STUDIES OF THE SKELETON OF THE SHEEP

III. THE RELATIONSHIP BETWEEN PHOSPHOROUS INTAKE AND RESORPTION AND REPAIR OF THE SKELETON IN PREGNANCY AND LACTATION

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(With Plates 1, 2 and 3 and Two Text-figures)

In Part II of this series an experiment was described in which the amounts of mineral matter withdrawn from the skeleton during pregnancy and lactation and the proportion recovered during a subsequent dry period were determined (Benzie, Boyne, Dalgarno, Duckworth, Hill & Walker, 1956). Radiological observations of the skeletons were made, and these, together with the results of bone analyses were compared with changes in serum calcium values. The experiment reported here was similar to that reported in Part II except for the basal diet which was designed to provide a low-phosphorus intake instead of the low-calcium intake of Part II.

METHODS

The plan of this experiment is set out in Table 1. The basal ration was designed to provide between 1·0 and 1·5 g. of phosphorus daily. This was converted to a ration of moderate phosphorus content by adding 3·0 g. of phosphorus (as disodium hydrogen phosphate) daily. The two contrasting types of ration are referred to below as 'low-phosphorus' and 'moderate-phosphorus' rations, respectively.

Sixty Cheviot ewes, of $3\frac{1}{2}$ years of age, were randomized according to live weight and date of tupping into six groups, each of ten ewes. Cast ewes were not used, and care was taken to ensure that all animals had sound mouths at the beginning of the experiment to permit observation of the effect of resorption of mandibular bone on the mobility of the incisors.

The basal diet for most of the experimental period was 100 g. daily of chopped oat straw and 800–1400 g. daily of concentrates. The concentrate mixture fed during the first part of the experiment consisted of 79 % dried sugar-beet pulp, 10 % maize gluten meal, 8 % maize meal, 1 % blood meal, 1 % meat meal and 1 % common salt. Two ml. of cod-liver oil were poured on to the daily concentrate ration of each ewe. Because of the low energy value and rather poor palatability of this mixture, certain modifications in the diet were necessary. During late gestation, ewes that began to refuse food were given a small amount of chopped turnip (1–2 lb. daily) until appetite improved. These quantities were very small when calculated as a daily intake of air-dry material over the whole experiment. In early lactation the concentrate mixture was modified slightly, maize meal being replaced by a larger amount of flaked maize, maize gluten meal being increased and dried sugar-beet pulp being reduced by a corresponding amount. The new mixture was 70 % dried sugar-beet pulp, 12 % maize gluten meal, 15 % flaked maize, 1 % blood meal, 1 % meat meal and 1 % common salt. Also in early lactation fresh potatoes were introduced, 600 g. being fed daily for about 10 weeks. All feedingstuffs were analysed for calcium and phosphorus content.

Quantities of food, and of calcium and phosphorus, offered daily at different times throughout the experiment are set out in Table 2, together with estimates of the daily provision of total digestible nutrients (T.D.N.) and digestible crude protein. While

Table 1. *The experimental treatment of the six groups of Cheviot ewes*

Group	Level of dietary phosphorus	Period for which it was fed	Interval between parturition and killing days
A	—	(Control killed in early gestation)	−120
B	Moderate	From early gestation to mid-lactation	+60
C	Moderate	From early gestation to mid-dry period	+180
D	Low	From early gestation to mid-lactation	+60
E	Low	From early gestation to mid-dry period	+180
F	Low	From early gestation to mid-lactation	—
	Moderate	From mid-lactation to mid-dry period	+180

the amounts of food offered were adjusted so that they approximated to the United States National Research Council (1945) recommended energy and protein allowances for pregnant and lactating sheep there were times, particularly during late pregnancy and early lactation, when it was considered desirable to restrict the food offered to the amount that was consumed completely by all, or nearly all, the animals. While avoiding the large-scale losses of appetite that cause difficulty in interpreting results, it was unavoidable that the mean daily intake of T.D.N. was below the recommended allowance during late pregnancy and the dry period.

Blood inorganic phosphorus and serum calcium concentrations were determined at 4-weekly intervals using the methods cited in Part I (Benzie, Boyne, Dalgarno, Duckworth, Hill & Walker, 1955).

Radiographs were taken of the skeleton at the same stages of the experiment and using the same apparatus and exposures as described in Part II (Benzie *et al.* 1956).

RESULTS

Food consumption and calcium and phosphorus intakes

These data are set out in Table 3.

The mean total food intakes were similar in groups killed at comparable times, largely because the amounts of food offered were equalized among the groups.

The mean daily phosphorus intake from early gestation to mid-lactation was 1·3–1·4 g. on the low-phosphorus ration and 4·2–4·5 g. on the moderate-phosphorus ration, and from mid-lactation to mid-dry period 1·5 g. on the low-phosphorus ration and 4·4–4·5 g. on the moderate-phosphorus ration.

Mortality, live weights and carcass weights of ewes

These data are set out in Table 4.

Animals that failed to survive to the date when they were due for slaughter, and those in which milk production ceased in less than 6 weeks after lambing were discarded. There were 9 remaining in groups C and D, 8 in groups B and F and 7 in group E.

The live weights of all animals decreased considerably during late pregnancy and early lactation, the decrease in ewes on the low-phosphorus ration (groups D, E and F) being significantly greater ($P < 0{\cdot}05$) than the decrease in ewes on the moderate-phosphorus ration (groups B and C). Between mid-lactation and the end of the experiment, ewes fed on the low-phosphorus ration to mid-lactation and moderate-phosphorus thereafter (group F) gained significantly ($P < 0{\cdot}05$) more weight than those fed the low-phosphorus ration throughout (group E). The decrease in mean live weight between early

Table 2. *Quantities of food and of calcium and phosphorus offered daily at different times throughout the experiment, together with calculated mean quantities of total digestible nutrients* (T.D.N.) *and digestible crude protein* (D.C.P.) *offered and a feeding standard, in grammes*

	Straw	Concentrates	Potatoes	Calcium	Phosphorus group B	C	D	E	F	Nutrients offered T.D.N.	D.C.P.	Nutrients recommended* T.D.N.	D.C.P.
Mid-pregnancy	100	800	—	5·4	4·0	4·0	1·0	1·0	1·0	649	75	—	—
Late pregnancy	100	1000	—	6·4	4·3	4·3	1·3	1·3	1·3	801	94	1043	100
Mid-lactation	100	1400	600	8·8	4·9	4·9	1·9	1·9	1·9	1233	149	1223	136
Dry period	100	800	—	5·4	—	4·1	—	1·1	4·1	647	82	769	77

* Recommended Nutrient Allowances, National Research Council, 1945.

Table 3. *Mean weights of rations offered daily, together with means of rations eaten, and the net calcium and phosphorus intakes, in grammes*

Group	Mean wt. of ration offered*	Mean wt. of ration eaten*	Net calcium intake	Net phosphorus intake: Early gestation to mid-lactation	Mid-lactation to mid-dry period
B	1178	1086	6·6	4·2	—
C	1195	1158	6·7	4·5	4·4
D	1178	1080	6·5	1·4	—
E	1200	1112	6·0	1·3	1·5
F	1234	1144	6·0	1·3	4·5

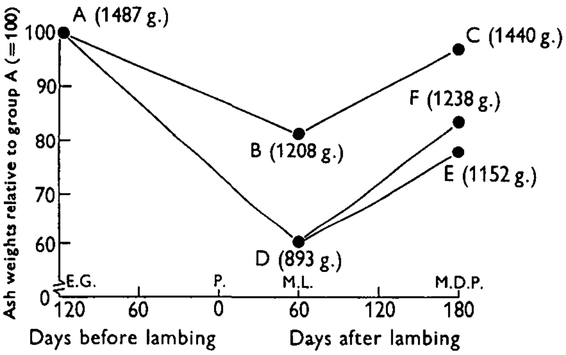
* These mean weights include the potatoes calculated to an air-dry basis of 10 % moisture. They also include the air-dry equivalent of the turnips fed to restore failing appetite. Together, these amounted to less than 1 % of the average feed intake over the whole experimental period.

Table 4. *Mortality, mean live weights and mean carcass weights*

	Group						Residual standard deviation
	A	B	C	D	E	F	
No. of ewes tupped	10	10	10	10	10	10	—
No. of ewes surviving to lambing	—	10	10	10	9	10	—
No. of ewes surviving to mid-lactation	—	8	9	9	8	8	—
No. of ewes surviving to the mid-dry period	—	—	9	—	7	8	—
Mean live weights in early gestation (lb.)	156	156	156	152	149	151	—
Mean live weights at mid-lactation (lb.)	—	125	132	120	114	110	—
Mean live weights at the mid-dry period (lb.)	—	—	143	—	124	128	—
Fall in live weight, from early gestation to mid-lactation (lb.)	—	31	24	32	35	41	11·7
Fall in live weight from early gestation to the mid-dry period (lb.)	—	—	13	—	25	23	11·9
Gain in live weight from mid-lactation to mid-dry period (lb.)	—	—	11	—	10	18	6·0
Mean carcass weights (lb.)	82·2	55·3	74·7	50·6	58·9	62·4	—

Table 5. *Reproductive performance of ewes and growth of their lambs*

	Groups				
	B	C	D	E	F
Mean number of lambs born, per ewe	1·88	2·11	1·89	1·71	2·00
Mean number of lambs reared to 4 weeks of age, per ewe	1·88	1·78	1·78	1·57	2·00
Mean weight of lambs at birth (lb.)	10·2	9·5	9·7	9·9	10·0
Mean weight of lambs at 4 weeks of age (lb.)	19·8	19·7	21·1	21·9	19·3
Mean weight of lambs per ewe at 4 weeks of age (lb.)	37·1	35·1	37·5	34·4	38·6



Text-fig. 1. Mean ash weights of the whole skeleton for each group relative to the ash weight of group A. E.G., early gestation; P., parturition; M.L., mid-lactation; M.D.P., mid-dry period.

gestation and the end of the experiment which took place in all groups was least in group C, greatest in group E and intermediate in group F, but these differences were not statistically significant. All these differences were reflected in the carcass weights.

Reproductive performance and growth of lambs

These data are set out in Table 5.

There was no significant difference among the groups in numbers of lambs born or reared, in birth weights or gains from birth to 4 weeks.

Bone analysis

The weight of ash and the percentage ash of the skull (without mandibles, nasal bones and teeth), mandible (without teeth), the 7 cervical vertebrae, different parts of the radius and metacarpal, and the total ash weight of the skeleton are set out in Tables 6 and 7 respectively. The total ash content of the skeleton is also shown in Text-fig. 1, with mean values expressed as percentages of the values for group A, the ewes of which were killed in early gestation.

Total skeletal ash of ewes fed on the moderate-phosphorus ration until mid-lactation (group B) was 19 % less than total skeletal ash of ewes killed in early gestation (group A), the difference being highly significant (A and B comparison). In ewes on the moderate-phosphorus ration throughout the experiment (group C) total ash weight of the skeleton was a little less than the value for ewes killed in early gestation (group A) but the difference was not statistically significant (A and C comparison).

When the low-phosphorus ration was fed to mid-lactation (group D) total skeletal ash was 40 % less than the value for ewes killed in early gestation (group A). This value for group D was also significantly lower than the value for group B ewes fed on the moderate-phosphorus ration and killed in mid-lactation (B and D comparison). The total weight of ash in the skeletons of ewes fed on the low-phosphorus ration throughout the experiment (group E) was significantly greater than the value for ewes fed on the same ration but killed in mid-lactation (D and E comparison), but was still 23 % less than the value for ewes killed in early gestation (group A). This difference was also significant.

The skeletons of ewes given in succession two levels of phosphorus (group F), low to mid-lactation and moderate thereafter, contained a little more ash than the skeletons of ewes fed low phosphorus throughout (group E), but the difference was not significant. The group F value was 17 % lower than the group A value, the difference being highly significant (A and F comparison).

Bones and parts of bones previously found sensitive to resorption (Part I) were again resorbed more than others in ewes fed the moderate-phosphorus ration to mid-lactation (group B). Decreases in ash weight between early gestation and mid-lactation in these animals were significant for all bones or parts of bones except the shaft of the radius and the shaft and proximal end of the metacarpal (A and B comparison, Table 6).

Repair was complete or almost complete in most bones of ewes fed the moderate-phosphorus ration to the mid-dry period (group C), though ash weights of the mandible and proximal end of the radius were still significantly lower than corresponding ash weights at the beginning of the experiment (A and C comparison).

Resorption in ewes fed the low-phosphorus ration to mid-lactation (group D) was again more severe in the cervical vertebrae, skull and mandible than in the shafts of long bones, but in animals of this group decreases in ash weight were highly significant for all bones and parts of bones which were ashed separately (A and D comparison). On comparing ash weights of individual bones from ewes fed on the moderate-phosphorus ration to mid-lactation with those of ewes fed the low-phosphorus ration to mid-lactation it is evident that in the skeleton of low-phosphorus ewes the shafts of long bones were being heavily drawn upon in the face of the severe phosphorus deficiency (B and D comparison).

There was some repair in all bones or parts of bones except the metacarpal shaft in ewes fed the low-phosphorus ration throughout the experiment (group E), but the effect was greater in some bones than others. Repair was greatest in the cervical vertebrae, followed by the skull and the mandible and finally the shafts of long bones in which no significant repair was found (D and E comparison).

In animals changed from the low-phosphorus to the moderate-phosphorus ration at mid-lactation (group F), repair was greater in most bones or parts of bones than in animals fed the low-phosphorus ration throughout (group E), exceptions being found in the shafts of radius and metacarpal where repair did not take place on either diet. While considerable repair took place in the skeletons of group F ewes particularly in the cervical vertebrae ash weights of all bones were significantly lower than in the control animals (A and F comparison), with the single exception of the 7th cervical vertebra.

Decreases and increases in the weights of ash in different bones and parts of bones were associated with changes in the percentages of ash in most cases. Between early gestation and mid-lactation there were large decreases in the percentages of ash in the cervical vertebrae and skull irrespective of phosphorus intake. The decreases were slightly less in the mandibles and the ends of the radii and metacarpals, again irrespective of phosphorus intake. There was little or no change in the percentages of ash in the shafts of the radii and metacarpals.

In ewes fed the moderate phosphorus ration and killed at the end of the experiment (group C) the percentage ash values of all bones except the mandible were equal or almost equal to those of ewes killed in early gestation (A and C comparison). In ewes fed the low-phosphorus ration throughout (group E) some recovery in percentage ash took place in most bones (D and E comparison), though this was incomplete, and no recovery was found in the mandible and radius shaft. When the low-phosphorus ration was fed to mid-lactation and the moderate-phosphorus ration thereafter (group F) recovery in percentage ash was greater than when the low-phosphorus ration was fed throughout (group E), but most bones did not recover to the percentage ash values in ewes killed at the beginning of the experiment (A and F comparison). Highly significant differences between percentage ash values of groups A and F ewes were found for the mandible, skull and first cervical vertebra.

Blood analysis

Mean values for whole blood inorganic phosphorus and serum calcium concentrations of ewes of groups C, E and F are shown in Text-fig. 2.

The concentration of whole blood inorganic phosphorus fell rapidly at the start of the experiment. Values for ewes fed on the low-phosphorus ration were significantly lower than those of ewes on the moderate-phosphorus ration when the first samples were taken about 3 weeks after commencement of experimental feeding.

Throughout the experiment blood inorganic phosphorus values in ewes fed the low-phosphorus ration (group E) were significantly lower than the values in ewes fed on the moderate-phosphorus ration (group C). Values for group F (fed the low-phosphorus ration until mid-lactation and moderate-phosphorus ration thereafter) were similar to

Table 6. *Mean ash content of total skeleton, of certain individual bones and parts of bones of ewes fed on diets of different phosphorus content, together with the significance of differences*

Group	Treatment
A	Killed in early gestation
B	On moderate phosphorus intake and killed in mid-lactation
C	On moderate phosphorus intake and killed in mid-dry period
D	On low phosphorus intake, and killed in mid-lactation
E	On low phosphorus intake and killed in mid-dry period
F	On low phosphorus intake to mid-lactation and then on moderate phosphorus intake until killed in mid-dry period

	Mean ash weights in g.						Significance of differences between groups*						Residual standard deviation
Group	A	B	C	D	E	F	A and B	B and D	A and D	A and C	A and F	D and E	
Skull (without mandibles, nasal bones and teeth)	161·6	118·8	152·4	87·7	119·1	131·2	SSS	SSS	SSS	–	SSS	SSS	11
Mandible (without teeth)	43·2	32·9	39·0	25·8	29·8	34·5	SSS	SSS	SSS	SS	SSS	S	3·3
Cervical vertebra													
1	19·8	15·3	20·0	11·3	15·4	18·1	SSS	SSS	SSS	–	S	SSS	1·8
2	20·6	15·7	21·3	11·2	16·1	18·5	SSS	SSS	SSS	–	S	SSS	1·9
3	17·9	13·0	17·8	9·1	13·4	15·3	SSS	SSS	SSS	–	SS	SSS	1·8
4	17·2	12·7	16·9	9·2	12·9	15·0	SSS	SSS	SSS	–	SS	SSS	1·7
5	15·5	11·7	15·7	8·3	12·3	13·9	SSS	SSS	SSS	–	S	SSS	1·5
6	14·8	11·8	14·9	8·1	12·0	13·6	SSS	SSS	SSS	–	S	SSS	1·3
7	11·1	8·5	11·4	6·7	9·2	10·2	SSS	SS	SSS	–	–	SSS	1·3
Radius													
Proximal end	7·6	6·2	6·8	4·6	5·7	6·0	SSS	SSS	SSS	S	SSS	SS	0·8
Shaft	17·5	16·7	17·0	13·9	14·5	14·5	–	SSS	SSS	–	SSS	–	1·6
Distal end	6·4	5·4	6·2	4·0	5·1	5·3	SS	SSS	SSS	–	SSS	SS	0·6
Metacarpal													
Proximal end	4·0	3·8	3·9	3·0	3·4	3·4	–	SSS	SSS	–	SS	–	0·4
Shaft	10·1	10·3	9·9	8·8	8·6	8·6	–	SSS	SSS	–	SS	–	0·9
Distal end	5·7	4·9	5·4	3·8	4·5	4·7	SS	SSS	SSS	–	SSS	S	0·6
Whole skeleton	1487	1208	1440	893	1152	1238	SSS	SSS	SSS	–	SSS	SSS	110

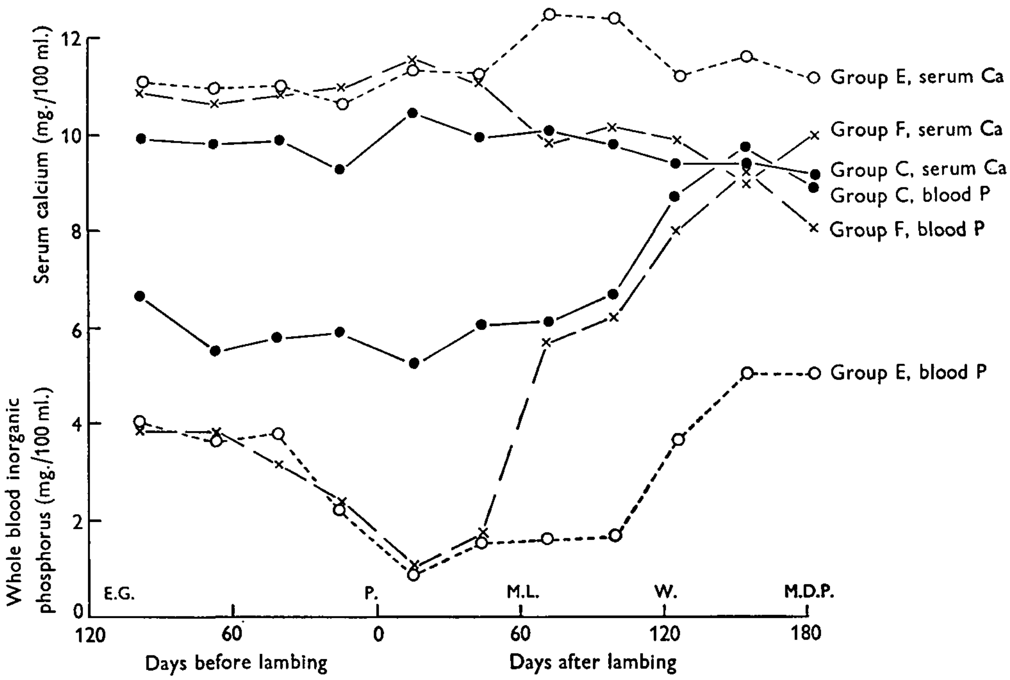
* – Indicates 'not significant'; S indicates $P<0{\cdot}05$; SS indicates $P<0{\cdot}01$; SSS indicates $P<0{\cdot}001$.

Table 7. *Mean percentage ash of certain individual bones and parts of bones of ewes fed on diets of different phosphorus contents, together with the significance of differences*

Group	Treatment
A	Killed in early gestation
B	On moderate phosphorus intake and killed in mid-lactation
C	On moderate phosphorus intake and killed in mid-dry period
D	On low phosphorus intake, and killed in mid-lactation
E	On low phosphorus intake, and killed in mid-dry period
F	On low phosphorus intake to mid-lactation and then on moderate phosphorus intake until killed in mid-dry period

	Mean percentage ash						Significance of differences between groups*						Residual standard deviation
Group	A	B	C	D	E	F	A and B	B and D	A and D	A and C	A and F	D and E	
Skull (without mandibles, nasal bones and teeth)	62·6	60·3	61·7	58·3	59·6	60·8	SSS	SSS	SSS	—	SSS	S	1·1
Mandible (without teeth)	68·6	66·8	67·2	65·3	64·9	66·0	SS	SS	SSS	S	SSS	—	1·2
Cervical vertebra													
1	61·9	57·7	61·2	54·7	57·4	59·2	SSS	SSS	SSS	—	SSS	SS	1·5
2	61·4	57·3	61·1	53·9	57·4	59·4	SSS	SSS	SSS	—	S	SSS	1·6
3	61·5	56·6	61·1	52·8	57·6	59·6	SSS	SSS	SSS	—	S	SSS	1·6
4	61·2	56·7	61·2	54·1	57·7	59·7	SSS	SSS	SSS	—	S	SSS	1·4
5	61·1	56·8	60·8	53·0	57·2	59·4	SSS	SSS	SSS	—	S	SSS	1·4
6	60·7	56·5	60·8	52·2	57·0	59·6	SSS	SSS	SSS	—	—	SSS	1·4
7	61·2	57·6	61·0	54·3	57·1	59·8	SSS	SSS	SSS	—	—	SS	1·7
Radius													
Proximal end	65·2	63·0	65·4	62·1	62·8	64·5	SSS	—	SSS	—	—	—	1·3
Shaft	67·0	66·5	66·9	66·8	66·4	67·0	—	—	—	—	—	—	0·8
Distal end	63·2	60·6	62·9	56·6	59·2	61·7	SS	SSS	SSS	—	—	SS	1·8
Metacarpal													
Proximal end	66·6	65·3	66·5	64·2	65·3	65·9	SS	S	SSS	—	—	S	1·0
Shaft	68·8	68·1	68·8	68·5	68·9	68·7	S	—	—	—	—	—	0·7
Distal end	64·0	60·9	63·2	59·2	61·4	62·4	SSS	SS	SSS	—	S	SS	1·5

* — Indicates 'not significant'; S indicates $P < 0·05$; SS indicates $P < 0·01$; SSS indicates $P < 0·001$.



Text-fig. 2. Mean serum calcium and whole blood inorganic phosphorus values for groups C, E and F. E.G., early gestation; P., parturition; M.L., mid-lactation; W., weaning; M.D.P., mid-dry period.

group E values until mid-lactation, and similar to group C values from mid-lactation to the mid-dry period.

Within groups there were significant changes in blood inorganic phosphorus concentration throughout the experiment. In ewes fed the moderate-phosphorus ration throughout (group C) values remained fairly uniform, around 6 mg./100 ml., until after weaning when there was a significant increase to about 9 mg./100 ml. In ewes fed the low-phosphorus ration throughout the experiment (group E) there was a fairly uniform fall in phosphate concentration from early gestation to mid-lactation at which point very low mean values, around 1 mg./100 ml., were found. The concentration increased by only a very small amount between early lactation and weaning, but after weaning there was a substantial and highly significant increase, reaching values in the region of 5 mg./100 ml., which are generally regarded as normal.

Differences in serum calcium values among groups were relatively small compared with differences in blood inorganic phosphorus values. They varied inversely with the phosphorus values.

Radiological observations

Among radiographs taken of the living animal only those of the radius and tibia gave a consistent picture of resorption and repair. Attention is confined here to the radius, treatment differences being more marked in the radius than in the tibia.

In Part I of this series radiographic features of resorption in the radius were described using flesh-free bones (Benzie *et al.* 1955). These features were observed in the present experiment in radiographs taken of the radius in the living animal as well as in those taken of the flesh-free bone. Radiographs of the radius taken in mid-gestation, at lambing, in mid-lactation and the mid-dry period (only groups C, E and F remained after mid-lactation) of each animal were given a value from 1 to 10. A bone showing no evidence of resorption, that is having a thick, dense cortex of fairly uniform appearance from endosteal to periosteal surface and having an extensive closely woven network of cancellous tissue at each end was given a value 10, and a bone showing very extensive resorption, that is having a thin cortex with many longitudinal striations and barely any cancellous tissue at the ends was given a value 1. The lowest value given to any bone of this experiment was 3 and the highest 10. Mean values for each group at different stages of the experiment are set out in Table 8.

At lambing a small and similar amount of resorption had taken place in all groups. By mid-lactation resorption had increased in all groups and was clearly greater in groups D–F (fed on the low-phosphorus ration) than in groups B and C (fed on the moderate-phosphorus ration. During the period mid-lactation to mid-dry period, repair, which took place in all three groups (C, E and F), was greater in groups C and F which were fed on the moderate-phosphorus than in group E which continued on the low-phosphorus ration. However, even in groups C and F repair was not complete when judged by the appearance of the bone at the beginning of the experiment.

This general picture of resorption and repair seen in radiographs of the bone of living animals resembles closely that seen in radiographs of bones in the flesh-free state. Radiographs were taken of the radius, cervical vertebrae (3rd, 4th and 5th) and the mandible of each animal and using the radiographic features of resorption which were described for each of these bones in Part I, a value between 10 and 1 was given to each bone. From the mean values which are presented in Table 9, it is evident that in each series of bones the effects of treatment on resorption and repair were similar to those described above for the radius taken in the living animal. The data do not reveal any marked differences among the bones though they do suggest, and it was generally agreed by the observers, that the evidence of resorption was rather more striking in the mandible than in the radius and vertebrae.

An illustration of group differences is given in Pls. 1 and 2, using the mandible. The values (mean of two observers) given to these bones were: group A 10·0, group B 6·2, group C 9·5, group D 3·5, group E 7·0 and group F 8·0, the corresponding group means being 10·0, 5·8, 9·0, 3·5, 6·0 and 8·2, respectively. In Pl. 3 the interdental bone of mandible A (Pl. 1) and of mandible D (Pl. 2) is reproduced slightly larger than actual size (× 1½) to illustrate the striations and uneven structure indicative of resorption which can be seen when the bone is studied at actual size or slightly magnified. This detail is even more marked in the original radiographs than in these slightly magnified reproductions.

Table 8. *Mean radiological values for the radius taken in the living animal**

	Stage of the experiment			
Group	Mid-gestation	Lambing	Mid-lactation	Mid-dry period
A	10·0	—	—	—
B	9·3	8·1	6·7	—
C	9·3	8·0	7·6	8·8
D	9·4	7·0	4·9	—
E	9·1	7·8	5·4	7·0
F	10·0	8·4	6·1	8·7

* Based on a scale from 1 to 10 where high values represent good bone.

able 9. *Mean radiological values for the radius, cervical vertebrae and mandible taken in the flesh-free state**

Group	Radius	Cervical vertebrae (3, 4 and 5)	Mandible
A	9·8	8·5	10·0
B	6·2	6·2	5·8
C	8·8	9·2	9·0
D	4·2	5·2	3·5
E	7·2	7·2	6·0
F	8·5	8·8	8·2

* Based on a scale from 1 to 10 where high values represent good bone.

DISCUSSION

In this experiment the degree of resorption in group B ewes was much greater than expected in view of the results for group B of the previous experiment. Both groups had apparently adequate calcium, phosphorus and vitamin D intakes but loss of skeletal ash was 18·8 % (279 g.) in the present experiment (Part III) and only 6·5 % (80 g.) in the previous experiment (Part II). There were a number of differences between these groups but none appears to explain fully this discrepancy. Thus at the start of the experiment the animals used were of different ages (3½ years, Part III; and cast ewes 5½ and 6 years, Part II), they were of different live weights (156 lb., Part III; and 134 lb., Part II) and different skeletal ash weights as indicated by those of group A (1487 g., Part III; and 1256 g., Part II). Furthermore, although there was a slightly greater milk yield in Part III compared with Part II, indicated by live weights of lambs per ewe at 4 weeks (37·1 lb., Part III; and 34·4 lb., Part II) this was not sufficient to account for more than a small proportion of the difference in resorption.

The calcium, phosphorus and vitamin D intakes of group B in each experiment were apparently adequate, though in Part III where resorption was greatest calcium was derived almost entirely from sugar-beet pulp and there is no information on the availability of this source of calcium. However, as serum calcium values were normal throughout the experiment and repair of the skeleton was complete in ewes fed on this ration to the mid-dry period it is unlikely that poor availability of calcium was responsible for the increased resorption.

A striking difference between the two groups B was in the loss of body tissue between early gestation and mid-lactation. The difference in carcass weight between group A ewes (killed at the beginning of the experiment) and group B ewes (killed in mid-lactation) was much greater in Part III (27 lb.) than in Part II (7½ lb.), and this greater loss of body tissue was associated with the greater loss of skeletal ash. While there is little evidence to support a physiological association here, it is possible that the dietary difference responsible for the loss of body tissue also caused the greater loss of skeletal ash. In an experiment with adult rats those fed on a low protein ration had lower body weights and lower femur ash weights than similar animals fed on a high protein ration (Eastremera & Armstrong, 1948). Thus it appears that a low protein intake in Part III could have caused the increased loss of body tissue and the increased resorption, but it is not possible at present to say which dietary differences were responsible for the results discussed here. Further work is in progress to elucidate this matter.

The percentage loss of ash from the whole skeleton during pregnancy and lactation in group D of Part II (18·2) and group B of Part III (18·8) were similar, and the distribution of loss among bones analysed separately was also similar in the two groups. The cause of resorption was different in these groups, suggesting that in general the distribution of resorption throughout the skeleton is independent of the agent responsible for resorption.

In group D of the present experiment resorption was very severe, corresponding to a loss of 40 % of skeletal ash, and involved the shafts of long bones as well as other parts of the skeleton. Losses from the shafts were small compared with the loss from other parts of the skeleton (radius shaft 20·6 %, metacarpal shaft 16·9 %, skull 45·7 % and fourth cervical vertebra 46·5 %), but they were highly significant. Such resorption did not take place in any group of Part I or Part II. This effect on the compact tissue of long bone shafts may be characteristic of resorption in the face of a low-phosphorus intake, but is probably characteristic of the degree of resorption rather than the specific factor agent responsible for resorption. This view is supported by an experiment with rats during lactation on diets containing different levels of calcium (Ellinger, Duckworth, Dalgarno & Quenouille, 1952): with diets containing 0·29, 0·54 and 0·79 % calcium there was only slight resorption and no significant effect on the shafts of long bones but with a diet containing 0·04 % calcium there was very severe resorption and the shafts were affected almost as much as the ends of long bones.

Resorption of the skeletons of ewes fed on a low-phosphorus ration (group D of the present experiment) was much greater than resorption of the skeletons of ewes fed on a low-calcium ration (group D, Part II). The quantity of bone resorbed depends upon the amounts of calcium and phosphorus required, in this case for maintenance and milk formation, the amount of calcium or phosphorus released per unit weight of bone resorbed, the daily intake of calcium and phosphorus and the availabilities of these. In these two groups the quantities of calcium (Part II) and of phosphorus (Part III) required were probably similar, but the amount of bone resorption required to supply a certain amount of phosphorus is about twice that required to supply the same amount of calcium. Thus other things being equal resorption in ewes fed on a low-phosphorus ration would be about twice as great as resorption in ewes fed on a low-calcium ration. There is no information on the availability of calcium in the Part II basal diet nor on the availability of phosphorus in the Part III basal diet, but as the degree of resorption on the low-phosphorus ration was just over twice that on the low-calcium ration, it appears that the amount of phosphorus available from the low-phosphorus ration was a little lower than the amount of calcium available from the low-calcium ration. In accounting for the severe resorption in Group D it is realized that no mention has been made of the phosphorus accruing from the breakdown of soft tissue nor of the, as yet unidentified, cause of resorption which was evident in group B. The matter is obviously complex but the chief cause of greater resorption in ewes fed on a low-phosphorus ration than in ewes fed on a low-calcium ration lies in the smaller amount of phosphorus compared with calcium, released per unit weight of bone resorbed.

For the same reason a greater amount of bone repair can take place with a low-phosphorus than with a low-calcium ration. This difference was obtained between groups E of the two experiments, the increase in ash weight (group E–group D) being 259 g. on low-phosphorus and 71 g. on low-calcium. The difference is clearly greater than can be accounted for on the basis of the amounts of each element required for bone formation but this was no doubt the cause of a large part of the difference. A notable difference between the skeletons of these groups at the start of recovery was the severely depleted state of one group (E of Part III) compared with the moderately depleted state of the other (E of Part II). Whether, under similar conditions, a severely depleted skeleton would recover more rapidly or at the same rate as a moderately depleted skeleton is not known, but the results of these experiments do not support the view that repair of a severely depleted skeleton is necessarily slower than repair of a moderately resorbed skeleton.

In view of the difference in phosphorus intake during the period mid-lactation to mid-dry period between groups E and F (1·5 g. and 4·5 g. respectively) the difference in repair of the skeleton was small (259 g. ash in group E and 345 g. ash in group F). This suggests that the quantity of phosphorus available for bone formation in group F was considerably greater than the amount which could be used for bone repair in these animals. The mean rate of bone repair in group F was greater than in any group so far investigated, and at almost 5 g. per day during the period mid-lactation to mid-dry period, may represent the maximum in an adult sheep.

The skeletons of groups E and F, which were affected in every part in mid-lactation and which were not completely repaired at the end of the experiment, provide information on the relative rates of repair of different parts of the skeleton. In Table 10, repair of each bone (E − D or F − D) is given as a percentage of the loss (A − D) for that particular bone, that is as a percentage of the amount required for complete repair. In both groups repair of the vertebrae was greatest, followed by the skull, mandible, and ends of the long bones and finally the long bone shafts. The supplementary phosphorus given to group F increased repair in all bones except the shafts of the radius and the shaft and proximal end of the metacarpal and the improvement due to added phosphorus was greatest in the mandible. It is evident that in general, those bones which are most readily resorbed are also those most readily repaired, and that once compact tissue has been affected, which is only when total skeletal resorption is very severe, it repairs very slowly even when there are ample quantities of bone forming nutrients present.

The physiological basis of skeletal resorption in the mature animal is moderately well established in calcium but not in phosphorus deficiency. In animals fed on a low-calcium diet the parathyroid gland is stimulated and the increased quantity of hormone produced causes resorption of bone, liberating calcium to meet requirements and, incidentally, phosphorus which is excreted. It is generally believed that no comparable process operates in phosphorus deficiency. Mitchell (1947) states, 'in the presence of enormous stores of phosphorus in the bones, the animal (on a low-phosphorus intake) is living on a hand to mouth basis so far as this element is concerned'. This view is not supported by results of the present experiment, and in earlier work on low-phosphorus rations (Du Toit, Malan & Roussouw, 1930; Du Toit, Malan & Groenewald, 1931, 1932; Martin & Peirce, 1934) observations made on ewes during reproduction and lactation suggested that resorption did take place to provide phosphorus for milk production. More recently Reinach, Louw & Groenwald (1952) found negative phosphorus balances in 18-month-old wethers fed on a low-phosphorus ration, and the quantities of phosphorus lost daily, as well as the large negative calcium balances, indicated that a net loss of bone was taking place. That resorption takes place in the face of low-phosphorus intakes in adult sheep, particularly when requirements are high, is clearly established by results of the present experiment, but no information was obtained on the processes involved. The results of experiments with other species on the parathyroid hormone indicate that this would not be involved in resorption caused by a low-phosphorus intake, though in the only experiment, known to the authors, on parathyroid activity in sheep the effects of the hormone were not similar in all respects to those found in other species (Lotz, Talmage & Comar, 1954), and therefore it remains possible that the parathyroid could be involved. Apart from the observation that a low blood inorganic phosphorus concentration probably stimulates the processes of resorption at an early stage, nothing can be said at present concerning the mechanism by which resorption takes place in phosphorus deficiency.

Blood inorganic phosphorus concentrations were affected markedly by phosphorus intake and by the stage of pregnancy, lactation and dry period as described earlier. When blood phosphorus values were low the skeleton was severely resorbed, but the converse relationship was not always true. Thus blood phosphorus was normal for group B in mid-lactation when the skeleton was moderately resorbed (18·8 % loss of skeletal ash), and for group E at the end of the experiment when repair of the skeleton was by no means complete (22·5 % less ash than group A). Furthermore, when phosphorus was added to the diet of group F ewes in mid-lactation normal blood phosphorus values were reached in less than 1 month, whereas repair of the skeleton was incomplete when the animals were killed 4 months after phosphorus feeding began. The conclusions reached from these results and from the results of Parts I and II on low-calcium rations are similar: low serum calcium or blood phosphorus values are generally indicative of reduced skeletal

Table 10. *The percentage repair of individual bones and the whole skeleton after severe resorption*

	Repair in Group E $\frac{(E-D)\,100}{(A-D)}$	Repair in Group F $\frac{(F-D)\,100}{(A-D)}$
Skull	42·5	58·9
Mandible	23·0	50·0
Cervical 1	48·2	80·0
2	52·1	77·7
3	48·9	70·5
4	46·3	72·5
5	55·6	77·8
6	58·2	82·1
7	56·8	79·5
Radius		
Proximal end	36·7	46·7
Shaft	16·7	16·7
Distal end	45·8	54·2
Metacarpal		
Proximal end	40·0	40·0
Shaft	−15·4	−15·4
Distal end	36·8	47·4
Whole skeleton	43·6	58·1

reserves, but normal or near normal values are not always a reliable guide to mineral status of the skeleton. If some guidance is being sought from blood analyses it appears from the results of these experiments that samples should be taken in late-lactation, that is shortly before weaning when the effects of a low-phosphorus intake on blood phosphorus and of a low-calcium intake on serum calcium are marked. Samples taken in early lactation would probably show the effects of phosphorus intake better than samples taken in late-lactation, but the early-lactation samples are unlikely to show any effect of calcium intake.

As indicated earlier, the radiological features of resorption for each bone were similar to those described in Part I. One of the striking features of resorption in the mandible is the decrease in amount and density of alveolar bone, and the purpose of using 3½-year-old ewes in this experiment, instead of cast ewes as in previous experiments, was to observe the effect of resorption on the mobility of incisor teeth. Occlusal radiographs of the incisors and surrounding tissues showed that considerable resorption of alveolar bone took place, particularly in those ewes fed on the low-phosphorus ration, but this did not cause any marked increase in mobility of the teeth. However, the diet of these animals consisted of a concentrated meal and chopped straw, and it remains possible that had they been grazing the severe resorption of the alveolar bone, together with the strains imposed by grazing, might have led to a different result.

Radiological observations gave a similar picture of resorption and repair as ash analyses, that is, there was moderate resorption followed by almost complete repair of the skeletons of ewes fed on the moderate-phosphorus ration (groups B and C), and severe resorption followed by moderate repair of the skeletons of ewes fed on the low-phosphorus ration (groups D and E). The radiological observations also showed that in ewes fed on the low-phosphorus ration to mid-lactation and the moderate-phosphorus ration thereafter (group F) resorption was severe at mid-lactation, but repair was almost complete at the end of the experiment. This result differed slightly from that given by ash analyses, which showed that at the end of the experiment repair in group F was by no means complete judging by ash values of either group A or group C. This difference between the results of ash analyses and radiological observations was most marked in the radius where ash values for group F were about 15 % less than those of group A and C and similar to those of group E, while the radiological observations indicated that the radii of group F animals resembled those of group A and group C animals and were considerably better than those of group E animals. However, apart from this feature of repair in group F there was close agreement between the results of ash analyses and the radiological observations.

In the previous experiment of this series (Part II) radiographs taken of the skeleton in the living animal did not consistently reveal features of resorption even when a moderate degree of resorption took place (groups D and E). On the other hand, in the present experiment a similar degree of resorption, as indicated by ash analysis, was detected in almost every animal (groups B and C). It is probable that moderate resorption such as took place in groups D and E of Part II and groups B and C of the present experiment is near to the borderline for detection in the living animal and the rather more uniform series which were obtained in the present experiment compared with those of the previous one, made detection just possible. Also there were considerably greater losses of live weight in this experiment than in the previous one, giving a thinner covering of flesh to the bones which allowed greater detail of the bone structure to be seen. However, it can be said from the results of this and the previous experiment that:

(i) Severe resorption, corresponding to a loss of about 40 % of skeletal ash was detected readily in radiographs taken of the living animal.

(ii) Slight resorption, corresponding to a loss of skeletal ash of less than 20 % could not be detected with confidence in the living animal.

(iii) Degrees of resorption between these values could no doubt be detected in the living animal though difficulty might be experienced occasionally when resorption corresponding to a loss of skeletal ash of about 20 % was being investigated.

(iv) The radius has so far proved the most useful bone for detecting resorption in the living animal.

In this experiment where the low-phosphorus feeding was begun in early gestation, severe resorption and low blood phosphorus values were not accompanied by reduced numbers and live weights of lambs, though it is probable that were such low-phosphorus rations fed over several reproductive cycles, lamb production would be affected.

SUMMARY

1. The ash content of the skeleton of Cheviot ewes fed a daily ration containing about 4·5 g. of phosphorus fell by 18·8 % between mid-gestation and mid-lactation, and 2 months after the end of lactation the loss was fully replaced.

2. In ewes fed a daily ration containing about 1·5 g. of phosphorus the loss of skeletal ash was 39·9 % at mid-lactation, and this was not replaced 2 months after the end of lactation. When the phosphorus intake was raised in mid-lactation from 1·5 to 4·5 g. repair was greater but was still not complete.

3. Resorption was greater in bones rich in

cancellous tissue, such as the cervical vertebrae, than in those rich in compact tissue, such as the shafts of long bones, but when severe resorption took place significant losses were found in the shafts of long bones as well as in other bones.

4. Whole blood inorganic phosphorus values were very low, particularly during lactation, in ewes fed on the low-phosphorus ration. When extra phosphorus was fed from mid-lactation onwards blood phosphorus values rose to normal in less than four weeks, a much more rapid recovery than that which took place in the skeleton.

5. Resorption of the skeletons of ewes on both moderate and low-phosphorus rations could be detected using radiographs taken of the radius in the living animal at mid-lactation, and severe resorption found in ewes fed on the low-phosphorus ration could be distinguished readily from the milder resorption found in ewes fed on the moderate-phosphorus ration.

We wish to acknowledge the assistance given in this work by Miss E. Gehlert, Miss M. McIntosh and Mr I. Robb.

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EXPLANATION OF PLATES

Plate 1

Radiograph of flesh-free mandibles, one from each of the groups A, B and C ($\times \frac{1}{2}$). Group A mandible (top) shows no sign of resorption, while group B mandible (centre) is less dense and indicates moderate resorption. Group C mandible (bottom) shows some improvement over the group B bone but is not as dense as the group A bone, indicating incomplete repair.

Plate 2

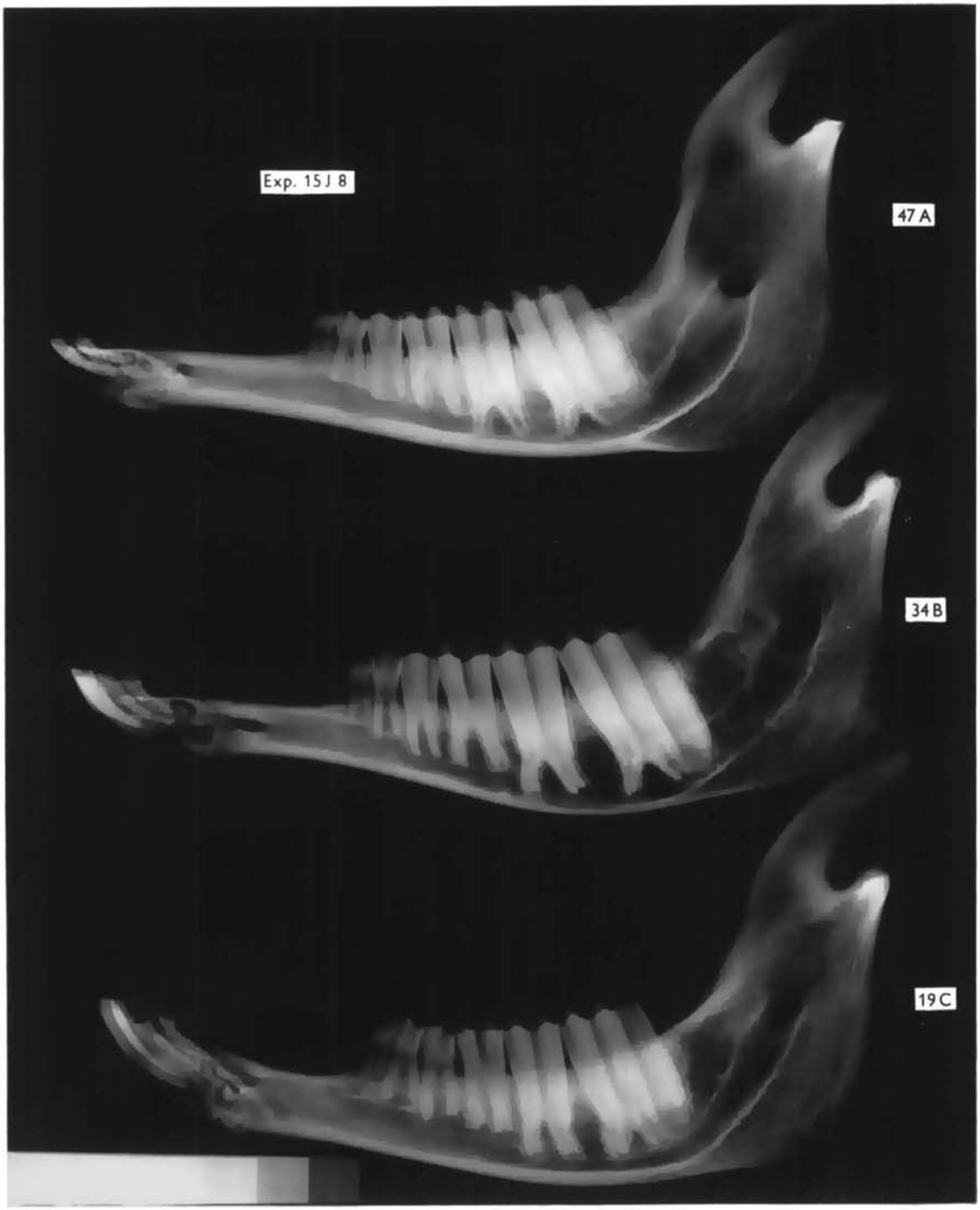
Radiograph of flesh-free mandibles, one from each of the groups D, E and F ($\times \frac{1}{2}$). Group D mandible (top) is typical of severe resorption, having a low density and poor bone structure. Group E mandible (centre) shows some repair compared with the group D bone. Group F mandible (bottom) reveals greater repair than the group E mandible but repair is not complete by comparison with the group A mandible.

Plate 3

(*a*) The interdental space and premolars of the group A mandible of Pl. 1 ($\times 1\frac{1}{2}$). The cortex is dense and there is a large quantity of alveolar bone.

(*b*) The interdental space and premolars of the group D mandible of Pl. 2 ($\times 1\frac{1}{2}$). The cortex is thinner than the cortex of the group A mandible and has dark (light) striations indicative of resorption. There is also less alveolar bone than in the mandible of group A.

(*Received* 13 *November* 1957)



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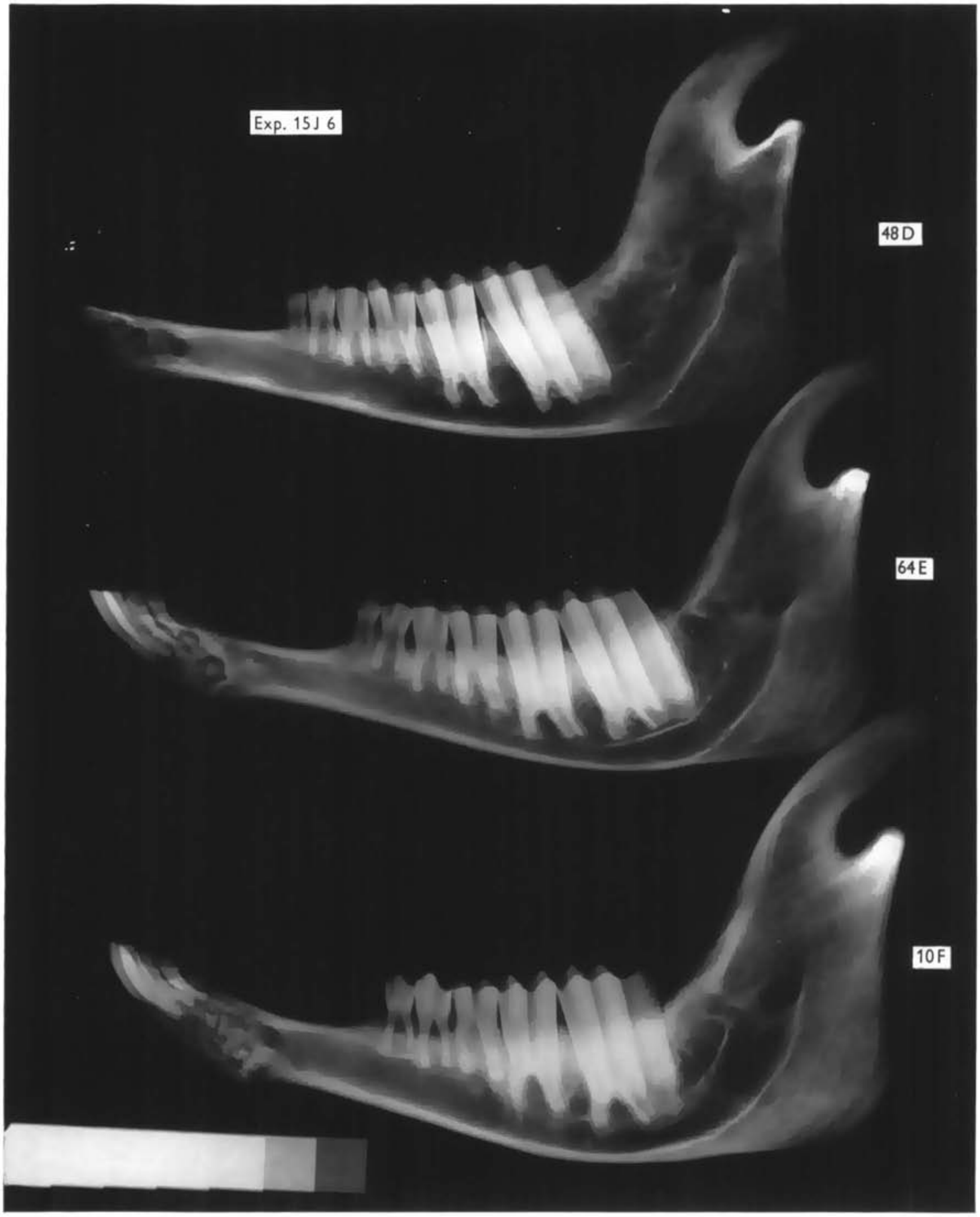
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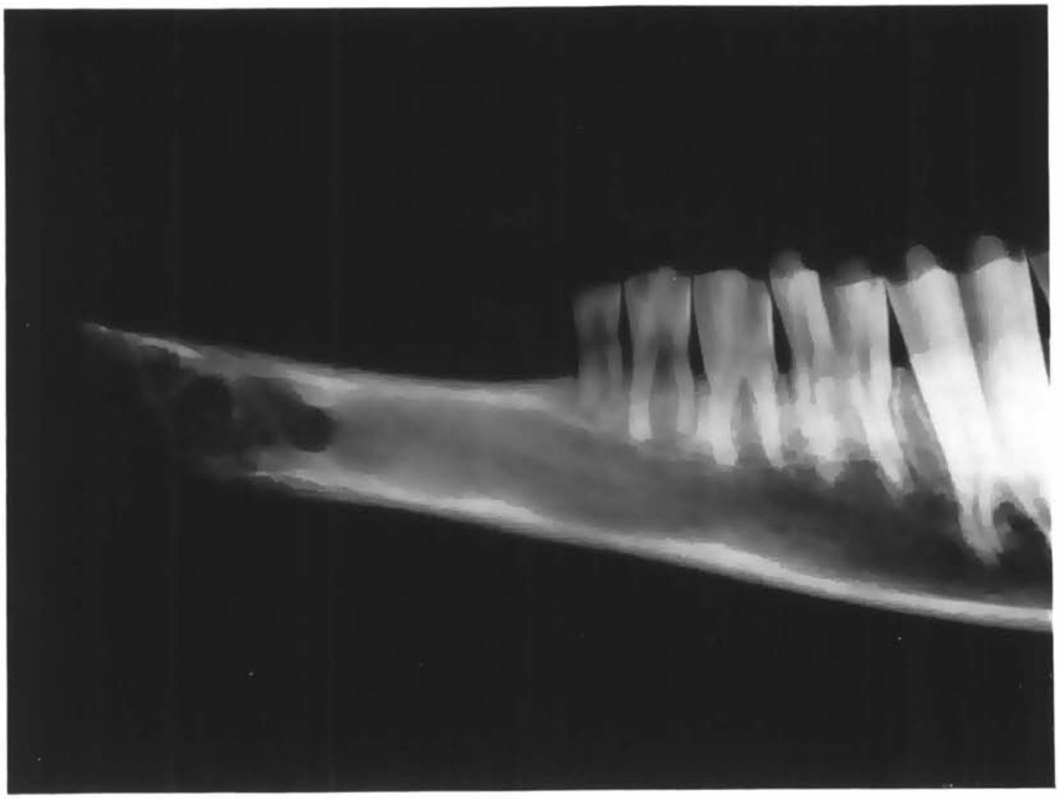

3a

3b