32
Entire rams	8	8	8	8	32
Spayed ewes	8	8	8	8	32
Entire ewes	8	—	—	—	8
Total	32	24	24	24	104

Measurements

Lambs were weighed weekly and length of right and left fore cannon bones was measured every 3 weeks to the nearest mm using vernier calipers. At the end of 180 days (at approximately 35 weeks of age, mean live weight 39 to 45 kg), the lambs were slaughtered and beheaded by severing the atlanto-occipital joint, teat lengths were measured and the warm carcasses, heads, testes and uteri were weighed then stored at −20 °C. The carcass was split along the midline using a band saw. The following measurements were taken from the thawed left half-carcasses before dissection; soft tissue depth over the 11th rib at 11 cm from midline of backbone (GR), A (length) and B (width of eye muscle, *m. longissimus thoracis et lumborum*, LD) and C (fat thickness over the 12th rib). A, B and C measurements were carried out according to the methods of Pálsson (1939). Whole right half-carcasses were minced and subsampled for chemical analysis. Subsamples were weighed, freeze-dried for 4 days, reweighed (to determine water content), ground and analysed for protein and fat content following the procedures of AOAC (1984). The frozen left half-carcasses were thawed and the cervical, thoracic, lumbar and sacral regions of the vertebral column were identified according to Getty (1975). Each region was measured in length and the number of vertebrae was recorded. The 1st and 12th ribs and their corresponding vertebral spines were exposed and each length of rib and height of spine was measured. The radius and ulna, humerus, femur and tibia were dissected out and measured in length and the humerus was weighed. For each of these bones maximum diameter at mid-point was measured to the nearest mm using vernier calipers. Femur bone was sawn transversely at mid-point and maximum thickness of cortex at this site (FMCT) was measured using the same calipers. The 12th rib was dissected out, separated from adhering tissue, dried in an oven at 110 °C for 24 h, then incinerated in a muffle furnace at 550 °C for 16 h. Ash proportion (ash weight divided by dry matter weight, g/kg) and ash to organic matter ratio (A:R), calculated as ash weight divided by (dry matter weight minus ash weight), were determined. Muscles, *m. biceps brachii* (BIC) and *m. extensor carpi radialis* (ECR), were exposed and

measured for length (from origin to insertion) and maximum girth (at thickest point) *in situ* then dissected out and weighed. *M. splenius* was also dissected out and weighed.

Statistical methods

Experimental data were analysed utilizing orthogonal polynomial contrasts (Alvey *et al.* 1980) for differences between control and treated animals. The same analysis was used to study the following contrasts; entire *v.* gonadectomized; castrated males *v.* spayed females; linear and quadratic dose responses within wether, ram and spayed ewe lambs; sex effect (rams *v.* entire ewes). The logarithmic form of the Gompertz equation:

$$\log e W = \log e A^{-be^{-kt}}$$

(where W is size at time t and A its ultimate value, k is the rate constant and b is time zero (Richards 1959)) was fitted to adjusted mean cannon bone lengths using optimization procedures of the Genstat statistical package (Alvey *et al.* 1980). The equation coefficients and their standard errors plus the residual standard deviation were used to compare elongation of cannon bones between different experimental groups during the experimental period. Other bone data were adjusted to mean initial radius length by analysis of covariance. Data from wether and spayed ewe lambs were pooled and analysed by use of orthogonal polynomial contrasts for differences between control and oestradiol-treated animals and for linear and quadratic dose responses.

RESULTS

The general trend was for faster live-weight gain in male lambs and for reduced fatness with oestradiol treatment.

Effectiveness of oestradiol implants

Dose of oestradiol (calculated as mean weight loss from implants) was 2·6, 6·0 and 16·4 mg (i.e. 14·4, 33·3 and 91·1 μg/day, assuming constant rate of release) for low, medium and high dose groups respectively (Table 2). Oestradiol concentration in plasma of implanted wethers was elevated immediately after implantation but was near to pre-implantation values at the end of the experiment (Table 3).

Effects of sex – entire males v. entire females

Entire ram lambs tended to have a higher rate of live-weight gain than entire ewe lambs (coefficient k, Table 4). Although the rate of elongation of cannon bones was not different to that of entire female lambs (Tables 5 and 7), the ram lambs tended to have a longer vertebral column, thicker limb bones with greater diameter:length ratios and generally heavier and larger bones (Table 5). The only significant differences, however, were longer 12th vertebral spine, thicker tibia bone and heavier humerus (Tables 5 and 6). Ram lambs had significantly thicker BIC and ECR muscles and heavier BIC and splenius muscles than ewe lambs (Tables 8 and 9). In addition, they had heavier head and greater protein and water content than entire ewe lambs, whereas the latter animals had greater carcass and non-carcass fat content (Tables 10 and 11).

Effects of sex – castrated males versus spayed females

Control wether lambs tended to have slower live-weight gain (coefficient k, Table 4) and had less non-carcass fat than control spayed female lambs (Tables

Table 3. *Mean plasma oestradiol-17β concentration (pg/ml) ± S.E.M. of non-implanted lambs (control) and wether lambs implanted with silicone rubber implants containing oestradiol-17β recorded at 3 stages of the trial* (n = *8*)

Group	Day of experiment		
	Pre-implantation	1	175
Control wethers	30*	—	—
Control rams	20 ± 8	—	24 ± 4
Control spayed ewes	43*	—	—
Control entire ewes	13 ± 3	—	26 ± 7
Low dose wethers	—	136 ± 44	29 ± 8
Medium dose wethers	43*	102 ± 47	42 ± 12
High dose wethers	30*	167 ± 60	31 ± 13

* Oestradiol concentration was obtained from pooled samples, collected at least 1 week after gonadectomy in the case of wethers and spayed ewes.

Table 2. *Loss of weight (mg) from small (L), medium (M) and large (H) silicone rubber implants containing oestradiol-17β, after 180 days subcutaneous implantation in lambs* (n = *8*)

Group	L (initial content 6 mg oestradiol)	M (initial content 22 mg oestradiol)	H (initial content 52 mg oestradiol)
Entire rams	2·5	6·0	22·7
Wethers	2·6	6·1	11·7
Spayed ewes	2·8	6·0	14·7

Table 4. *Coefficients A, b and k (and their standard errors, S_a, S_b and S_k) from Gompertz equations fitted to mean live weights (adjusted to initial live weight) of control and treated wether, ram, spayed ewe and entire ewe lambs implanted with silicone rubber implants containing low, medium or high doses of oestradiol-17β* (n = 8) (*rsd = residual standard deviation*)

Group	A (antilog)	(S_A)	B	(S_b)	k	(S_k)	rsd
Control wethers	36·21	0·0237	0·1566	0·0185	0·0225	0·0095	0·0236
Low dose wethers	40·10	0·0464	0·1756	0·0246	0·0154	0·0080	0·0274
Medium dose wethers	40·36	0·0439	0·1823	0·0234	0·0123	0·0049	0·0204
High dose wethers	41·01	0·0549	0·1768	0·0281	0·0140	0·0079	0·0282
Control rams	38·52	0·0332	0·1692	0·0222	0·0201	0·0099	0·0284
Low dose rams	37·93	0·0327	0·1611	0·0212	0·0193	0·0095	0·0270
Medium dose rams	40·48	0·0349	0·1809	0·0201	0·0160	0·0062	0·0236
High dose rams	40·00	0·0296	0·1772	0·0200	0·0192	0·0076	0·0256
Control spayed ewes	37·48	0·0275	0·1609	0·0191	0·0200	0·0085	0·0243
Low dose spayed ewes	40·21	0·0360	0·1805	0·0217	0·0169	0·0071	0·0264
Medium dose spayed ewes	40·23	0·0373	0·1743	0·0221	0·0171	0·0078	0·0270
High dose spayed ewes	42·82	0·0356	0·1935	0·0208	0·0164	0·0016	0·0252
Control entire ewes	37·85	0·0235	0·1682	0·0159	0·0186	0·0060	0·0181

10 and 11). Also, the wether lambs had longer vertebral column, longer 1st vertebral spine and larger and heavier humerus bone, but shorter 1st rib (Tables 5 and 6). In the case of muscles, wether lambs had longer, thinner and lighter ECR but heavier splenius muscles than spayed females (Tables 8 and 9).

Effects of gonadectomy – general

In male lambs, castration reduced final live weight (coefficient A, Table 4 and Table 10), weight of the head, A measurement of the LD muscle and protein and water content in the carcass, whereas it increased C measurement of fat depth and fat content (Tables 10 and 11). Ovariectomy in females had very little effect on live-weight or on most body components except the uterus (Tables 10 and 11). Spayed ewe lambs had shorter teats than entire ewes and their uteri were one third of the weight of those of entire ewes (Tables 10 and 11).

Effects of gonadectomy – male lambs

In male lambs castration increased growth in length of cannon bones but reduced length of the 12th vertebral spine and length of tibia (Tables 5, 6 and 7). Castration significantly reduced the girth of BIC and ECR muscles and the weight of ECR and splenius muscles (Tables 8 and 9).

Effects of gonadectomy – female lambs

Ovariectomy increased elongation of cannon bones and increased length of the vertebral column, 12th vertebral spine and radius diameter:length ratio (Tables 5, 6 and 7) but did not affect any of the muscle measurements (Tables 8 and 9).

Effects of oestradiol implants – general

Oestradiol treatment tended to increase final live weight in gonadectomized lambs of both sexes and had minor growth stimulatory effects in entire rams (Tables 10 and 11, coefficient A in Table 4). For many skeletal components there was a positive linear relationship with the dose of the hormone implant although in a few cases, within sex class, there appeared to be a quadratic response (Tables 5 and 6).

Effects of oestradiol implants – gonadectomized lambs

Oestradiol treatment reduced ultimate length of cannon bones (coefficient A, Table 7). Cannon bones of treated animals gained less in absolute terms during the treatment period and consequently were shorter at the end of the experiment than in control lambs (Tables 5 and 6). Oestradiol-treated wether and spayed ewe lambs tended to have longer vertebral column and 12th rib than their relative controls. However, there was no significant effect of oestradiol on length of medial limb bones, i.e. radius and ulna, humerus, femur and tibia (Tables 5 and 6). Oestradiol treatment tended to increase diameter of limb bones in gonadectomized lambs of both sexes, with the effect being significant in the case of the femur (Tables 5 and 6) and it increased cortical thickness of the femur, ash proportion and ash:organic matter ratio of the rib in wethers (Tables 5 and 6). Treatment with oestradiol did not significantly affect muscle length in gonadectomized lambs but girth and weight of muscles, e.g. BIC muscle, were generally increased (Tables 8 and 9). Because of similar trends in the responses of animals to oestradiol treatment, data from castrated male and spayed female lambs were

Table 5. *Mean length (mm), diameter (mm) and other measurements of some bones adjusted to mean initial radius length* (n = *8*) *and difference between control* (*C*) *and treated* (*T*) *wether, ram, spayed ewe and entire ewe lambs implanted with low* (*L*), *medium* (*M*) *or high* (*H*) *dose silicone implants containing oestradiol-17β*

	Wethers					Rams					Spayed ewes					Entire ewes	
	C	L	M	H	C *v.* T	C	L	M	H	C *v.* T	C	L	M	H	C *v.* T	C	ESE
Bone length																	
Cannon bone length	148	146	146	144	*	145	144	145	145		148	143	144	145	***	146	1
Gain in cannon bone	13	12	11	10	*	11	10	10	11		13	9	10	10	***	12	1
Total vertebral column	814	825	821	837		824	820	816	844		811	816	834	838		807	12
1st rib	91	94	94	96		96	92	95	97		97	94	98	95		93	3
1st vertebral spine	46	45	44	43		46	45	48	48		39	40	39	41		42	2
12th rib	178	184	182	190		180	181	191	190		180	181	194	192		186	5
12th vertebral spine	21	22	21	23		25	23	18	23		23	21	23	24		20	1
Radius	137	137	137	136		137	135	137	136		136	135	136	136		136	1·31
Ulna	169	169	170	167		170	168	169	167		168	170	169	167		168	1·90
Humerus	121	121	122	122		122	122	122	122		119	120	122	120		121	1·27
Femur	154	155	154	157		157	153	156	156		154	153	156	155		155	1·81
Tibia	177	179	177	180		181	178	180	179		178	179	180	178		179	2·00
Bone diameter																	
Radius	16·7	17·7	17·6	17·6		17·7	18·3	17·2	18·2		17·3	16·9	17·6	18·1		16·6	0·57
Humerus	18·6	19·3	19·4	19·9		19·2	19·6	19·1	19·5		18·2	18·5	19·1	19·4		18·4	0·59
Femur	18·1	19·6	19·5	19·2	*	19·1	19·0	18·6	19·2		18·9	18·3	19·2	19·6	*	18·5	0·54
Tibia	15·2	15·5	15·3	15·5		15·7	15·8	15·5	15·5		15·3	14·8	15·3	15·8		14·9	0·39
Diameter : length ratio																	
Radius	0·12	0·13	0·13	0·13		0·13	0·13	0·13	0·13		0·13	0·12	0·13	0·13		0·12	0·00
Humerus	0·15	0·16	0·16	0·16		0·16	0·16	0·16	0·16		0·15	0·15	0·16	0·16		0·15	0·00
Femur	0·12	0·13	0·13	0·12		0·12	0·12	0·12	0·12		0·12	0·12	0·12	0·13		0·12	0·00
Tibia	0·09	0·09	0·09	0·09		0·09	0·09	0·09	0·09		0·09	0·09	0·08	0·09		0·08	0·00
Other measurements																	
FMCT (mm)	2·94	3·48	3·27	3·13	*	3·02	3·23	3·20	3·58		3·62	3·17	3·33	3·64		3·17	0·24
12th rib ash (g/kg)	528	559	556	552	*	535	548	561	556		552	556	554	565		534	1·04
12th rib (A : R)	1·12	1·27	1·25	1·24	*	1·16	1·22	1·28	1·25		1·23	1·27	1·24	1·31		1·16	0·05
Humerus wt. (g)	89·3	94·2	96·1	94·6		95·3	94·4	92·6	92·2		85·8	82·2	90·8	91·9		83·8	3·2

ESE, estimated standard error.
* $P < 0{\cdot}05$, *** $P < 0{\cdot}001$.

Table 6. *Significance of oestradiol dose responses and effect of sex and castration analysed by orthogonal polynomial contrasts in skeletal measurements of wether* (*W*), *ram* (*R*), *entire* (*E*) *and spayed ewe* (*SE*) *lambs*

	Gonadectomy			Linear			Quadratic			
Parameter	Males R *v.* W	Females E *v.* SE	W *v.* SE	W	R	SE	W	R	SE	R *v.* E
Bone length										
Cannon bone					*	*			*	
Gain in cannons	*				*	*			*	
Total vertebral col.		*	*			**			*	
1st rib			***							
1st vertebral spine			***	*	***	*	*	***	**	
12th rib				**		***	*		**	
12th vertebral spine	***	*			*			*	*	***
Radius			*							
Ulna										
Humerus			*				*			
Femur										
Tibia	*				*					
Bone diameter										
Radius					*					
Humerus			*	*	*		*			
Femur						*				
Tibia										*
Diameter:length ratio										
Radius		*				**			*	
Humerus						**				
Femur						**				
Tibia										
Other measurements										
FMCT						*			*	
12th rib ash				*		**	*		*	
12th rib A:R				*		**	**		*	
Humerus weight			***				***	**		***

* $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.

Table 7. *Coefficients A, b and k* (*and their standard errors,* S_A , S_b *and* S_k) *from Gompertz equations fitted to mean cannon bone length* (*adjusted to mean initial cannon bone length by analysis of covariance*) *and residual standard deviation* (*rsd*) *of control and treated wether, ram, spayed ewe and entire ewe lambs implanted with silicone rubber implants containing low, medium or high doses of oestradiol-17β* (n = *8*) (*rsd = residual standard deviation*)

Group	A (antilog)	(S_A)	B	(S_b)	k	(S_k)	rsd
Control wethers	161·6	6·98	0·1876	0·0417	0·0039	0·0013	0·4967
Low dose wethers	156·2	7·94	0·1537	0·0488	0·0046	0·0023	1·1061
Medium dose wethers	149·1	2·09	0·1041	0·0124	0·0092	0·0026	0·7013
High dose wethers	147·0	2·43	0·0920	0·0149	0·0096	0·0036	0·9419
Control rams	149·9	3·19	0·1162	0·0194	0·0078	0·0027	0·8855
Low dose rams	148·7	3·80	0·1072	0·0235	0·0070	0·0030	0·8769
Medium dose rams	149·2	2·53	0·1100	0·0156	0·0069	0·0019	0·5334
High dose rams	150·6	3·70	0·1195	0·0226	0·0068	0·0024	0·7828
Control spayed ewes	165·7	8·23	0·2114	0·0484	0·0034	0·0011	0·6387
Low dose spayed ewes	148·4	3·91	0·1069	0·0243	0·0072	0·0032	1·9813
Medium dose spayed ewes	146·6	3·15	0·0931	0·0196	0·0094	0·0045	0·2518
High dose spayed ewes	147·9	2·15	0·1013	0·0131	0·0089	0·0026	0·7289
Control entire ewes	151·5	2·94	0·1243	0·0177	0·0075	0·0021	0·7210

Table 8. *Mean length, girth, weight, and girth:length ratio of some dissected muscles of control (C) and treated (T) wether, ram, spayed ewe and entire ewe lambs implanted with low (L), medium (M) or high (H) dose implants containing oestradiol-17β*

	Wethers					Rams					Spayed ewes					Entire ewes	
	C	L	M	H	C *v.* T	C	L	M	H	C *v.* T	C	L	M	H	C *v.* T	C	ESE
M. biceps brachii (BIC)																	
Length (mm)	157	159	157	159		159	161	164	158		156	154	160	157		154	2·94
Girth (mm)	65·6	67·6	69·2	70·9	*	70·9	70·2	70·0	71·0		65·9	66·9	67·7	70·7	*	66·7	1·83
Girth:length ratio	0·42	0·43	0·44	0·45		0·44	0·43	0·43	0·45		0·42	0·43	0·43	0·45		0·43	0·01
Weight (g)	28·7	30·3	31·7	33·1	*	31·6	32·7	32·0	31·5		28·2	29·3	30·5	32·9	*	28·4	1·49
Concentration in carcass (g/kg)	35·6	35·3	38·5	38·4		38·6	40·7	38·1	36·9		33·9	34·9	37·2	37·1	*	35·2	0·16
M. extensor carpi radialis (ECR)																	
Length (mm)	180	179	181	177		180	177	181	177		179	173	173	175		176	2·60
Girth (mm)	75·2	80·4	78·9	78·0		80·2	78·7	76·1	77·8		76·0	76·3	75·8	78·0		73·5	2·49
Girth:length ratio	0·42	0·45	0·43	0·44		0·45	0·44	0·42	0·44		0·42	0·44	0·44	0·44		0·42	0·01
Weight (g)	32·6	36·4	36·0	35·8		38·0	37·6	36·1	35·7		34·6	32·0	32·4	36·4		34·3	2·14
Concentration in carcass (g/kg)	44·3	42·4	43·7	41·4		46·2	46·4	42·8	41·8		41·6	38·1	39·3	41·1		42·4	0·02
M. splenius																	
Weight (g)	7·5	7·3	7·4	9·6		15·4	13·9	10·4	9·8		5·6	7·5	6·5	7·1		6·0	1·70
Concentration in carcass (g/kg)	9·6	8·7	9·3	11·2		18·5	17·0	12·0	11·3	*	6·7	8·9	8·0	8·1		7·5	0·19

* $P < 0{\cdot}05$.

Table 9. *Significance of effect of gonadectomy, oestradiol dose response and sex on muscle data analysed by orthogonal polynomial contrasts in wether (W), ram (R), entire ewe (E) and spayed ewe (SE) lambs*

	Gonadectomy			Linear			Quadratic			
	R *v.* W	E *v.* SE	W *v.* SE	W	R	SE	W	R	SE	R *v.* E
M. biceps brachii (BIC)										
Length					*			**		
Girth	**				**		**	*		*
Girth : length ratio							*			
Weight					*		***	**		*
Proportion of carcass										
M. extensor carpi radialis (ECR)										
Length		***		*		**			***	
Girth	*	*								**
Girth : length ratio										
Weight	*	*						*		
Proportion of carcass										
M. splenius										
Weight	***	**		***	***				**	***
Proportion of carcass										

* $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.

pooled and analysed using orthogonal polynomial contrasts. The results, which are shown in Tables 12 and 13, substantiate the inhibitory effect of oestradiol treatment on growth of cannon bones and its stimulatory effects on the vertebral column, 12th rib, BIC muscle and ECR muscle in these animals.

Oestradiol treatment increased teat length, carcass protein and water content and decreased fat content in the wether and spayed ewe lambs (Tables 10 and 11). In addition, spayed ewe lambs treated with oestradiol had significantly longer teats, heavier warm carcass, head, and uteri weights, and had less fat and more protein and water content in the carcass than their controls (Tables 10 and 11).

Effects of oestradiol implants – entire ram lambs

Treatment of ram lambs with oestradiol had some minor stimulatory effects on skeletal development (Table 5 and 6), it reduced weight of the splenius muscle (Tables 8 and 9) and testes, and it increased teat length (Tables 10 and 11).

DISCUSSION

These results show that the effects of gender and sex steroids on musculo-skeletal growth of sheep vary for different regions of the body, probably depending on the relative state of maturity of each region. In general, entire males had heavier muscles and distal limb bones than females, and oestradiol treatment of rams, wethers and spayed ewes stimulated growth of central skeletal regions but inhibited growth in distal limbs. This can be interpreted as an acceleration of the differential maturation of the skeleton where the central skeletal core ceases to increase in size later than the distal limbs. In the current study this effect of maleness and oestradiol was manifested as a comparative elongation of the vertebral column and an inhibition of growth (early cessation) of the cannon bones and associated muscles.

Effectiveness of oestradiol implants

Loss of weight by the oestradiol-containing implants and plasma oestradiol concentrations indicated that treated animals had received substantial amounts of oestradiol during the experimental period. This was borne out by increases in teat length and uterine weight and the reduction of testes weight in the implanted lambs.

Effects of exogenous oestrogens

In the present study the high dose of oestradiol resulted in an increase of 19%, 8% and 21% live weight in treated wether, ram and spayed ewes, respectively, above that of their controls. These values are comparable to those of Muir (1985) who stated that oestrogens may stimulate live-weight gain by 10–20% in ruminants. However, gains in live weight in the present experiment were not exceptionally high (159 g/day maximum) compared with other data for growth rates of lambs on pasture (e.g. 230 g/day, Everest & Scales 1983). In experiments which have reported greater stimulation in live weight of lambs treated with oestradiol than recorded here, high quality feeds were used. For example Galbraith *et al.*

Table 10. *Mean fresh weights of some non-carcass components, carcass measurements and carcass chemical composition values* (n = *8*) *and difference between control* (*C*) *and treated* (*T*) *wether, ram, spayed ewe and entire ewe lambs implanted with low* (*L*), *medium* (*M*) *or high* (*H*) *dose silicone implants containing oestradiol-17β*

	Wethers					Rams					Spayed ewes					Entire ewes	
	C	L	M	H	C *v.* T	C	L	M	H	C *v.* T	C	L	M	H	C *v.* T	C	ESE
Teat length (mm)	8·5	17·7	17·4	17·0	***	8·2	13·7	15·2	16·9	***	9·9	12·9	16·7	17·9	***	12·5	1·1
Final live weight (kg)	39·4	42·6	40·7	43·0		41·7	40·4	42·6	43·2		40·4	40·5	42·8	44·7	**	40·0	1·3
Live-weight gain (g/day)	124	145	133	147		139	130	145	150		131	132	147	159	**	128	7·6
Warm carcass weight (kg)	16·9	18·2	17·1	17·9		17·4	17·1	18·0	18·2		17·3	17·5	17·5	18·8	**	17·1	0·7
Organ weight (g)																	
Head	2250	2420	2400	2310		2460	2460	2550	2540	*	2170	2070	2340	2480	*	2190	90
Testes						339	246	118	97	***						4·1	2·7
Uterus											10·0	19·0	22·7	19·9	***	32·3	2
Fat depot weight (g)																	150
Total non-carcass fat	1389	1376	1207	1175		1267	1066	1255	1142		1739	1505	1392	1473	*	1585	
Carcass measurements (mm)																	
GR measurement	8·6	9·3	5·7	7·5		6·7	5·3	8·5	8·5	*	10·2	9·6	6·5	8·4		8·7	1·3
LD A measurement	51·1	54·4	53·2	52·6		54·6	54·0	53·3	52·8		53·0	51·4	54·8	54·8		53·7	1·5
LD B measurement	23·3	24·9	24·3	23·8		24·6	22·8	23·9	23·9		25·2	23·7	24·4	27·2		24·7	1·4
C measurement	5·4	4·1	2·6	3·7	*	3·4	3·0	4·1	4·5		5·1	5·0	3·7	3·8		4·8	0·9
Chemical composition (g/kg on DM basis)																	
Carcass fat	570	552	499	516	**	524	492	533	533		573	583	521	516	**	576	1·75
Carcass protein	340	351	395	379	**	381	408	366	370	*	333	334	378	384	**	336	1·4
Carcase water	513	525	541	543	*	540	551	519	534		500	508	549	540	**	510	1·2

* $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.

Table 11. *Significance of oestradiol dose responses and effect of sex and castration analysed by orthogonal polynomial contrasts in live weight growth and body composition of wether (W), ram (R), entire (E) and spayed ewe (SE) lambs*

	Gonadectomy			Linear			Quadratic			
Parameter	Males R *v.* W	Females E *v.* SE	W *v.* SE	W	R	SE	W	R	SE	R *v.* E
Final teat length		*		***		***	***	**	***	***
Final live weight						***	**		***	
Warm carcass weight									*	
Head weight	*		*		***			***		**
Total non-carcass fat weight			**	*	***	*		**		*
Testes weight								**		
Uterus weight		***								
GR measurement								**		
LD A measurement	*									
LD B measurement									*	
C measurement	*						*	**		
Carcass fat	*		*		*		***	***		**
Carcass protein	**				*		***	***		**
Carcass water	*						***	**		*

* $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.

(1990) used a pelleted concentrate feed and reported live-weight gains of 257 g/day in treated and 206 g/day in control wether lambs; a 25% increase in the treated group.

Reports in the literature on the effects of oestradiol on live-weight growth of sheep are inconsistent but are often based on dissimilar experimental conditions. For instance Galbraith *et al*. (1990) treated lambs at 18 kg (approximately 10 weeks old), whereas in studies reported by Hunter *et al*. (1987) and Bass *et al*. (1989), animals were treated from 4–6 weeks of age. Therefore, contradictory responses to oestradiol treatment may arise from differences in ages (stages of maturity) of animals. Growth rate in sheep increases sharply in early postnatal life, reaching a maximum when the animal achieves about 20% of its mature weight and declines thereafter (Butterfield 1988). Other studies have shown that oestrogens are not very effective in stimulating live-weight gain in lambs of 0–5 months old (J. J. Bass, personal communication). It is possible to speculate that treatment with oestrogens in early postnatal life has little effect on live-weight growth simply because these animals are already growing at a high rate. However, with the progress of maturity, i.e. when animals have heavier weights and growth rates slow down, effects of treatment with oestrogens or other growth promoters may become more pronounced.

Oestradiol treatment reduced weight of carcass fat and non-carcass fat, especially in wether and spayed female lambs, which is in agreement with other studies on fat deposition in ruminants (Galbraith & Topps 1981; Bass *et al*. 1989). The latter authors proposed the use of oestrogens in sheep not only to promote growth but also to prevent overfatness in lambs. The present study confirms this view and indicates that increased live-weight gain also could be achieved through the use of oestrogens in pasture-fed sheep. Lack of a fat-reducing effect of oestradiol in the rams can be explained by the fact that they were leaner than the other lambs to start with; the fat levels in spayed ewes and wethers only approaching values as low as those of the rams at the high dose oestradiol treatment.

It is possible that compounds with sex steroidal activity were present in the pasture consumed by lambs in this study, either as naturally occurring phyto-oestrogens or from excretion of oestradiol or its metabolites by the lambs which were treated with the oestradiol-containing implants. However, this concern can be largely allayed by the data for teat length in non-implanted wether lambs (mean = 8·5 mm, Table 10) which are similar to those (mean = 8·2 mm) of wether lambs at equivalent live weights which had not been exposed to oestrogens in the study reported by Galbraith *et al*. (1997). The sensitivity of this parameter to oestrogens is well demonstrated by the lengths recorded in the oestradiol-treated wethers in the present study (see Table 10).

Effects of sex and gonadectomy

Increased growth in length of cannon bones following castration suggests that lack of androgens may have delayed the epiphyseal closure of these early maturing

Table 12. *Skeletal data (means, n = 8) of non-implanted lambs (C) and lambs (T) implanted with silicone rubber implants containing different doses (L, M, H) of oestradiol (data pooled from wether and spayed ewe lambs); comparison between control and treated lambs, and response to dose of oestradiol in treated lambs*

	Dose						
	C	L	M	H	ESE	C *v.* T	Dose response†
Bone length (mm)							
Cannon bones final	147	144	144	144	0·8	***	
Gain in cannon bones	13	11	10	10	0·8	***	
Total vertebral column	812	820	826	838	9·1	*	
1st rib	94	94	96	95	2·1		
1st vertebral spine	42	43	42	42	1·4		
12th rib	179	183	187	191	3·4	*	*
12th vertebral spine	22	22	22	23	0·7		
Radius	137	136	137	136	0·9		
Ulna	169	170	170	167	1·4		
Humerus	120	120	122	121	1·0		
Femur	154	154	155	156	1·4		
Tibia	177	179	178	179	1·4		
Bone diameter (mm)							
Radius	17·0	17·4	17·5	17·9	0·4	*	
Humerus	18·3	19·0	19·3	19·6	0·4	**	
Femur	18·4	19·0	19·3	19·4	0·4	*	
Tibia	15·2	15·2	15·3	15·7	0·2		
Diameter : length ratio							
Radius	0·124	0·127	0·128	0·132	0·003	*	
Humerus	0·153	0·157	0·158	0·162	0·003	*	
Femur	0·120	0·123	0·125	0·125	0·003	*	
Tibia	0·086	0·085	0·086	0·087	0·001		
Other measurements							
Humerus weight (g)	87·4	88·0	93·4	93·0	2·8		
FMCT (mm)	3·3	3·3	3·3	3·4	0·16		
12th rib ash (g/kg)	540	557	555	559	0·66	**	
12th rib (A : R)	1·178	1·262	1·249	1·274	0·032	**	
Muscle measurements							
M. biceps brachii (BIC)							
Length (mm)	156	156	158	158	2·24		
Girth (mm)	66	67	68	71	1·16	**	**
Girth : length ratio	0·422	0·431	0·435	0·449	0·01	*	
Weight (g)	28·4	29·8	31·1	32·9	1·00	***	**
Concentration in carcass (g/kg)	3·5	3·5	3·8	3·8	0·01	*	*
M. extensor carpi radialis (ECR)							
Length (mm)	179	176	177	176	2·07		
Girth (mm)	76	78	77	78	174		
Girth : length ratio	0·421	0·444	0·436	0·443	0·01	*	
Weight (g)	33·5	34·1	34·3	36·0	1·50		
Concentration in carcass (g/kg)	4·1	4·0	4·3	4·1	0·01		
M. splenius							
Weight (g)	6·5	7·4	7·0	8·3	0·80		
Concentration in carcass (g/kg)	0·82	0·87	0·87	0·95	0·01		

FMCT, femur maximum cortical thickness; ESE, estimated standard error.
* $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.
† Only linear dose responses shown, quadratic responses are all non-significant.

limb bones. Bones of castrated male lambs were also smaller in diameter which resulted in lower values for their diameter : length ratios. The decrease in cortical thickness, bone weight and ash proportion indicates retardation of bone deposition and mineralization. Stimulation of skeletal growth of ewe lambs as a result of spaying was not marked but was concordant with previous findings on growth of distal limbs in spayed heifers (Hubard Ocariz *et al.* 1970; Brannang 1971*b*).

Table 13. *Means of growth and body composition parameters* (n = *8*) *of non-implanted lambs* (*C*) *and lambs* (*T*) *implanted with silicone rubber implants containing different doses* (*L*, *M*, *H*) *of oestradiol-17β* (*data pooled from wether and spayed ewe lambs*); *comparison between control and treated lambs, and response to dose of oestradiol in treated lambs*

	Dose						Dose response	
	C	L	M	H	ESE	C *v.* T	Linear	Quadratic
Final teat length (mm)	9·18	15·20	16·94	17·31	0·70	***	***	
Final live weight (kg)	39·84	41·47	41·60	43·79	0·97	**	*	
Live-weight gain (g/day)	127	138	139	154	6	**	*	
Warm carcass weight (kg)	17·02	17·80	17·27	18·34	0·05			
Head weight (g)	2200	2240	2370	2400	70	*	*	
Total non-carcass fat weight	1566	1436	1294	1332	117	*		
GR measurement	9·4	9·4	6·1	8·7	1·0			
LD A measurement	52	53	54	54	1·0			
LD B measurement	24	24	24	26	1·0			
C measurement	5·3	4·6	3·1	3·7	0·6	**		*
Carcass fat (g/kg)	572	567	509	516	1·2	***	***	**
Carcass protein (g/kg)	337	343	387	381	1·0	***	***	**
Carcass water (g/kg)	507	516	540	541	0·8	***	**	

ESE, estimated standard error.
* $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.

Effects of castration in reducing muscular growth in male lambs were especially marked in the splenius muscle which has been observed previously (Brannang 1971*b*). In addition splenius muscle development was inhibited by oestradiol treatment in entire ram lambs. This muscle has been recognized as a target tissue for sex hormones in males of some species. For example, it showed a marked increase in size prior to the rut in deer stags (Tan & Fennessy 1981; Field *et al.* 1985). Seasonal changes in this muscle appear to be positively related to blood levels of testosterone in male deer (Field *et al.* 1985) which may explain the negative effect of castration on this muscle. Also the high dose of oestradiol used in this experiment may have inhibited secretion of luteinising hormone (LH) and consequently reduced blood levels of testosterone. This is supported by the marked reduction in weight of testes which could be attributed to lowered secretion of LH as a consequence of the negative feedback action of oestradiol (Shanbacher & Ford 1977). Thus testosterone may be regarded as an essential factor in determining the large splenius weight in rams and the response of this muscle to oestradiol could be explained by a reduction in the secretion of testosterone.

Factors that determine sexual dimorphism in mammals are not well understood although they involve both genetic and hormonal mechanisms (Short 1980). In the present experiment, lack of androgens affected growth of sheep more than lack of oestrogens. However, it was not possible to define a critical age at which sex hormones have most effect on growth.

Effects of oestrogens in gonadectomized animals

In this experiment, oestradiol treatment generally inhibited longitudinal growth of distal limb bones (cannon bones), stimulated that of vertebral column and ribs, but had little effect on linear growth of medial and proximal limb bones. There are few other reports of effects of oestrogens on specific bones in sheep. For example, oestradiol-17β implants increased rib size in lambs (Galbraith *et al.* 1997). Stimulation on the one hand and inhibition on the other of bone growth following administration of oestrogens has been explained by the premise that low doses of oestrogens stimulate longitudinal bone growth, while large doses inhibit it (Suzuki 1958; Silberberg & Silberberg 1972; Short 1980). In light of the results of the present study this explanation can no longer be supported. Firstly, the effects of oestradiol which were recorded here were generally linearly dose-related. Secondly, studies in meat animals show a disto-proximal and cranio-caudal pattern of skeletal maturation which results in differential maturity of the different regions of the skeleton (reviewed by Davies *et al.* 1984). Consequently, an interpretation of the present results is that oestradiol has accelerated closure of epiphyseal plates of early maturing bones (cannon bones), so that further elongation was limited, and it has stimulated longitudinal growth of late maturing bones (vertebral column and rib) where it did not cause premature closure of the epiphyseal plates. Differential growth patterns in normally growing skeletons were attributed to differences in rates of cell division or to differences in cartilage cell

population in epiphyseal plates (Burwell 1986). In an actively growing bone, oestrogens, which have anabolic effects, may increase the rate of cell division, thus producing longer bones. In bones approaching maturity such stimulation accelerates cessation of epiphyseal plate activity and will curtail elongation, i.e. cause a relative shortening of bones. Oestrogenic effects on bone may also be modified by the differential regulation of steroid receptors. For instance, in active bones, oestrogen receptors may be upregulated in comparison with those in bones where epiphyseal activity is slowing down.

In the present experiment there was little effect of oestradiol on final length of limb bones, which is in contrast to the effects reported previously (Wilkinson *et al*. 1955; Shroder & Hansard 1959). However, data from serial radiographs (not presented) indicated that the tibia bone was longer in oestradiol-treated wethers than controls at days 61 and 134. This suggests that there may have been early stimulation of linear growth by the hormone, the effect of which had disappeared towards the end of the trial.

In contrast to its minimal effects on their length, oestradiol tended to increase diameter (and thus, diameter : length ratio) and cortical thickness of limb bones, with the effect being significant in the case of the femur. These findings suggest that stimulation of periosteal growth was not accompanied by an equivalent amount of bone resorption on the medullary surface. General inhibition of bone resorption by oestrogens is recognized as a classic effect of these compounds and, thus, they are widely utilized for the treatment of osteoporosis in humans.

Treatment with oestradiol produced little effect on muscle length. This may be explained by the lack of effect on length of limb bones to which these muscles were attached. Growth in length of muscles follows that of intimately related bones (Hooper 1978*a*, *b*). In contrast to the lack of response of muscle length, girth of *m. biceps brachii* and consequently girth : length ratio were stimulated by oestradiol treatment which must have altered the shape of this muscle, i.e. made it relatively thicker for its length. Such remodelling of muscle shape may result from the direct anabolic effects of oestrogens on skeletal muscle (Galbraith & Topps 1981).

In the present experiment the most marked stimulatory effects of oestrogens on bone growth (especially on linear growth of vertebrae) were in spayed ewe lambs, more so than in wethers, whereas entire males showed little response at all. Some studies of the effects of oestrogens on live-weight growth in sheep have indicated that castrated male ruminants are the most responsive to oestrogen treatment (Galbraith & Topps 1981; Muir 1985; Hancock *et al*. 1991). However, according to Bradfield (1968), animals of different sexes take different growth routes to reach maturity. Consequently, variation in response to oestrogens may result from applying the treatment to animals of the same age but at different stages of maturity. In addition, Bradfield (1968) reported that exogenous oestrogens had more pronounced effects on growth at the lower slaughter weights (36·3 kg) where lambs of all sex/castration combinations except the entire males responded, whereas the spayed ewes were the only group of lambs to respond to treatment at the higher weight (45·4 kg). The study of Bradfield (1968) is concordant in both respects (sex class of responders and live weight at slaughter) with the present case and has a similar result.

On the basis of the results described in the present paper, it may be concluded that the sex steroids have an essential role in controlling skeletal growth in sheep. However, the effects of these hormones need to be interpreted with attention to degree of maturity of animals, differential pattern of skeletal maturation and dose of the hormone. Growth of early maturing parts of the skeleton was inhibited by the administration of exogenous oestrogens whereas that of late maturing parts of the skeleton was stimulated.

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REFERENCES

Alvey, N. G. *et al*. (1980). *GENSTAT: a General Statistical Program*. Lawes Agricultural Trust (Rothamsted Experimental Station). Parts I & II.

AOAC (1984). *Official Methods of Analysis. 14th edition*. Virginia: Association of Official Analytical Chemists.

Bass, J. J., Fowke, P. J., Duganzich, D. M. & Peterson, A. J. (1989). Effects of different doses of 17β-oestradiol on growth and carcass composition of wether and ewe lambs. *Journal of Agricultural Science, Cambridge* **113**, 183–187.

Bradfield, P. G. E. (1968). Sex differences in the growth of sheep. In *Growth and Development of Mammals* (Eds G. A. Lodge & G. E. Lamming), pp. 92–108. London: Butterworths.

Brannang, E. (1971*a*). Studies on monozygous cattle twins XXIII. The effect of castration and age of castration on the development of single muscles, bones and special sex characters. Part II. *Swedish Journal of Agricultural Research* **1**, 69–78.

Brannang, E. (1971*b*). Studies on monozygous cattle twins. XXIV. Some notes on the effect of ovariectomy. *Swedish Journal of Agricultural Research* **1**, 79–82.

Burwell, R. G. (1986). The growth of bone. In *Control and Manipulation of Animal Growth* (Eds P. J. Buttery, N. B. Haynes & D. B. Lindsay), pp. 53–65. London: Butterworths.

Butterfield, R. M. (1988). *New Concepts of Sheep Growth*. University of Sydney, Australia.

Davies, A. S. (1979). Musculoskeletal growth gradients: A contribution to quadrupedal mechanics. *Anatomia Histologia Embryologia* **8**, 164–167.

DAVIES, A. S., TAN, G. Y. & BROAD, T. F. (1984). Growth gradients in the skeleton of cattle, sheep and pigs. *Anatomia Histologia Embryologia* **13**, 222–230.

EVEREST, P. G. & SCALES, G. H. (1983). Pre and post weaning growth rates of ewes and lambs in the South Island. In *Lamb Growth* (Ed. A. S. Familton), pp. 41–46. Canterbury, New Zealand: Lincoln College.

FIELD, R. A., YOUNG, O. A., ASHER, G. W. & FOOTE, D. M. (1985). Characteristics of male fallow deer muscle at a time of sex-related muscle growth. *Growth* **49**, 190–201.

GALBRAITH, H. & TOPPS, J. H. (1981). Effect of hormones on the growth and body composition of animals. *Nutrition Abstracts and Reviews – Series B* **51**, 521–540.

GALBRAITH, H., HATENDI, P. R., ALDERSON, E. M. & SCAIFE, J. R. (1990). The effect of cimaterol and oestradiol-*17*β alone or combined on growth and body composition of wether lambs. *Animal Production* **51**, 311–319.

GALBRAITH, H., SINGH, S. B. & SCAIFE, J. R. (1997). Response of castrated male sheep to oestrogenic and androgenic compounds implanted alone or in combination. *Animal Science* **64**, 261–269.

GETTY, R. (Ed.) (1975). *Sisson and Grossman's The Anatomy of the Domestic Animals. 5th edition*. Philadelphia: Saunders.

HAMMOND, J. (1932). *Growth and Development of Mutton Qualities in Sheep*. London: Oliver and Boyd.

HANCOCK, D. K. L., WAGNER, J. F. & ANDERSON, D. B. (1991). Effects of estrogens and androgens on animal growth. In *Growth Regulation in Farm Animals* (Eds A. M. Pearson & T. R. Dutson), pp. 255–297. London: Elsevier.

HOOPER, A. C. B. (1978*a*). Muscles and bones of large and small mice compared at equal body weights. *Journal of Anatomy* **127**, 117–123.

HOOPER, A. C. B. (1978*b*). Bone length and muscle weight in mice subjected to genetic selection for the relative length of the tibia and radius. *Life Sciences* **22**, 283–286.

HUBARD OCARIZ, J. L., LITTLEJOHN, A. & ROBERTSON, I. S. (1970). A comparison of entire and ovariectomized beef heifers treated with ethylestrenol. *Journal of Agricultural Science*, *Cambridge* **74**, 349–356.

HUNTER, R. A., DAVEY, J. B. & BUTTERY, P. J. (1987). Fractional rate of protein synthesis in liver and in individual muscles of lambs: effect of time of sampling following the use of the continuous infusion technique. *Journal of Agricultural Science*, *Cambridge* **108**, 511–514.

MUIR, L. A. (1985). Mode of action of exogenous substances on animal growth: an overview. *Journal of Animal Science* **61**(Suppl. 2), 154–180.

PÁLSSON, H. (1939). Meat qualities in the sheep with special reference to Scottish breeds and crosses. *Journal of Agricultural Science*, *Cambridge* **29**, 544–626.

PETERSON, A. J., FAIRCLOUGH, R. J. & SMITH, J. F. (1975). Radioimmunoassay of estradiol-17β in bovine peripheral plasma with and without chromatography. *Steroids* **25**, 487–496.

RICHARDS, F. J. (1959). Flexible growth function for empirical use. *Journal of Experimental Botany* **10**, 290–300.

SHANBACHER, B. D. & FORD, J. J. (1977). Gonadotropin secretion in cryptorchid and castrate rams and the acute effects of exogenous steroids treatment. *Endocrinology* **100**, 387–393.

SHORT, R. V. (1980). The hormonal control of growth at puberty. In *Growth in Animals* (Ed. T. L. J. Lawrence), pp. 24–45. London: Butterworths.

SHRODER, J. D. & HANSARD, S. L. (1958). Effects of orally administered stilbestrol upon growth and upon calcium and phosphorus metabolism in lambs. *Journal of Animal Science* **17**, 343–352.

SILBERBERG, M. & SILBERBERG, R. (1972). Steroid hormones and bone. In *The Biochemistry and Physiology of Bone, 2nd edition. Volume II* (Ed. G. H. Bourne), pp. 401–484. New York: Academic Press.

SPENCER, G. S. G. (1985). Hormonal systems regulating growth. A review. *Livestock Production Science* **12**, 31–46.

SUZUKI, H. K. (1958). Effects of estradiol-17-beta-n-valerate on endosteal ossification and linear growth in the mouse femur. *Endocrinology* **63**, 743–747.

TAN, G. Y. & FENNESSY, P. F. (1981). The effect of castration on some muscles of red deer (*Cervus elaphus* L.). *New Zealand Journal of Agricultural Research* **24**, 1–3.

TANDLER, J. & KELLER, K. (1910–1911). Uber den einfluss der Kastration auf den Organismus. IV. Die Korperform der Weiblichen Fruhkastraten des Rindes. *Archives fur Entwicklungsmechanik der Organismen* **31**, 289–306.

WALLACE, L. R. (1948). The growth of lambs before and after birth in relation to the level of nutrition. Parts I, II & III. *Journal of Agricultural Science*, *Cambridge* **38**, 93–153, 243–302, 367–401.

WILKINSON, W. S., O'MARY, C. C., WILSON, G. D., BRAY, R. W., POPE, A. L. & CASIDA, L. E. (1955). The effect of diethylstilbestrol upon growth, fattening, and certain carcass characteristics of full-fed and limited-fed western lambs. *Journal of Animal Science* **14**, 866–877.

ZONDEK, B. (1936). Impairment of anterior pituitary function by follicular hormone. *Lancet* **231**, 842–847.