STUDIES OF THE SKELETON OF THE SHEEP

IV. THE EFFECTS AND INTERACTIONS OF DIETARY SUPPLEMENTS OF CALCIUM, PHOSPHORUS, COD-LIVER OIL AND ENERGY, AS STARCH, ON THE SKELETON OF GROWING BLACKFACE WETHERS

By D. BENZIE, A. W. BOYNE, A. C. DALGARNO, J. DUCKWORTH, R. HILL and D. M. WALKER

The Rowett Research Institute, Aberdeen, Scotland

(With Plates 9 and 10 and Seven Text-figures)

Defects of bones and teeth are found in many classes of animals, and are often of dietary origin. These defects are particularly common in young animals where the rates of bone growth and tooth development are rapid and where, in consequence, the deleterious effects of dietary deficiencies and of imbalance among nutrients are greatest. Exposure to these risks is least among those classes of farm animals that receive most or all of their feed in the form of mixed rations compounded according to what is known of nutritional requirements. Exposure is greatest in animals that depend mainly or exclusively on natural grazing for their nourishment. As far as the live-stock of the United Kingdom is concerned hill sheep are thus the class of farm animals most exposed to the risks of disorders of the bones and teeth. Records exist of several bone and tooth disorders occurring in hill sheep in this country: 'cappi' (Bowes 1932; Bosworth & Stewart, 1932–33; Thomlinson, 1953–54; Fraser & Stamp, 1957; Cresswell, 1958), 'bent leg' (Elliot, Orr, Wood & Crichton, 1926; Dunlop, 1954), 'cripples' (Stewart, 1933; Piercy, 1934*a*, *b*), osteoporosis (Butler, Nisbet & Robertson, 1957) and early loss of incisor teeth (Wallace, 1955; Fraser & Stamp, 1957). Workers in New Zealand have drawn attention to analogous disorders in sheep—rickets (Fitch, 1943; Ewer & Bartrum, 1948), 'bowie' (Fitch, 1954; Cunningham, 1957) and excessive wear of incisor teeth (Barnicoat, 1957).

In an earlier study (Duckworth, Godden & Thomson, 1943) it was found that the degree of rickets produced in growing Blackface and half-bred wether lambs was related to the extent to which growth was permitted; animals growing rapidly on a vitamin D deficient ration developing a much more severe disorder, as judged by bending of the legs and stiffness of gait, and by changes in blood composition, than others growing more slowly, rates of growth being controlled by the levels of dietary energy allowed. Animals whose growth was completely prevented showed neither leg deformity nor disturbed blood composition.

In the earlier investigation opinions on the degree of skeletal damage were based on the clinical appearance of the animals and the composition of their blood. In our more recent studies, reported in Parts I, II and III of this series (Benzie, Boyne, Dalgarno, Duckworth, Hill & Walker, 1955, 1956; Benzie, Boyne, Dalgarno, Duckworth & Hill, 1959), we followed the changes in the mineral status of the ewe during pregnancy, lactation and the subsequent 'dry' period, on rations either adequate or deficient in calcium or phosphorus, by blood analysis and radiography during life and with chemical analysis and radiography of the isolated skeleton after death. In this work it was found that there were great differences among structurally dissimilar bones in their liability to resorption, and within a single bone analogous metabolic differences among structurally divergent regions. Bones rich in cancellous tissue were always resorbed to a disproportionately high degree when significant withdrawals were made from the skeleton as a whole. In long bones the regions rich in spongiosa were always more greatly resorbed than the compact bone of the shaft. Blood composition was not a good guide to the status of the skeleton, affording little indication of the degree of damage inflicted by dietary deficiencies. Data on blood composition were easier to interpret when the stage of the pregnancy–lactation cycle and the status of the skeleton were taken into consideration.

In the present investigation the effects and interactions of four dietary variables on bone development in wether hoggs were studied in an experiment using a factorial design. These were calcium, phosphorus, cod-liver oil as a source of vitamin D, and energy supplements in the form of starch.

METHODS

Housing, management, feeding, treatment of carcasses and methods of analysis were similar to those described in Part I (Benzie *et al.* 1955).

Fifty-four Blackface wether lambs were purchased in September when about 5 months old, and allowed to graze good lowland pasture until the end of November. The heaviest and lightest were discarded to leave forty-eight which were housed and fed in individual pens, direct sunlight being excluded. The animals were divided at random among the sixteen treatments shown in Table 1, so that there were three on each.

The daily basal ration consisted of 100 g. chopped oat straw and 450 g. of a concentrate mixture consisting of 40 parts flaked maize, 10 parts maize gluten meal, 5 parts decorticated extracted groundnut meal and 1 part common salt. This basal diet allowed an intake of 0·4 g. of calcium per day, and was also very low in vitamin D. It was moderately low in phosphorus. The daily allowance contained sufficient protein to meet the requirements for normal growth, but provided only sufficient energy, when unsupplemented with starch, for maintenance, or for only very slight growth. Supplements were added, either alone or in combination as required in the plan shown in Table 1, in amounts of 8·6 g. disodium hydrogen phosphate (2 g. P), 7·5 g. calcium carbonate (3 g. Ca), 170 g. starch and 2 ml. cod-liver oil. The starch, which was given as an ingredient of the concentrate mixture, was gradually increased in quantity to 200 g. daily, the mean amount offered daily during the experiment being 170 g. At each feeding other supplements were placed on the concentrates in each individual trough and mixed in thoroughly. Samples of straw and concentrates were taken monthly for determination of calcium and phosphorus, and food refusals were weighed daily.

The animals were weighed every 2 weeks. Blood samples were taken every 4 weeks for determinations of serum calcium and whole blood inorganic phosphorus concentrations.

Radiographs were taken of most parts of the skeleton at the beginning and the end of the experiment, using the X-ray unit described in Part II of this series (Benzie *et al.* 1956). The radiographic ratings used for the living animal are set out in Table 2. Radiographs of flesh-free bone were taken on standard films at 36 in. from the anode with an output of 70 kV. and 50 mA. for 1·0 sec.

To standardize the evaluation of radiographs of bones by different independent observers a system of grading was devised. In the case of the radius a maximum value of 10 points was awarded for a bone of good quality throughout, the distribution of these points among different regions of the bone being as shown in Table 3. In the case of the mandible a maximum value of 28 points was awarded for a bone of good quality throughout. the distribution of these points among different regions of the bone also being

Table 1. *Plan of the experiment showing the treatment given to each group*

		Added calcium		No added calcium	
		Added starch	No added starch	Added starch	No added starch
Added phosphorus	Added cod-liver oil	CaPDSt*	CaPD	PDSt	PD
	No added cod-liver oil	CaPSt	CaP	PSt	P
No added phosphorus	Added cod-liver oil	CaDSt	CaD	DSt	D
	No added cod-liver oil	CaSt	Ca	St	Nil

Daily supplements offered: * Ca = 3 g. calcium; P = 2 g. phosphorus; St = 170 g. starch; D = 2 ml. cod-liver oil.

Table 2. *Positions for radiography of the living animal, together with radiographic ratings and films used*

Region of skeleton	Position	Radiographic ratings: mA.	kV.	Film focus distance (in.)	Exposure (sec.)	Intensifying screen	Potter–Bucky diaphragm	Film
Thorax	Lateral	200	80	42	0·20	Yes	Yes	Standard
Skull	Oblique lateral 30°	200	70	36	0·15	No	No	Ilfex
Radius	Anterior–posterior	100	70	36	0·12	No	No	Ilfex
	Lateral	100	70	36	0·12	No	No	Ilfex
Tibia	Posterior–anterior	100	70	36	0·12	No	No	Ilfex
	Lateral	100	70	36	0·12	No	No	Ilfex
Pelvis	Dorso-ventral	200	70	36	0·20	Yes	Yes	Standard

shown in Table 3, and after the values for individual sections of the mandible had been summed, the total was divided by 2·8, for easier comparison with the results for the radius. A preliminary examination of the radiographs was made to determine, for each region of the radius and mandible, the highest quality status, for which full points would be awarded, and the lowest quality status, for which no value would be awarded. In the final assessing of bone quality the radiographs were presented in a random order to each of three (for the mandible radiographs) or four (for the radius radiographs) independent observers. Only one part of each bone was assessed at a time, the remainder of the bone being masked. A statistical study of the assessment of bone quality from radiographs will be published separately (Boyne & McDonald).

After about 6 months on the experimental rations the animals were killed and the skeletons were prepared for analysis. In order to distribute treatments as well as possible throughout the 2 months required for this work the animals were divided into three groups each of sixteen, containing one animal from each dietary group. They were killed in random order within each group.

RESULTS

General health and mortality. Only one animal was discarded, number 503 of group PDSt.* This was lame, had a high temperature and refused to eat early in the experiment. When it was eventually killed, in an emaciated condition, lesions of chronic broncho-pneumonia were found.

Eight other animals were either slightly lame or did not eat well during the latter part of the experiment. No specific disease other than that attributable to treatment could be found, and therefore these animals were included in the study, although they were killed at the beginning of the slaughter programme instead of on the dates allocated. These eight animals were all from groups receiving added starch, either as the only supplement or with one other: two of these animals were from group St, three from group PSt, two from group CaSt, and one from group DSt.

Food intakes. The data are set out in Table 4, together with calculated intakes of total digestible nutrients (T.D.N.). The provision of the phosphate supplement had little effect on appetite, but in contrast to this, the provision of starch caused significant reduction in the intake of the basal diet, while calcium and cod-liver oil supplements improved intakes.

There was a significant interaction of calcium and starch supplements on appetite, intakes being unaffected by calcium supplements in the absence of starch and increased in the presence of starch. Similarly, there was a marked interaction between starch and cod-liver oil on appetite. Food refusals were found to be related to live-weight increases and also to the weights of ash in the cervical vertebrae and in some ribs. Since the food refusals were considered to be a consequence of the diets fed it was decided not to adjust group means for differences in food intakes. However, it is of interest to note in which bones the degree of mineralization was affected by total food intake.

Live-weight changes. The mean live weight of the animals at the beginning of the experiment was 65 lb., with a range of 55–75 lb. All animals lost weight when initially housed and gained thereafter until the original weight was reached. This was followed by either a continued increase or a second decrease, depending upon treatment. As stated earlier, the allowance of concentrates and straw

* In the text, as in Table 1, the code letters designating dietary groups show the supplements given, with Ca = calcium, P = phosphorus, St = starch, and D = cod-liver oil.

Table 3. *Points awarded in relation to the appearance of different parts of the radius and mandible in assessing the radiological values of these bones from radiographs*

Bone	Range of points awarded	Basis of assessment
Radius:		
Epiphyseal cartilage	4	Cartilage uniformly thin
	0	Cartilage thick
Cortex	4	Broad, dense, uniform structure
	0	Narrow, low density, irregular structure with many striations especially towards endosteal surface
Proximal end	1	Much cancellous tissue with close reticular structure
	0	Little cancellous tissue with open structure
Distal end	1	As for proximal end
	0	As for proximal end
Maximum	10	
Mandible		
Coronoid process	5	Dense and of uniform structure
	0	Low density and irregular structure
Condyle	5	Dense
	0	Low density
Cortex below condyle	5	Broad, dense, uniform structure
	0	Narrow, low density, irregular structure with many striations especially towards endosteal surface
Cortex below third molar	5	As for cortex below condyle
	0	As for cortex below condyle
Cortex of interdental bone	5	As for cortex below condyle
	0	As for cortex below condyle
Alveolar bone	3	Large amount orientated to form good tooth socket
	0	Little evidence of any alveolar bone
Maximum	28	

comprising the basal diet had been designed to permit, when completely consumed, only a small increase in weight during the experiment.

The mean gain in live weight for all animals was 7·1 lb. during the study. As shown by the data set out in Table 5, the greatest overall effects were those of calcium and starch. But these effects were not uniform, as either supplement in the absence of the other had a meagre effect on growth whereas their effect in combination was very large. Cod-liver oil had a less marked effect, and that of phosphorus was not statistically significant.

Bone ash. Mean group ash weights for the whole skeleton, overall treatment means, main effects and interactions are set out in Table 6. The 'mean group ash weights' are the mean values for the three sheep in each dietary group. The 'overall treatment means' are the means of all values for all animals to which was given or from which was withheld a particular dietary supplement; for example the data from all eight dietary groups receiving calcium (CaPDSt, CaPSt, CaDSt, CaSt, CaPD, CaP, CaD and Ca) were combined and averaged to give the 'with Ca' overall treatment mean, while the data from the remaining eight dietary groups were combined and averaged to give the 'without Ca' overall treatment mean. The main or overall effects are the differences between these pairs of overall treatment means.

The provision of supplementary calcium increased the content of mineral matter in the skeleton by almost a half. The overall effects of supplements of cod-liver oil and phosphorus were significant and positive but much smaller. Starch, overall, did not affect the quantity of ash in the skeleton.

There was a significant interaction between calcium and cod-liver oil, over the whole skeleton the sum of increases in mean skeletal ash weights caused by calcium without cod-liver oil (groups Ca, CaP, CaSt and CaPSt *v.* groups Nil, P, St and PSt) and cod-liver oil without calcium (groups D, PD, DSt and PDSt *v.* groups Nil, P, St and PSt), being only 209 g. (177 + 32 g.) compared with the determined increase of 308 g. (753–445 g.) when both supplements were included together (groups CaD, CaPD, CaDSt and CaPDSt *v.* groups Nil, P, St and PSt), giving a positive interaction of 50 g., i.e. $\frac{1}{2}$(308–209 g.). If the effect of two factors in combination is greater than the sum of their separate effects the interaction is said to be positive, and if the combined effect is less than the sum of the separate effects it is said to be negative. In other words adding cod-liver oil failed to produce a significant increase in the mineral content of the skeletons of sheep on the calcium-poor diets, but additions of calcium to calcium-poor diets improved mineral deposition in the skeleton whether cod-liver oil was provided or not, a bigger effect being produced when calcium was given with cod-liver oil than without it.

There was also a significant positive interaction between starch and cod-liver oil, in their effect on total skeletal ash, amounting to 54 g. In the absence of starch supplements, cod-liver oil had no significant effect on the quantity of minerals in the skeleton, while the provision of a starch supplement in the absence of cod-liver oil significantly depressed skeletal ash. However, when both were present

Table 4. *Mean daily intakes of the basal diet and of calcium, phosphorus, cod-liver oil, starch, and total digestible nutrients, by main treatments*

Groups	Basal diet: Concentrates (g.)	Basal diet: Straw (g.)	Calcium (g.)	Phosphorus (g.)	Cod-liver oil (ml.)	Starch (g.)
With Ca	431	66	3·3	2·2	1·0	78
Without Ca	387	65	0·4	2·0	0·9	63
With P	415	66	1·9	3·0	1·0	72
Without P	403	63	1·8	1·1	0·9	69
With D	429	68	1·9	2·2	1·9	77
Without D	389	63	1·8	2·0	—	64
With St	374	53	1·7	1·9	0·9	141
Without St	443	77	2·0	2·2	1·0	—

Significant interactions in food intakes (g.) of T.D.N. per day

	+St	−St			+St	−St	
+Ca	474	376	98 SSS	+D	476	375	101 SSS
−Ca	394	363	31	−D	392	363	29
	80 SSS	13	34 S		84 SSS	12	36 S

In this and subsequent tables: S = significant at the 5 % level; SS = significant at the 1 % level; SSS = significant at the 0·1 % level.

Table 5. *Live-weight changes, showing overall treatment means, main effects and interaction; in pounds*

Overall treatment means and main effects

With Ca	11·8	With P	8·4
Without Ca	2·4	Without P	5·8
Main effect	9·4 SSS	Main effect	2·6
With D	9·8	With St	11·1
Without D	4·4	Without St	3·0
Main effect	5·4 SS	Main effect	8·1 SSS

Interactions and partial effects

	+St	−St			+St	−St	
+Ca	19·3	3·1	16·2 SSS	+D	13·1	6·5	6·6 SS
−Ca	4·3	1·6	2·7	−D	9·2	−0·4	9·6 SSS
	15·0 SSS	1·5	6·8 SSS		3·9	6·9 SS	−1·5

	+D	−D	
+Ca	13·2	10·4	2·8
−Ca	6·4	−1·5	7·9 SS
	6·8 SS	11·9 SSS	−2·6

there was a highly significant increase in the amounts of mineral deposited in the skeleton. Other interactions among supplements were not significant.

In Table 7 are set out the levels of significance and the direction of the main effects and first-order interactions on the quantity and percentage of ash in individual bones and in various parts of selected bones.

The overall effect of adding calcium on the weight of ash and on the percentage of ash in bones and parts of bones was positive and highly significant throughout almost the whole skeleton. The overall effect of giving cod-liver oil was to increase significantly the weight of ash everywhere, except for the posterior ribs and the distal end of the radius, and to increase the percentage of ash in the head, the anterior vertebrae, and the ends of some of the long bones. The overall effect of adding phosphorus was to increase the ash content of the appendicular skeleton and ribs; there was no uniform effect on the axial skeleton. Its overall effect on the percentage of ash in bone was irregular, but distributed in a manner resembling its effect on ash weight. The overall effect of adding starch to the ration was to reduce the percentage of ash, and to leave the ash weight unaltered in most of the skeleton. However, the response of the mandible was exceptional in that the addition of starch reduced its content of mineral matter but did not alter the percentage of ash.

There was a positive interaction between calcium and cod-liver oil in the weight of ash of the axial skeleton, the pelvis, and some of the long bones, and in the percentage of ash in some of the ribs. The inter-

Table 6. *Mean group ash weights of the whole skeleton, overall treatment means, main effects and interactions (g.)*

Mean group ash weights

Group	Skeletal ash	Group	Skeletal ash	Group	Skeletal ash	Group	Skeletal ash
CaPDSt	804	CaPD	732	PDSt	527	PD	452
CaPSt	633	CaP	682	PSt	428	P	519
CaDSt	766	CaD	709	DSt	438	D	490
CaSt	522	Ca	650	St	410	Nil	425

Overall treatment means and main effects

With Ca	687	With P	597
Without Ca	461	Without P	551
Main effect	226 SSS	Main effect	46 SS
With D	615	With St	566
Without D	534	Without St	582
Main effect	81 SSS	Main effect	−16

Interactions

	+D	−D			+St	−St	
+Ca	753	622	131 SS	+D	634	596	38
−Ca	477	445	32	−D	498	569	−71 SS
	276 SSS	177 SSS	50 SS		136 SSS	27	54 SS

action between cod-liver oil and starch was detected in the ash weight of the posterior vetrebrae, the pelvis and most parts of the leg bones, where it was also positive. The other interactions were seldom significant, and these instances of significance were erratically distributed throughout the skeleton, except for the third-order interaction, CaPDSt, which was negative for ash weight in most of the long bones. This interaction may be interpreted as follows: if starch was present or if phosphorus was present the addition of cod-liver oil improved the utilization of calcium, if neither starch nor phosphorus was present the addition of cod-liver oil failed to improve the utilization of calcium, and if both phosphorus and starch were simultaneously present the addition of cod-liver oil again failed to improve the utilization of calcium, as far as these long bones were concerned.

Among the individual bones of the skeleton the mandible was of particular interest for a number of reasons. These included the features observed radiologically and described below, and the naturally occurring disorder of the mandible also described below. In addition, the reaction of the mandible to variations in dietary treatment, while resembling the reaction of the skeleton as a whole (e.g. in the highly significant main effects of calcium and cod-liver oil, and in the first-order interaction of starch and cod-liver oil), was unique in showing a strong detrimental effect of starch on its weight of ash. Not shown in Table 7 is a highly significant second-order interaction of calcium, cod-liver oil and starch in the weight of ash in the mandible.

The mean ash weights for the mandibles of the sheep in the sixteen dietary groups are set out in Table 8, together with overall treatment means, main effects, two significant first-order interactions, and the significant second-order interaction. This table shows the overall effect of starch in reducing the weight of mandibular ash, and the effect of calcium and cod-liver oil which increased it. The starch effect was significant at the 0·1% level, but it relied mainly for its significance upon the extent to which it reduced the weight of mandibular ash in the absence of cod-liver oil when a calcium supplement was included in the diet, as may be seen from the marginal differences in the second-order interaction in Table 8. Similarly, the cod-liver oil effect was most striking by far when calcium and starch were both added to the diet (17·9 g. compared with 11·6 g.). Calcium increased ash weight in all combinations with other supplements, but was least effective when the diet contained starch and no cod-liver oil.

Radiological observations. In radiographs taken of all parts of the skeleton during life the main effects of treatments were observed, but most clearly, as in previous experiments (Parts I, II and III of this series), in radiographs of the radius, particularly those taken in the anterior–posterior position. Attention is confined here, as far as radiographs of the live animal are concerned, to those of the radius, although radiographs of the tibia, when taken in the lateral position, were almost as informative as those of the radius, as was found in our earlier studies of skeletal resorption in lactating ewes.

In radiographs of bones in the flesh-free state taken at the end of the experiment the effects of treatments were, as would be expected, also evident, differences being demonstrated much more

Table 7. *The levels of statistical significance of main effects and of first-order interactions of dietary treatments in the weight of ash and the percentage of ash in different bones and parts of bones of growing Blackface wethers**

	Main effects								Interactions											
	Ca		P		D		St		CaP		CaD		CaSt		PD		PSt		DSt	
Bone	Wt.	%	Wt.	%	Wt.	%	Wt.	%	Wt.	%	Wt.	%	Wt.	%	Wt.	%	Wt.	%	Wt.	%
Skull	3	3	.	.	3	2	.	−3	.	1	1	.	.	.	.	.	.	.	1	.
Mandible	3	3	.	.	3	3	−3	.	.	.	.	.	−1	.	.	−1	.	.	2	.
Hyoid	3	3	1	.	1	1	.	.	.	.	3	.	1	.	−2	−2	.	.	1	1
Cervical																				
1	3	3	.	.	2	.	.	−1	.	.	1	.	.	.	.	.	.	.	.	.
2	3	3	.	.	3	1	.	−2	.	.	1	.	.	.	.	.	.	.	.	.
3	3	3	.	.	3	1	.	−2	.	.	1	.	.	.	.	.	.	.	.	.
4	3	3	.	1	3	1	.	−2	.	.	1	.	.	.	.	.	.	.	.	.
5	3	3	.	.	3	1	.	−2	.	.	1	.	.	.	.	.	.	.	.	.
6	3	3	1	.	3	1	.	−1	.	.	1	.	.	.	.	.	.	.	.	.
7	3	3	1	1	3	.	.	−2	.	.	1	1	.	.	.	.	.	.	.	.
Thoracic																				
1	3	3	1	1	3	1	.	−2	.	.	2	.	.	.	.	.	.	.	.	.
2	3	3	1	.	3	.	.	−2	.	.	2	.	.	.	.	.	.	.	.	.
3	3	3	1	.	2	.	.	−2	.	.	1	1	.	.	−1	.	.	.	.	.
4	3	3	1	.	3	.	.	−2	.	.	2	.	.	.	.	.	.	.	.	.
5	3	3	1	.	2	.	.	−2	.	.	2	.	.	.	.	.	.	.	1	.
6	3	3	.	.	2	.	.	.	.	.	2	.	.	.	.	.	.	.	1	.
7	3	3	.	.	2	1	.	−2	.	.	2	.	.	.	.	.	.	.	.	.
8	3	3	.	.	3	.	.	−2	.	.	2	.	.	.	.	.	.	.	2	.
9	3	3	.	1	2	.	.	−1	.	.	2	.	.	.	.	.	.	.	2	.
10	3	3	.	.	2	.	−1	−2	.	.	3	.	.	.	.	.	.	.	1	.
11	3	3	.	.	3	.	.	−2	.	.	3	.	.	.	.	.	1	.	1	.
12	3	3	.	.	3	.	.	−2	.	.	3	.	.	.	.	.	1	.	1	.
13	3	3	.	1	3	.	−1	−2	.	.	3	.	.	.	.	.	.	.	1	.
Lumbar																				
1	3	3	1	.	3	.	.	−2	.	.	2	.	.	.	.	.	.	.	2	.
2	3	3	.	1	3	.	.	−1	.	.	2	1	.	.	.	.	.	.	2	.
3	3	3	1	1	3	.	.	−2	.	.	2	.	.	.	.	.	.	.	2	.
4	3	3	1	2	3	.	.	−2	.	.	1	.	.	.	.	.	.	.	2	.
5	3	3	.	1	3	.	.	−2	.	.	1	.	.	.	.	.	.	.	2	.
6	3	3	1	2	3	.	.	−1	.	.	1	.	.	.	.	.	.	.	2	.
Sacrum	3	3	.	1	3	.	.	−2	.	.	1	.	.	.	.	.	.	.	3	.

Rib																				
1	3	3	.	1	1	.	.	−2	.	.	1	2	.	.	.	.	.	.	.	.
2	3	3	1	1	1	.	.	−2	.	.	2	1	.	.	.	.	.	.	.	.
3	3	3	1	.	1	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.
4	3	3	2	.	1	.	.	−1	.	.	2	.	.	.	.	.	.	.	.	.
5	3	3	2	1	1	.	.	−2	.	.	2	.	.	.	.	.	.	.	.	.
6	3	3	3	1	2	.	.	−1	.	.	2	1	.	.	.	.	.	.	.	.
7	3	3	3	1	1	.	.	−1	.	.	2	2	.	.	.	.	.	.	.	.
8	3	3	2	2	1	.	.	−2	.	1	1	1	.	.	.	.	.	.	.	.
9	3	3	2	1	.	.	.	.	.	.	1	2	.	.	.	.	.	.	.	.
10	3	3	2	2	.	.	.	.	.	.	1	1	.	.	.	.	.	.	1	.
11	3	3	2	2	.	.	.	.	.	.	1	1	.	.	.	.	.	.	1	.
12	3	3	1	1	.	.	.	−3	.	1	.	.	.	.	.	.	.	.	1	.
13	3	3	.	.	.	.	.	−2	.	.	.	.	.	.	.	.	.	.	.	.
Sternum	3	3	1	.	1	.	.	−1	.	.	.	.	.	.	.	.	.	.	.	.
Scapula	3	3	2	2	1	.	.	−1	.	.	1	.	.	.	.	.	.	.	.	.
Humerus																				
Proximal end	3	3	.	1	2	1	.	.	.	.	.	.	.	.	.	.	.	.	1	.
Shaft	3	2	1	1	1	.	.	−2	.	.	.	.	.	.	.	.	.	.	.	.
Distal end	3	3	2	3	3	.	.	−1	.	.	.	.	.	.	.	.	1	.	2	.
Radius																				
Proximal end	3	3	1	1	3	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.
Shaft	3	3	1	1	1	.	.	−1	.	.	.	.	.	.	.	.	.	.	1	.
Distal end	3	3	.	.	.	.	.	−1	.	.	1	.	.	.	.	.	.	.	1	.
Ulna	3	3	1	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.
Metacarpal																				
Proximal end	3	3	2	.	1	.	.	−3	.	.	.	.	.	.	.	.	.	.	.	.
Shaft	3	3	2	.	2	.	.	−2	.	.	.	.	.	.	.	.	.	.	.	.
Distal end	3	3	1	.	2	2	.	−3	.	1	.	.	.	1	.	.	.	.	1	.
Pelvis	3	3	2	2	3	.	.	−2	.	.	2	.	.	.	.	.	.	.	3	.
Femur																				
Proximal end	3	3	1	1	3	.	.	.	.	.	1	.	.	.	.	.	.	.	1	.
Shaft	3	.	2	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.
Distal end	3	3	1	3	3	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.
Tibia																				
Proximal end	3	3	2	2	3	1	.	.	.	.	1	.	.	.	.	.	.	.	3	.
Shaft	3	1	2	3	3	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.
Distal end	3	3	2	2	3	.	.	.	.	.	1	.	.	.	.	.	.	.	3	.
Metatarsal																				
Proximal end	3	2	2	1	3	.	.	−2	.	.	.	.	.	.	.	.	.	.	1	.
Shaft	3	2	3	1	3	.	.	−1	.	.	.	.	.	.	.	.	.	.	3	.
Distal end	3	3	2	.	3	2	.	−2	.	.	.	.	.	.	.	.	.	.	3	.

* In each column the level of significance of the change in weight of ash is given first, followed by the corresponding figure for percentage of ash. No sign precedes the figure when the difference caused by treatment is a gain in weight or in percentage of ash, but a decrease is shown by a negative sign. 1 = $P < 0{\cdot}05$; 2 = $P < 0{\cdot}01$; 3 = $P < 0{\cdot}001$.

clearly in these than in those of the same bone taken in the living animal at the end of the experiment. This was the case particularly for the bones of the axial skeleton, where the presence of intervening flesh and wool caused variable absorption of X-rays leading to irregular intensity in the image on the radiographic film. This makes it difficult to discriminate among small treatment effects and interactions, although large overall effects are readily seen.

Pl. 9 shows the radius, taken *in vivo* at the beginning and at the end of the experiment, for one animal, A, on a diet (CaPD) that promoted good bone development and for a second animal, B, on a diet (P) that allowed only very poor bone development. The bones of both animals (radiographs A 1 and B 1) at the beginning of the experiment showed no abnormality; there was no abnormal thickening of the epiphyseal cartilage and the cortex was moderately dense and uniform in appearance from the endosteal to the periosteal surface. In general, these bones radiographed at the beginning of the experiment lacked density, but not to an extent that is unusual in commercial hill stock of 7 months of age.

Radiographs of radii taken *in vivo* at the end of the experiment and illustrated in Pl. 9 (A 2 and B 2) demonstrate the chief effects of treatments on this bone. In A 2 the epiphyseal cartilage has a normal appearance similar to that at the beginning of the experiment (A 1) while in B 2 the cartilage is abnormally thick. The cortex in A 2 is dense and uniform from the endosteal to the periosteal surface, but in B 2 the bone is less dense throughout and with many striations, in the cortex, that show dark in the original radiographs and light in positive prints. These striations in the diaphysis are found predominantly towards the endosteal surface, but some are also found deeper in the cortical tissue. These lesions are most pronounced in the medial side of the proximal diaphysis in the region of insertion of the brachial muscle. Differences exist between A 2 and B 2 in the quantities of cancellous tissue in the ends of the bones, more being present in A 2 than in B 2. However, these differences in cancellous bone are less easily evaluated than the features described above for the epiphyseal cartilage and the diaphysis, particularly when differences are smaller than those illustrated by A 2 and B 2.

Since the radiographs of the radius taken at the beginning of the experiment were closely similar in appearance, assessments of bone quality were made by examination of the final *in vivo* radiographs of this bone. As these assessments, made by the four independent observers using the method described above, were in close agreement only a single statistical analysis of the means of their radiological values for the radius was made. Similarly, the mean assessments of the three observers for the flesh-free mandible were used in statistical analysis.

The mean radiological values for the radius are set out in Table 9. Supplements of calcium and cod-liver oil each had a large and highly significant overall effect on the radiological values for the radius. Supplements of starch, on the other hand, produced a bone of poorer radiological value. The overall effect of supplementing with phosphorus was not significant. There was a negative interaction between starch supplementation and calcium supplementation, starch producing a significant reduction of bone quality when a calcium supplement was added to the ration, but not when it was withheld. There was also a negative interaction between cod-liver oil and phosphorus supplements, the effect of cod-liver oil being greater in the absence of phosphorus supplements than in their presence.

The mean radiological values for the mandible in the flesh-free state are set out in Table 10. As with the radius, supplements of calcium and, to a lesser extent, cod-liver oil improved the quality of this bone, while the giving of starch supplements reduced quality to some extent. Supplements of phosphorus were without effect. The interactions between starch and calcium and between cod-liver oil and phosphorus, noted in radiographic observations on the radius taken *in vivo*, were also found for the mandible. In the mandible the interaction between cod-liver oil and starch was also found to be significant.

Analysis of the radiographic scores for the mandible brought to light the interaction involving cod-liver oil, calcium and starch, which was detected in the analysis of the ash weights and is set out in Table 8. In the absence of cod-liver oil the addition of starch countered the beneficial effect of adding calcium to the diet. In the presence of cod-liver oil it failed to do so.

In Pl. 10 are shown radiographs of three mandibles chosen to illustrate some features of the bone which may be altered by the different dietary treatments to which the sheep have been submitted. The mandibles are: A, from group CaP in which the quality of the mandible was high; B, from group CaPSt in which the mandibles were of poor quality, but superior to those of some groups (e.g. Nil and St); C, from group CaPDSt in which the quality of the mandible was, again, high. This set of three mandibles thus also serves to show how the damaging action of starch added to a calcium supplemented ration is countered by adding a source of vitamin D.

In the region of the coronoid process, the condyle and the mandibular notch, mandible A exhibits an anatomical structure superior to that of mandible C. Mandible A shows the normal contour of the coronoid process, the mandibular notch and the condyle, while in mandible C the coronoid process is flattened and the mandibular notch assumes a more circular

Table 8. *Mean group ash weights of the right mandible (without teeth), overall treatment means, main effects and interactions (g.)*

Mean group ash weights

Group	Mandible ash	Group	Mandible ash	Group	Mandible ash	Group	Mandible ash
CaPDSt	18·4	CaPD	18·1	PDSt	9·5	PD	10·5
CaPSt	12·0	CaP	18·7	PSt	7·8	P	11·3
CaDSt	17·4	CaD	19·5	DSt	10·1	D	10·9
CaSt	11·2	Ca	17·7	St	7·7	Nil	7·5

Overall treatment means and main effects

With Ca	16·6	With P	13·3
Without Ca	9·4	Without P	12·8
Main effect	7·2 SSS	Main effect	0·5
With D	14·3	With St	11·8
Without D	11·7	Without St	14·3
Main effect	2·6 SSS	Main effect	−2·5 SSS

First-order interactions

	+St	−St			+St	−St	
+Ca	14·8	18·5	−3·7 SSS	+D	13·8	14·7	−0·9
−Ca	8·8	10·0	−1·2	−D	9·7	13·8	−4·1 SSS
	6·0 SSS	8·5 SSS	−1·2 S		4·1 SSS	0·9	1·6 SS

Second-order interactions

With cod-liver oil (+D)	+St	−St		Without cod-liver oil (−D)	+St	−St	
+Ca	17·9	18·8	−0·9	+Ca	11·6	18·2	−6·6 SSS
−Ca	9·8	10·7	−0·9	−Ca	7·8	9·4	−1·6
	8·1 SSS	8·1 SSS	0·0		3·8 SS	8·8 SSS	−2·5 SS

Table 9. *Mean group radiological values of right radius, overall treatment means, main effects and interactions*

Mean group radiological values

Group	Value points*	Group	Value points*	Group	Value points*	Group	Value points*
CaPDSt	8·0	CaPD	8·8	PDSt	4·6	PD	5·2
CaPSt	6·1	CaP	8·4	PSt	3·7	P	4·0
CaDSt	8·2	CaD	9·0	DSt	5·6	D	4·3
CaSt	4·3	Ca	8·0	St	1·6	Nil	1·9

Overall treatment means and main effects

	Points*		Points*
With Ca	7·6	With P	6·1
Without Ca	3·8	Without P	5·4
Main effect	3·8 SSS	Main effect	0·7
With D	6·7	With St	5·3
Without D	4·8	Without St	6·2
Main effect	1·9 SSS	Main effect	−0·9 S

Interactions

	+St	−St			+D	−D	
+Ca	6·6	8·6	−2·0 SS	+P	6·6	5·6	1·0 S
−Ca	3·9	3·8	0·1	−P	6·8	4·0	2·8 SSS
	2·7 SSS	4·8 SSS	−1·0 S		−0·2	1·6 SS	−0·9 S

* The method of arriving at the radiological values is described in the section dealing with Methods on pages 202 and 203.

Table 10. *Mean group radiological values of left mandible, overall treatment means, main effects and interactions*

Mean group radiological values

Group	Value points*	Group	Value points*	Group	Value points*	Group	Value points*
CaPDSt	8·0	CaPD	7·9	PDSt	2·9	PD	2·8
CaPSt	4·1	CaP	7·8	PSt	3·5	P	3·7
CaDSt	7·4	CaD	8·1	DSt	4·3	D	4·5
CaSt	3·5	Ca	7·2	St	2·2	Nil	1·1

Overall treatment means and main effects

	Points*		Points*
With Ca	6·8	With P	5·1
Without Ca	3·1	Without P	4·8
Main effect	3·7 SSS	Main effect	0·3
With D	5·8	With St	4·5
Without D	4·1	Without St	5·4
Main effect	1·7 SSS	Main effect	−0·9 SS

First-order interactions

	+St	−St	
+Ca	5·8	7·8	−2·0 SSS
−Ca	3·2	3·0	0·2
	2·6 SSS	4·8 SSS	−1·1 SS

	+St	−St	
+D	5·6	5·8	−0·2
−D	3·3	5·0	−1·7 SS
	2·3 SSS	0·8	0·8 S

	+D	−D	
+P	5·4	4·8	0·6
−P	6·1	3·5	2·6 SSS
	−0·7	1·3 S	−1·0 SS

Second-order interactions

With cod-liver oil (+D)

	+St	−St	
+Ca	7·7	8·0	−0·3
−Ca	3·6	3·6	0·0
	4·1 SSS	4·4 SSS	−0·2

Without cod-liver oil (−D)

	+St	−St	
+Ca	3·8	7·5	−3·7 SSS
−Ca	2·8	2·4	0·4
	1·0	5·1 SSS	−2·0 SSS

* The method of arriving at the radiological values is described in the section dealing with Methods on pages 202 and 203.

shape because of the bending forward of the condyle, which is also somewhat flattened. In mandible B the appearance in this region is much poorer, and there is severe flattening and distortion of the condyle. The vertical ramus below the condyle is much less dense in mandible B than in mandibles A and C. The coronoid process is flattened in mandible B.

In the region of the angle of the mandible, the horizontal ramus and the interdontal bone, the differences between mandibles A and C are not great. Mandible B is markedly poorer throughout this region, with a general lack of density in the bone and striations throughout all cortical bone.

An outstanding feature is the small amount of alveolar bone surrounding the molars in mandible B, compared with the amount present in mandibles A and C. Most striking of all is the virtual absence of *lamina dura* in the sockets of the first and second permanent molars in mandible B. In contrast to this the *lamina dura* is well defined in mandibles A and C, but is more compact in mandible C than in mandible A. In the alveolar bone of the primary (deciduous) pre-molars the same features are evident, a somewhat greater amount being present in mandible C than in mandible A, with but little in mandible B. The development of the secondary (permanent) third pre-molar is further advanced in mandible C than in mandible A, calcification of the crown of the tooth being well defined in mandible C. In mandible B the tooth crypt has formed but there is only very slight evidence of mineralization.

Lengths of long bones. The effects of treatments on the lengths of long bones were small, but some were statistically significant. Calcium increased the lengths of the humerus, radius, femur and tibia just over 2 % ($P < 0{\cdot}01$), but the increase of about 1 % in the metacarpal and metatarsal was not significant. Of the other supplements only cod-liver oil in the presence of starch gave consistent increases (between 3 and 4 %) and these were significant for all six long bones.

Blood analyses. Mean monthly serum calcium values for each treatment group are shown in Figs. 1–4.

The serum calcium values for all groups not receiving the calcium supplement decreased during the experiment (Figs. 1, 2). while values for all except two groups receiving calcium remained well within the normal range (Figs. 3, 4). The overall effect of the calcium supplement on serum-calcium values at the end of the experiment was highly significant.

The decrease in serum-calcium values in groups not receiving supplementary calcium was slightly greater in the presence than in the absence of supplementary phosphorus (Figs. 1, 2). This interaction between calcium and phosphorus supplements was significant.

The addition of cod-liver oil reduced slightly the fall in serum-calcium values which took place in all groups not receiving the calcium supplement (Figs. 1, 2), and prevented the fall which took place in groups fed calcium and starch supplements (Fig. 4). The overall effect of cod-liver oil in reducing or preventing the fall of serum-calcium values was significant, and there was a suggestion that starch depressed serum-calcium levels in hoggs receiving either a calcium supplement alone or calcium and phosphorus supplements together, and was prevented from doing so by the addition of cod-liver oil. However, this interaction among calcium, cod-liver oil and starch was not significant.

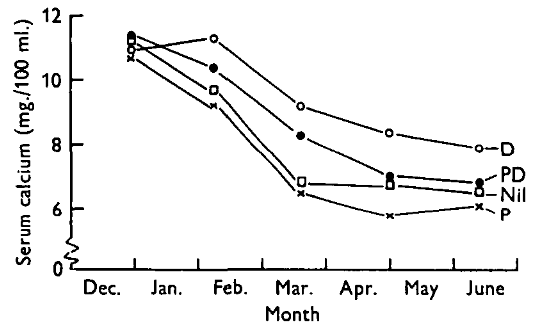

Fig. 1. Changes in the levels of calcium in the blood serum of hoggs of groups PD, P, D and Nil.

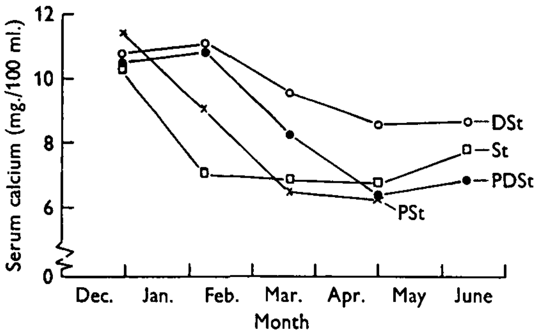

Fig. 2. Changes in the levels of calcium in the blood serum of hoggs of groups PDSt, PSt, DSt and St.

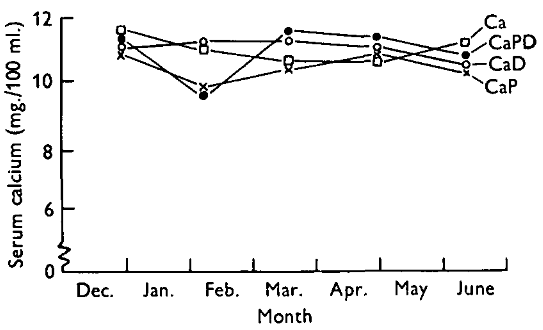

Fig. 3. Changes in the levels of calcium in the blood serum of hoggs of groups CaPD, CaP, CaD and Ca.

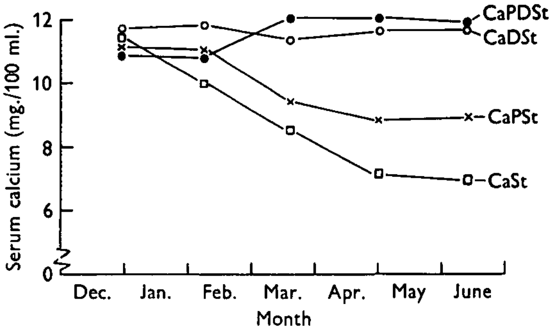

Fig. 4. Changes in the levels of calcium in the blood serum of hoggs of groups CaPDSt, CaPSt, CaDSt and CaSt.

In general, blood inorganic phosphorus values increased during the experiment, the increases being greatest for groups having low serum-calcium values.

DISCUSSION

The dietary treatments used in this experiment produced great differences in live-weight increase, in blood composition, in bone growth as measured by the amount of mineral matter in the skeleton, and in the quality of bone as judged from radiographs of the living animal and of bones in the flesh-free state. Differences in the percentage of ash in bone were proportionately less than differences in other measures among different treatment groups. The total weight of ash in the skeleton of animals that had been given diets favourable to body and bone growth was almost twice as great at the end of the experiment as that of animals that had received the poorest diets. Since animals were not killed at the beginning of the study to provide estimates of the amount of bone and bone minerals present at the time when they were transferred to the experimental diets, there is no way of estimating the actual accretions of bone and bone minerals promoted by the different dietary treatments.

Supplements of calcium and cod-liver oil had marked overall effects on live-weight increases and on bone growth, as measured by the quantities of minerals deposited in the skeleton as a whole, and in individual bones. The calcium supplements promoted a heavier deposition of minerals in the skeleton than did the cod-liver oil, and this greater deposition was associated with an increased density of bone, as shown by radiography and by the marked elevation in the percentages of ash in all parts of the skeleton. Cod-liver oil supplements improved the density of only a few bones.

Supplementation of the diets with starch had a marked overall effect on live-weight increase, but none on the deposition of minerals in the skeleton as a whole. Presumably, as the dietary provision of energy was increased, the organic framework of the bones increased in size concomitantly with the rest of the body so that, on account of mineral deposition not being proportionately increased, the percentage of ash fell in almost all of the axial skeleton and in many regions of the appendicular skeleton. Of particular interest was the atypical reaction in the mandible, where the provision of extra dietary energy resulted in a smaller bone in which the percentage of ash had not been reduced.

Adding phosphorus supplements to the diets had no overall effect on live-weight increase but a small overall effect on the total deposition of minerals in the skeleton. Among the bones of the skeleton this increase was found mainly in the weights of ash in appendicular parts. To a lesser extent bone density was improved in appendicular parts, and erratically in the axial skeleton. Since the deficiency of phosphorus was considerably less severe than that of calcium it was not to be expected that the benefits derived from giving phosphorus supplements would be as great as those derived from adding calcium to the diets.

There was a positive interaction between calcium and cod-liver oil, in that supplements of cod-liver oil exerted a beneficial effect on the deposition of minerals in the skeleton only in the presence of calcium supplements. This failure of cod-liver oil to improve mineral deposition in the absence of calcium supplements is not surprising, since the basal diet provided the animals with only 0·4 g. of calcium daily. In contrast, the raising of calcium intakes to 3·3 g. daily, without giving cod-liver oil, had a marked beneficial effect on the deposition of minerals in the skeleton and at the same time strongly stimulated general body growth. However, the full capacity of the skeleton for development was not satisfied by providing calcium alone, as was shown when cod-liver oil supplements were added along with it. In summary, the addition of cod-liver oil alone produced no increase in ash weight, the calcium supplement alone increased ash weight from 445 to 622 g. and their combined effect was to increase the weight of ash to 753 g. There was no comparable interaction in live-weight increases, the combination of calcium and cod-liver oil promoting the gains one would expect from the gains on either alone. The positive interaction between calcium and cod-liver oil was exhibited chiefly in the weight of ash of the axial skeleton, and of a few appendicular parts.

Cod-liver oil and starch interacted in their effects on the weight of ash in the skeleton, but not on the percentage of ash nor on live-weight increase. This interaction, which was positive, was mainly detected in the weights of ash in the head, the posterior vertebrae and the appendicular skeleton. From Tables 5 and 6, when one compares the effect of starch in the absence of cod-liver oil, it is seen to enhance live-weight increase, but to bring about a reduction in the deposition of minerals in the skeleton as a whole.

The most interesting feature of the study was the behaviour of the mandible, in that it revealed differences among treatment groups to a greater extent than any other part of the skeleton, whether judged by chemical composition or by radiography in the flesh-free state. Moreover, there was a close similarity in the first- and second-order interactions and in the partial effects between the analytical values for the mineral content of the bone and the values awarded on radiological evaluation. In addition, both the radiographic appearance and the mineral content of the mandible bore an obvious overall relationship to the terminal values for calcium in the serum. In Fig. 5 the radiological values obtained for the flesh-free mandibles are plotted against the corresponding serum-calcium levels, as means of the data obtained for each group on analysis of the blood samples drawn in May and June. Against the point plotted to represent the mean terminal status of each group, for serum-calcium and radiological value, are also given the supplements added to the basal diet and the mean value for ash content of the mandible. Thus, the point CaPDSt at the co-ordinates representing a mean serum-calcium value of 12·0 mg. per 100 ml. and a mean radiological value of 8·0 points refers to the treatment group receiving all four dietary supplements and having a mean (rounded-off) mandibular ash content of 18 g. From a clinical standpoint hoggs having serum-calcium values of 10–12 mg./100 ml. and radiological values of 8–10 points would be classified as normal in these respects.

The data in Fig. 5 show that mandibles of good quality, as measured by their content of minerals and by their radiographic appearance, were obtained in six treatment groups (Ca, CaP, CaD, CaPD, CaDSt, and CaPDSt) and that the hoggs of these groups had normal levels of serum-calcium in the terminal stage of the experiment, as indeed they had earlier shown. Among these six groups were four (Ca, CaP, CaD and CaPD) in which body growth was kept to a low level by withholding the starch supplement, yet the animals of these groups developed mandibles of weight and quality equal to the remaining two groups that had received the energy supplement (CaDSt and CaPDSt). However, if calcium were withheld, or if calcium and starch were both given while withholding cod-liver oil, there was serious damage to the mandible, shown by simultaneously reduced values for mineral content and for radiographic appearance, and by the associated serum-calcium level. No greater precision in the relationship between the radiographic appearance and the mineral content of the mandible on the one hand, and the serum-calcium content on the other, as mentioned above, is claimed beyond this general division of good from poor. Inspection of Fig. 5 shows that radiological values below 4·5 points were associated with mandibular ash weights of 12 g. or less and serum-calcium values under 9·0 mg./100 ml. Radiological values of 7 points and above occurred along with ash weights of 17 g. and over, and with serum-calcium values of above 10·0 mg. per 100 ml.

Some features of the second-order interaction among calcium, cod-liver oil and starch supplements are clearly shown in Fig. 5. The quality of the mandible and the mineral status of the animal in general were good when a calcium supplement was given (group Ca). This is shown by the mandible ash content (18 g.), the radiological value (7·2 points), and the serum calcium level (10·8 mg./100 ml.), and also by the mineral content of the whole skeleton, to which reference will be made later. Giving the caloric supplement in addition to the calcium supplement (group CaSt) caused a sharp reduction in the three values shown in Fig. 5, and in the total skeletal ash. The further supplementation with cod-liver oil (group CaDSt) caused a reversal of the position. The same interaction operated in groups CaP, CaPSt and CaPDSt, with the qualification that the serum-calcium level and the total skeletal ash content did not fall so precipitously in these circumstances, where phosphorus was present, as did the weight and quality of the mandible.

In Fig. 6 radiological values for the radius, taken *in vivo* at the end of the experiment, are plotted against the terminal values for serum calcium. Against the point plotted to represent each treatment group is given the form of dietary supplementation and the corresponding mean value for total ash in the skeleton. From the clinical standpoint of blood composition or radiographic appearance of the radius (and other bones taken *in vivo*) hoggs having serum-calcium values between 10 and 12 mg./100 ml. or radiological values between 8 and 10 points would be classified as normal, and the hoggs of groups Ca, CaP, CaD, CaPD, CaDSt and CaPDSt all satisfy both these criteria. The skeletons of the hoggs in the two groups that received starch supplements (CaDSt and CaPDSt) were heavier than those of the remaining four groups but were of no better quality, as judged by the present method of evaluating radiographs, in which differences in overall size are not specifically taken into consideration in awarding points for bone quality. Within these normal ranges there was a tendency for higher levels of bone ash to be associated with higher levels of serum-calcium, but radiological values did not appear to be associated

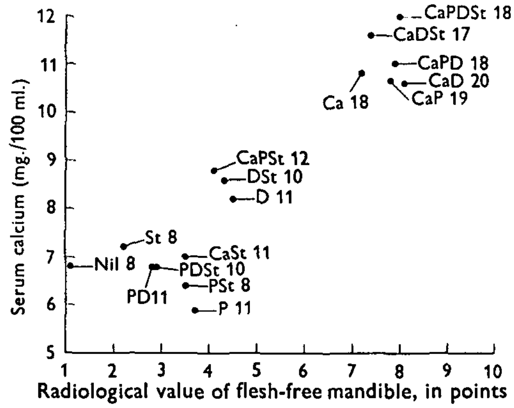

Fig. 5. The relationship between the level of calcium in blood serum and the quality of the mandible as seen in radiographs taken of this bone in the flesh-free state. Against each point is given the dietary treatment of the group, together with the mean value (g.) for mandibular ash.

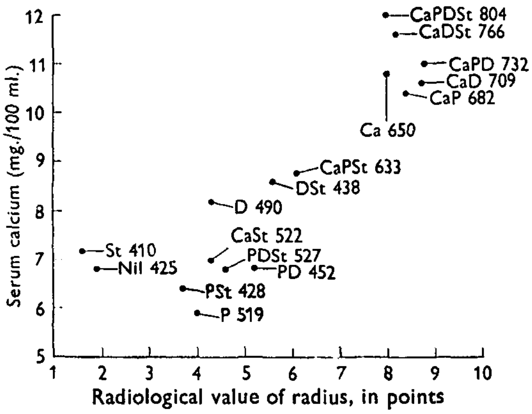

Fig. 6. The relationship between the level of calcium in blood serum and the quality of the radius as seen in radiographs taken *in vivo*. Against each point is given the dietary treatment of the group, together with the mean value (g.) for total skeletal ash.

with either. There was no relationship between serum-calcium levels and radiological values among these six groups.

The mineral status of the remaining ten groups was poorer, as indicated by terminal serum-calcium levels, by radiological values, and by the amounts of ash in the skeleton—except in group CaPSt, where the amount of skeletal ash was not seriously reduced in comparison with the amounts in hoggs of groups Ca and CaP. In these ten groups there was not a close association between the level of calcium in the serum and the quality of the radius as seen radiographically in the living animals. Thus, the lowest terminal mean value for serum-calcium (5·9 mg./100 ml.) was found in group P where the radiological value was 4·0 points, while the lowest radiological value (1·6 points) was found in group St where the serum-calcium level was 7·2 mg. Similarly, among these ten groups neither serum-calcium levels nor radiological values were closely related to the weights of ash in the skeletons. Among the sixteen groups, as a whole, there was a general tendency for radiological values to vary with the serum-calcium levels, with higher concurrent values of both to be associated with better mineral deposition in the skeleton, and conversely for lower concurrent values of both to be associated with poorer mineral deposition.

When the distribution of points in Fig. 5 is compared with the distribution of points in Fig. 6 a similarity in pattern is immediately apparent. By all three criteria used in Fig. 6 the skeleton as a whole was poorer if calcium had been withheld. In addition, the detrimental effect on the mandible of starch, in certain dietary combinations, was repeated for the skeleton as a whole. The interaction, involving starch, cod-liver oil and calcium, can be seen in Fig. 6, although for the skeleton as a whole it did not quite reach a level of significance, whereas in the mandible the interaction was most marked. Thus, when the diets were adequate in calcium or in calcium and phosphorus and when growth was stimulated by giving a good energy supply, but withholding a vitamin D supplement, there was a more severe reduction in mandibular development than was indicated by serum-calcium levels or general clinical appearance. In this connexion it will be recalled that the only symptom of bone disorder in this experiment was slight lameness in a small number of starch-fed animals. As one of the characteristic changes that took place in the mandible was a severe reduction in the amount of alveolar bone constituting the tooth sockets, it seems possible that changes of this nature that might predispose the animal to dental disorders later in life can take place during early growth without noticeable ill-health.

In Fig. 7 the radiological values of the radius, taken *in vivo*, are plotted against the radiological values for the flesh-free mandible for all dietary treatment groups. It may be noted that the largest treatment effects were detected radiographically in both bones so that such large effects on the mandible, taken in the flesh-free state, were indicated by the values obtained for the radius, taken *in vivo*.

As previously stated, in these hoggs there was no manifestation of rickets, using this term in its common clinical sense of entailing the deviation of the bones of the legs from their normal alignment owing to distortion at the joints. This distortion develops when the organic matrix at the epiphysis grows, but fails to pass through the succeeding stages of calcification and ossification, and there forms a band of cartilage and imperfectly hardened bone. This failure in calcification and ossification is caused by an inadequate supply of minerals, and perhaps other essentials, at the site of cartilage growth. In severe cases of rickets the joints are medially or laterally bent and the long bones are swollen at their zones of longitudinal growth, owing to localized broadening of the poorly mineralized organic matrix. This accumulation of unossified cartilage is not peculiar to the leg bones, but is found throughout the skeleton in regions of endochondral ossification. However, in the legs the consequences of imperfect bone formation are seen when the burden of the animal's weight causes distortion.

There are large differences among species in their susceptibility to rickets, and these can, perhaps, be explained in part by the great differences among the various species in the stage of maturity of their skeletons at birth. In the new-born lamb the com-

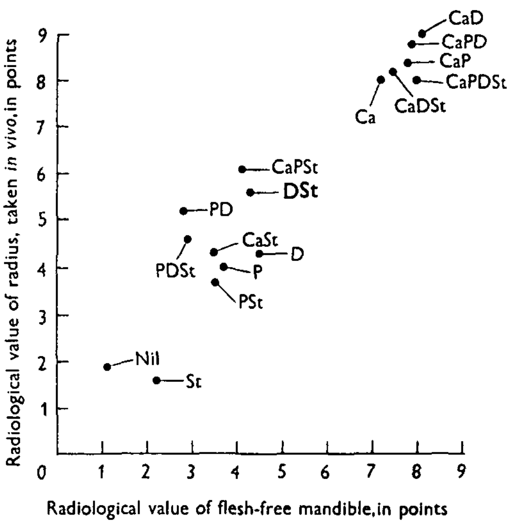

Fig. 7. The relationship between the radiological value of the radius, taken *in vivo*, and the radiological value of the flesh-free mandible. Against each point is given the dietary treatment of the group.

bined leg bones at birth are almost half their final, or adult, length (Hammond, 1932). About three-fifths of adult leg-bone length has been achieved in the new-born foal (Meek, 1901), about half in the new-born calf (Ragsdale, 1934), about one-quarter in the new-born piglet (McMeekan, 1940), and about one-sixth in the new-born rat (Donaldson, 1920). The leg bones of the newly hatched chick are about one-fifth of their final length (Wilson, 1954). Another important source of variation among mammalian species in susceptibility to rickets is the amount of additional leg growth made during the permitted suckling period. Information on this is not extensive, but in lambs of 5 months of age the combined leg bones have increased in length so that they are about four-fifths of adult length (Hammond, 1932). It is obvious that the risk of rickets developing is less in proportion to the amount of adult bone length achieved before the protective influence of suckling is withdrawn. When only small increases in bone length remain to be made after weaning there is less opportunity for a fully rachitic joint to develop.

Exposure to the risk of frank rickets through imperfect feeding is thus of limited duration in lambs, compared with such domestic species as the dog, the rat and the chick. Unless they are severe, shortages of essential nutrients in the diet of the pregnant female have little or no effect on foetal bone development. Similarly, the milk produced is of uniform composition, deficiencies in the diet affecting milk yield rather than milk composition. Consequently, unless the maternal diet is poor during late pregnancy and lactation, the lamb is exposed to the risk of rickets mainly in the later stage of the suckling period and after weaning when it depends on the grazing available or on the feed provided. Liability to rickets is also subject to a non-dietary influence in the form of ultra-violet radiation which affects vitamin D formation, either directly, in the animal itself, or indirectly, in the herbage on which it is grazing.

The extent to which lambs, and other young mammals, are exposed to the risk of developing frank rickets depends also on the rate of growth permitted after weaning. It was shown in the earlier study (Duckworth *et al.* 1943) that there was no clinical manifestation of rickets, no stiffness of gait, and only minor changes in the blood levels of calcium, inorganic phosphorus and phosphatase when growth was kept at a low level through restricting the supply of metabolizable energy in rations containing little vitamin D and only moderate amounts of calcium and phosphorus. In the present study the dietary treatment of groups Ca and CaP was similar to those just mentioned in the earlier study, and the earlier observations have been confirmed; not only was there no clinical evidence of rickets but no radiographic evidence of bone disease in the radius, the mandible, or in other bones examined but not reported on in the present communication. On the other hand, rapid growth on diets unbalanced in their content of nutrients closely involved in bone metabolism caused damage to the skeleton in both studies, and exemplified in the present one by the hoggs of groups CaSt and CaPSt.

Interpretation of published work on the causation of bone disease in growing sheep is made more difficult because of uncertainties about the requirements of calcium and phosphorus for normal growth. The early standard of Mitchell & McClure (1937), based on assessments of the amounts of calcium and phosphorus that need to be present in the diet to allow normal daily accretions of these elements in the body, set requirements at 1·6 g. for calcium and 1·5 g. for phosphorus in the diets of growing sheep, of 70–90 lb. live weight, consuming about 1 kg. of dry matter daily. The more recent estimates of the U.S. National Research Council's Sub-committee on Sheep Nutrition (1957) for replacement ewe lambs of 70 lb. live weight, are 3·0 g. of calcium and 2·6 g. of phosphorus daily; no explanation is given for raising the allowances above the earlier levels, beyond indicating that the general approach of the sub-committee involved the inclusion in their recommended allowances of margins of safety in the provision of nutrients. Auchinachie & Fraser (1932) showed that when growing sheep of 7 months of age received diets containing 1·9 g. of calcium and 4·8 g. of phosphorus growth was good, blood composition remained normal and bone development was apparently satisfactory, provided that the diet was supplemented with cod-liver oil as a source of vitamin D. If the vitamin D source was omitted the animals became severely rachitic, growth was poor and serum-calcium levels fell. If instead of giving the vitamin D supplement extra calcium was given, so that the daily intake rose to 7·0 g., there was no sign of rickets, blood composition remained normal, but growth was not stimulated to the same extent as when cod-liver oil was given.

Duckworth *et al.* (1943), working under conditions closely similar to those of Auchinachie & Fraser, found that with allowances close to those recommended by Mitchell & McClure—namely, of 1·6 g. of calcium and 1·9 g. of phosphorus daily—growth was good, blood composition remained normal and bone development was apparently satisfactory provided that cod-liver oil was given. If the vitamin D supplement was withheld rickets developed, provided, as said above, that the diet contained sufficient metabolizable energy for growth to be good. Comparable results were obtained by Andrews & Cunningham (1945) who fed diets containing higher levels of minerals—2·7 g. of calcium and 5·8 g. of phosphorus daily—and capable of supporting high rates of growth. They found that if no source of vitamin D were provided there were changes in the composition of the blood and in the histological appearance of the bone indicative of rickets.

The findings of Ewer (1951*a*) are of particular interest from the standpoint of calcium, phosphorus and vitamin D provision, rate of growth and the manifestation of rickets in growing sheep. He found that rickets, in the form of enlargement of the carpus, stiff gait, and thickening of the epiphyseal cartilage as seen radiographically, developed in sheep receiving rations that provided 8·9 g. of calcium and 0·3 g. of phosphorus daily when vitamin D was not given. However, rickets did not develop: (1) if the calcium intakes were reduced from 8·9 to 4·2 g. daily, without giving a vitamin D supplement; (2) if the phosphorus intakes were raised from 0·3 to 1·9 g. daily, without giving a vitamin D supplement; and (3) if the mineral allowances were unchanged but a vitamin D supplement was given. It is obvious that normal accretions of bone minerals could not take place in growing sheep receiving as little as 0·3 g. of phosphorus daily, and indeed the phosphorus balance studies of sheep on such diets published by Ewer (1951*b*) showed that there were, on average, slight negative phosphorus balances when vitamin D was withheld and only trivial gains in phosphorus when the vitamin was given. There are probably three reasons for the absence of evidence of bone disease, in the form of clinical rickets, in sheep on such low phosphorus diets and provided with vitamin D: (1) since the sheep were 10 months old when transferred to the experimental diets the amount of leg bone growth still to be made was small; (2) since the deficiency of phosphorus markedly reduced live-weight increase the leg-bone elongation would probably be reduced; and (3) since, as demonstrated radiographically, there was no thickening of the epiphyseal cartilage of the radius, enlargement of the carpus, with consequent stiffness of gait, could not develop. An analogous finding was made in the present experiment where giving vitamin D in the absence of calcium led to a markedly better quality of bone as assessed radiographically by the present method of awarding points, which involves deductions for thickening of the epiphyseal cartilage. In spite of this the deposition of minerals in the skeleton as a whole, and in the radius as an individual bone, was not increased. In Ewer's work, too, judging from his phosphorus balance measurements, there was little improvement in mineralization of the skeleton as a whole in spite of the improved radiographic appearance of the radius and the absence of gross clinical symptoms. In other work, where animals had access to sunlight or were provided with a dietary supplement of vitamin D, poor skeletons but not rickets were produced when diets were low in calcium (Franklin, 1934–35) or phosphorus (Martin & Peirce, 1934; Stewart, 1934–35).

Theiler (1934) reported findings that are at variance with the view that a simple deficiency of phosphorus or calcium, vitamin D being adequately supplied, causes a poorly mineralized skeleton but not rickets. He reported that rickets developed in lambs on the phosphorus-deficient veld of South Africa in spite of intense solar radiation. He considered that when ruminant stock are grazing on phosphorus-deficient herbage the shortage of phosphorus caused the lactating female's milk supply to be low thus forcing the young to come to depend on the herbage for nourishment to a greater extent than would be the case if the dam's milk production were higher. Since the herbage is inadequate in its phosphate content for growth of the young animal, as well as for sustaining normal lactation in its dam, the calf or lamb is exposed to phosphorus deficiency, leading, in spite of abundant solar radiation, to the development of rachitic skeleton. Work at this Institute, later to be published, supports Theiler's view. Lambs weaned at 2 and 4 months of age have been transferred to diets deficient in phosphorus but well supplied with vitamin D and have developed typical rachitic lesions, as seen radiographically, with swollen carpal joints, and distortion of the hind limbs.

The existence of an unidentified substance, or substances, interfering with bone metabolism has recently been suggested. Ewer & Bartrum (1948) produced rickets in growing sheep by allowing them to graze green oats or barley during winter. The calcium and phosphorus intakes appear to have been between 2 and 3 g. daily, which would be at or above requirement levels for sheep also receiving vitamin D. The bone disorder and the depressions in blood calcium and inorganic phosphorus could be prevented by dosage with vitamin D. This may have been a simple vitamin D deficiency, such as was encountered by Andrews & Cunningham (1945), although Ewer & Bartrum considered that a substance present in green herbage interefered with bone growth. Subsequently, support for the existence of such a substance, or substances, in the ether soluble fraction of green herbage was obtained from experiments with rats (Grant, 1954; Weits, 1956; Grant & O'Hara, 1957), but the suggestion that it is β-carotene or vitamin A could not be supported (Coates, Kon & Porter, 1956). More recent work has shown that the rachitogenic effect of vitamin A on rats is profoundly affected by the nature of the test diet on which the rats are fed (Grant, 1956). Accordingly the position remains obscure.

A condition somewhat similar to that reported for growing sheep on green oats and barley has been reported for a flock of ewe hoggs in the north of Scotland (Shanks & Donald, 1955). The hoggs grazed during 2 months of the autumn and early winter on a newly reseeded hill. Until mid-November growth was good, but eventually the animals were found to have developed bones that were thin and poorly mineralized. The main gross clinical symptom was not lameness, of which only a few cases were seen, but a slight displacement of the mandible that led to dental malocclusion. In about one-third of the flock the incisors did not close on the dental pad owing to abnormal engagement of the molars, and closure could not be forced even by the application of considerable manual force in severe cases. On radiographic examination the state of the skeletons of severely afflicted animals resembled closely the poorer skeletons of the present study, while the general form of the mandible—involving flattening of the coronoid process, distortion of the mandibular notch, flattening of the condyle, and rami that were thin, striated and lacking in density—was closely similar. More detailed observations on these animals and their subsequent dental history will be published later (Benzie, Dalgarno & Hill).

As far as bone disease in growing sheep is concerned the above survey of the literature and the results of the present study suggest:

(1) that there are combinations of energy, calcium, phosphorus and vitamin D intakes that support a rate of growth that falls short of realizing the animal's inherent potentialities, but although the skeleton is undersized in relation to age there is little or no damage to the skeleton demonstrable by radiography or chemical analysis, and no disturbance in blood levels of calcium and inorganic phosphorus;

(2) that there are other combinations of energy, calcium, phosphorus and vitamin D intakes that cause damage to the skeleton, and these include simple deficiencies of calcium, phosphorus and vitamin D accompanied by sufficient energy and protein to sustain good rates of growth;

(3) that the degree of damage varies considerably from one part of the skeleton to another and from one region to another within an individual bone, the nature of the damage depending upon the type of deficiency, but with zones of active growth usually showing the most marked effects, and there are dietary combinations that cause serious damage to the mandible including the supporting structures of the teeth;

(4) that the age at which the animal begins to eat an unbalanced diet, capable of causing bone disease, is of importance in determining the kind of damage that will predominate in the skeleton, younger animals being more liable to exhibit such gross clinical symptoms as frank rickets than older growing animals that are closer to the completion of skeletal development when they encounter such a diet;

(5) that some confusion in relating diet to bone disease has arisen through reliance on gross clinical examination and blood analysis as methods of indicating the status of the skeleton and that radiographic examination, including some method of quantitative assessment of the radiographs, is more informative.

SUMMARY

1. A factorial experiment on wether hoggs was carried out to examine the effects on bone growth and composition and on blood composition of four dietary factors. The factors were extra calcium, phosphorus, vitamin D, and energy in the form of starch, and each was fed in all possible combinations, so that there were sixteen dietary treatment groups in all. Each group consisted of three animals.

2. Serum calcium and blood inorganic phosphorus concentrations were determined monthly, and radiographs of most parts of the skeleton were taken at the beginning and end of the experiment. After about 6 months on the experimental diets the animals were killed and the ash weights and percentage of ash of different parts of the skeleton and of the whole skeleton were determined. Live-weight changes and food intakes were also measured.

3. The unsupplemented diet was markedly deficient in calcium, vitamin D and metabolizable energy, and moderately deficient in phosphorus.

4. Calcium supplementation was the most important single factor and increased the ash content of every bone measured. Cod-liver oil supplementation increased the ash content of almost all bones. Phosphorus had a similar but smaller effect. The ash weight was increased more when calcium and cod-liver oil were applied together than would be expected from the results of their independent application. There was a similar positive interaction between cod-liver oil and energy supplementations, but this interaction applied more to the long bones while the calcium:cod-liver oil interaction applied rather to the ribs and vertebrae.

5. Calcium supplementation also improved the percentage of ash more than any other factor, and did so for every bone. Starch supplementation was harmful almost everywhere. Phosphorus improved the lower vertebrae, the ribs and some of the long bones, while cod-liver oil improved the cervical vertebrae and the bones of the head. There was a positive interaction between calcium and cod-liver oil in some of the ribs, and a few other isolated interactions erratically distributed in the skeleton.

6. The mandible was different from other bones in its response to starch supplements. The addition of starch did not alter the percentage of ash but reduced the weight of ash in the mandible.

7. Calcium increased the length of the long bones, and cod-liver oil also increased their length, provided that starch was present.

8. The radiological values for the mandible and radius, obtained at the end of the experiment, agreed well with the results obtained for the weights of ash in these bones, but less well with the change in serum calcium that had taken place during the experiment.

We thank Mr R. Pearcy and Miss M. Smith for assistance in this work.

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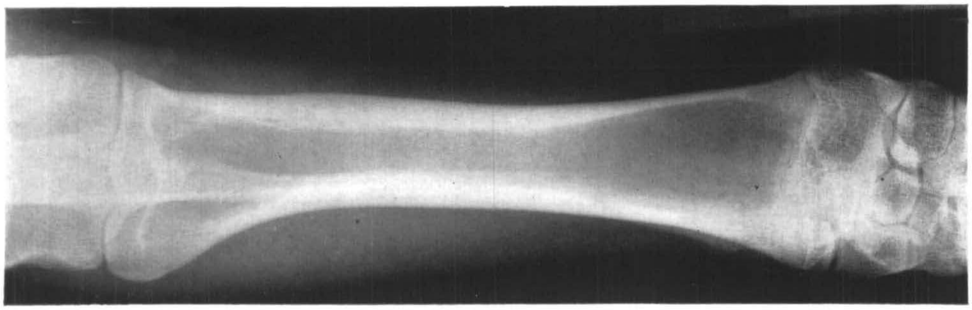
EXPLANATION OF PLATES

PLATE 9

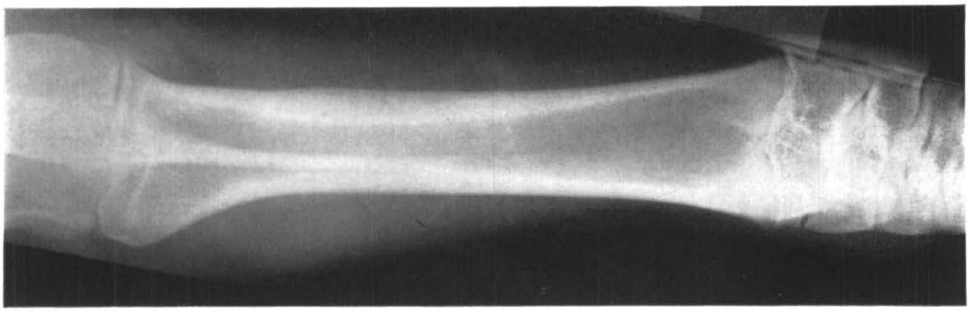
Radiographs of radii, taken *in vivo* (anterior-posterior position). A 1 was taken at the beginning of the experiment and A 2 at the end of the experiment, of a hogg fed throughout on a diet (CaPD) that promoted good bone development. B 1 was taken at the beginning of the experiment and B 2 at the end of the experiment, of a hogg fed throughout on a diet (P) that promoted only poor bone development. The bone (A 2) developed by the hogg on the good diet (CaPD) was normal in appearance, for age, having uniform density of cortical bone, from the endosteal to the periosteal surface, and a well-developed trabecular structure at the ends. The bone (B 2) developed by the hogg on the poor diet (P) lacked density and showed marked striations in the medial cortex, trabecular structure was poor, and fusion of the epiphysis was retarded.

PLATE 10

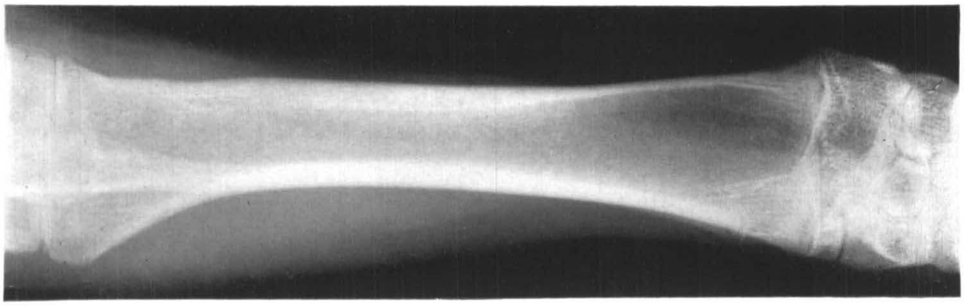
Radiographs of mandibles, taken in the flesh-free state at the end of the experiment. Mandible A is from a hogg of group CaP and shows the normal contour of the coronoid process, the condyle and the mandibular notch; the vertical and horizontal rami and the interdontal bone are of good quality, and the structure of the molar alveolar bone is also good. Mandible B is from a hogg of group CaPSt and shows poor quality throughout: the coronoid process and the condyle are



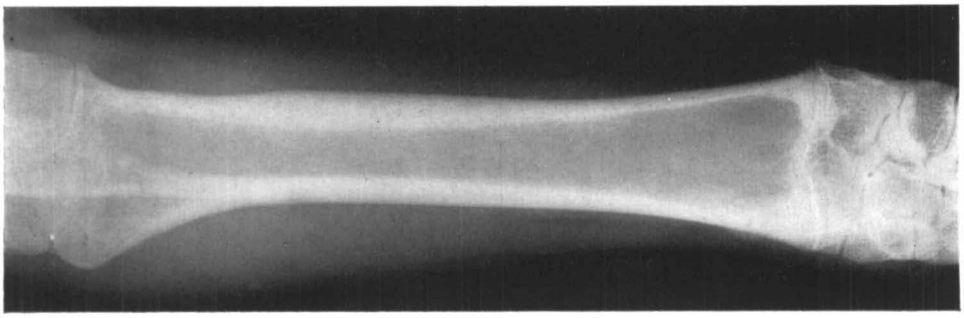
A 1



A 2

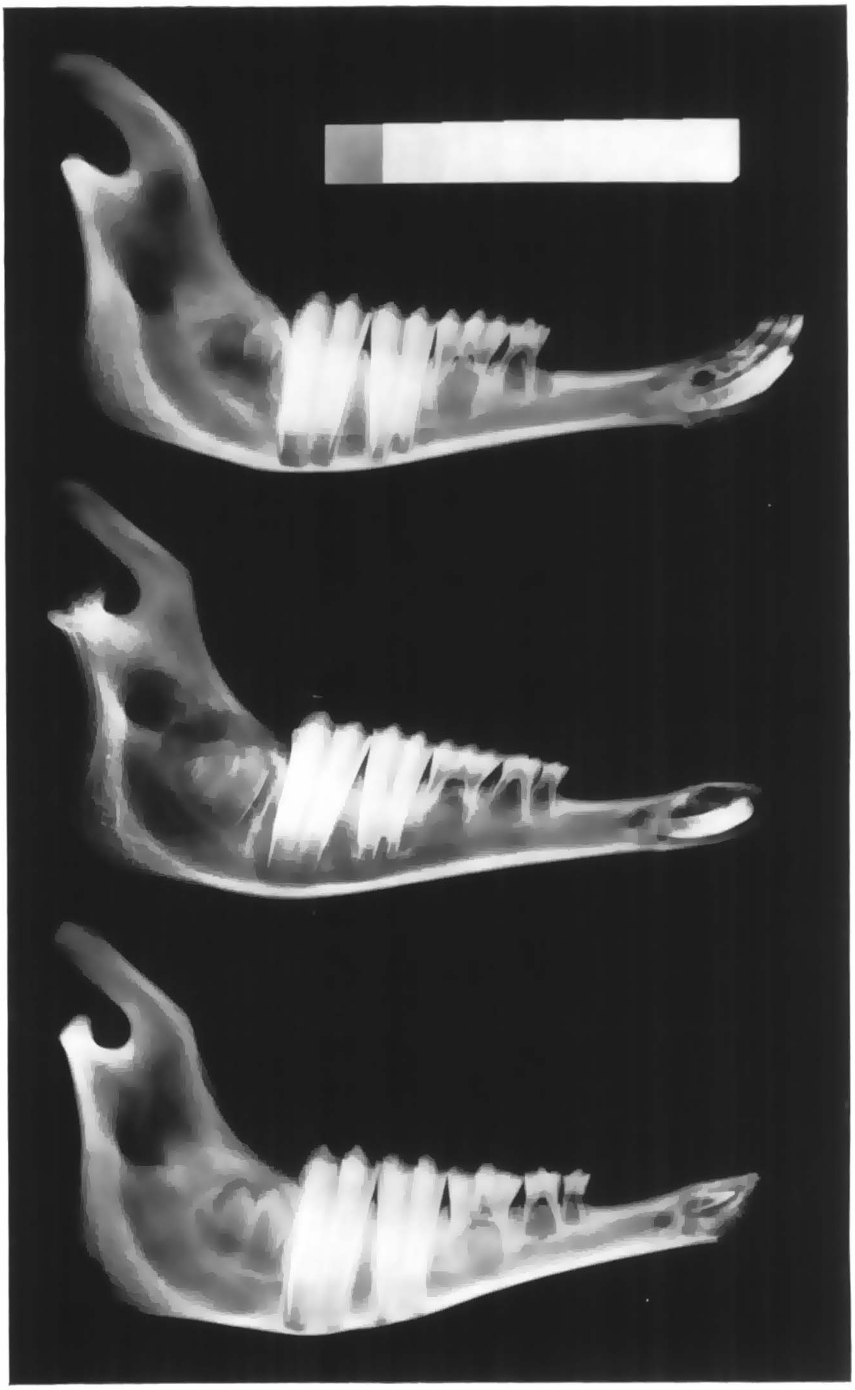


B 1



B 2

AGRICULTURE—BENZIE AND OTHERS



flattened and the mandibular notch is distorted; the vertical and horizontal rami and the interdontal bone lack density and striations are readily seen; there is little molar alveolar bone. Mandible C is from a hogg of group CaPDSt and is of good quality although there is a certain amount of distortion in the region of the coronoid process, the condyle and the mandibular notch; the vertical and horizontal rami and the interdontal bone are of good quality and similar to the same parts in mandible A; the molar alveolar bone and the *lamina dura* are closely similar in quality to those of A.

(*Received* 3 *June* 1959)