Severe undernutrition in growing and adult animals

8.* The dimensions and chemistry of the long bones

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(*Received* 22 *February* 1961—*Revised* 9 *June* 1961)

McMeekan (1940) showed that when the materials required for the growth of pigs are in short supply the bones take high priority in the allocation of the available nutrients. A similar result was obtained by Pomeroy (1941) in an experiment in which older pigs were given a submaintenance diet. These findings are in agreement with those of earlier workers who had found that the bones of other species, held on maintenance or submaintenance diets, continued to grow even at the expense of other tissues although admittedly at a much slower rate than those of well-nourished animals (Waters, 1909; Aron, 1911; Jackson, 1915, 1932). McMeekan (1940) found that the dimensions of the femur of his pigs were altered by underfeeding from an early age. Growth in 'thickness', which occurs 'late' in the development of the pig, was affected to a greater degree than growth in length.

McCance (1960) undernourished pigs and cockerels severely from an early age. This resulted in a gross retardation of growth and has been shown to alter profoundly the structure of their bones (Pratt & McCance, 1960). When these undernourished animals were restored to an ample supply of food, they rapidly gained weight and the normal structure of the bones was restored (Pratt & McCance, 1961). The strength and physical properties of these bones have been discussed by McCance, Dickerson, Bell & Dunbar (1962).

The present paper is concerned with the effect of undernutrition and its relief on the dimensions and chemical composition of the humerus of pigs and the femur of cockerels. The femur of cockerels undernourished after reaching maturity has also been investigated. The effect of undernutrition and its relief on the composition of the skeletal muscle of cockerels and of the soft tissues of the pig has been reported in previous papers in the series (Dickerson & McCance, 1960; Widdowson, Dickerson & McCance, 1960).

EXPERIMENTAL

The undernourished and rehabilitated animals used in this investigation were similar to those described by McCance (1960). The number of each kind of animal and their mean body-weights are shown in the tables. Rehabilitation in both the pigs and cockerels has been studied at two stages. Stage 1 in the pigs has been derived from

* Paper no. 7: *Brit. J. Nutr.* (1961), 15, 213.

a study of five animals which were killed at weights of 18·4–55·5 kg and stage 2 from a study of two animals weighing 159 and 209 kg. Stage 1 in the cockerels represents the state of affairs in seven animals after 15 weeks and stage 2 in four animals after 27 weeks.

Two kinds of controls were used for the undernourished animals of both species. These were (*a*) animals of the same weight but much younger, and (*b*) animals of the same age but much heavier. Since the composition of the fowl's femur did not change materially between 27 and 42 weeks of age, the values obtained in eleven animals aged 27–42 weeks have been averaged and the mean values are shown in the tables. These animals served as the 'age' controls for the cockerels undernourished from an early age, those undernourished after reaching maturity and the rehabilitated cockerels. The cockerels were $2\frac{1}{2}$ weeks old and weighed about 100 g each at the beginning of undernutrition. Since they gained some weight during the 25 weeks of underfeeding a third set of control animals was introduced to define the starting material. These were killed at the age and size at which the undernutrition of the others began. The cockerels undernourished from 27 weeks of age lost about a third of their body-weight in 15 weeks. The control animals were the same as, or similar to, those which had been used for a study of the effect of development on the composition of fowl skeletal muscle (Dickerson, 1960) and of the muscle and other soft tissues of the pig (Dickerson & Widdowson, 1960*a*; Widdowson & Dickerson, 1960). The effect of development on the composition of the humerus of the pigs and the femur of the fowl has been reported (Dickerson, 1961), and further information about the composition of the bones of the control animals and the description of the chemical methods may be found in that paper. The bones were measured as described by Dickerson & Widdowson (1960*b*).

RESULTS

The bones of the undernourished animals were smooth on the periosteal and endosteal surfaces and the cortex was very thin and brittle. The marrow cavity was enlarged owing to medullary erosion.

Table 1 shows that the humeri of the undernourished pigs were significantly heavier, longer and thicker (in each instance $P < 0·01$) than those of their corresponding

Table 1. *Effect of undernutrition on the dimensions of the pig's humerus*

(Values are means for the number of animals shown)

	'Weight' control	Undernourished	'Age' control
No. of pigs	6	6	3
Mean age (weeks)	4	46	43
Body-weight (kg)	6·58	6·55	189
Fresh weight of humerus (g)	25·9	31·6*	407
Length of humerus (cm)	7·87	9·00*	20·9
Thickness of humerus (cm)	1·17	1·55*	3·65
Ratio, thickness: length	0·149	0·171*†	0·176
weight: length	3·10	3·31	19·5

* Difference between undernourished and paired 'weight' control statistically significant ($P < 0·01$).
† Difference between undernourished and 'age' control not significant.

'weight' controls. They accounted, therefore, for a larger proportion of the body-weight in the undernourished animals than they did in well-nourished controls of the same size.

During normal growth and ageing the shaft of the pig's humerus grows relatively more in thickness, measured in an antero-posterior direction, than in length. Under-feeding did not prevent these changes taking place and the thickness:length ratio in the bones of the undernourished and well-nourished animals of the same age was the same. During normal growth, however, the weight:length ratio increases over six times, and undernutrition almost completely prevented any increase.

Table 2. *Effect of undernourishing cockerels (A) from 2½ weeks of age and (B) from 27 weeks of age on the dimensions of the femur*

(Values are means for the number of animals shown)

	A				
	At beginning of undernutrition	'Weight' control	Under-nourished	'Age' control	B
Age (weeks)	2½	4	27	27–42	42
No. of birds	12	9	11	7	9
Body-weight (g)	92·4	195	187	3720	2060
Fresh weight of femur (g)	0·50	1·17	1·46*	22·17	20·60
Length of femur (cm)	3·35	4·29	5·30*	10·89	11·14
Thickness of femur (cm)	0·25	0·36	0·39	1·09	1·04
Ratio, thickness:length	0·073	0·084	0·073*	0·098	0·094
weight:length	0·151	0·272	0·273†	2·01	1·64†

* Difference between undernourished and 'weight' control statistically significant ($P < 0·01$).
† Difference between undernourished and 'age' control statistically significant ($P < 0·01$).

Table 2 shows that the cockerels undernourished from 2½ weeks of age gained only some 95 g in 25 weeks. Well-nourished animals did this in 1½ weeks. The femurs of the undernourished cockerels, like the humeri of the undernourished pigs, were heavier, longer and thicker than those of well-nourished 'weight' controls (in each instance $P < 0·01$). The thickness:length ratio of the femur at 4 weeks of age was greater than it was at 2½ weeks ($P < 0·01$), but this effect of age and growth did not continue during undernutrition. In cockerels, as in pigs, undernutrition prevented the normal increase with age in the weight:length ratio. Underfeeding birds from 27 weeks of age did not alter the dimensions or shape of their bones, but it did significantly ($P < 0·01$) reduce the weight:length ratio.

Table 3 shows the effect of severe undernutrition in growing pigs and cockerels on the composition of cortical bone. The undernourished bone contained per 100 g of dry fat-free solids less total nitrogen, collagen ($P < 0·01$) and more calcium ($P < 0·01$) than the bone from the 'weight' controls. It also contained less total N and collagen ($0·02 < P < 0·05$) per 100 g of solids than the bone of the 'age' controls, but almost the same amount of calcium and phosphorus.

Table 3 also shows values for ratios of the major constituents. Undernutrition did not change the proportion of total N present as collagen in growing pigs. It delayed, without altogether preventing, the replacement of non-collagen N by collagen N which occurs during the postnatal growth of fowls. The Ca:N ratio of the cortical bone of the undernourished cockerels was almost the same as in normal bone of the same age, but in the pigs it was significantly higher ($P < 0.01$). Undernutrition in both species raised the Ca:collagen ratio to a value higher ($P < 0.01$) than that found in any of the controls. Underfeeding did not induce any significant change in the Ca:P ratios.

Table 3. *Effect of undernutrition in growing pigs and cockerels on the composition of cortical bone*

(Values are means for the number of analyses shown)

	Pigs			Cockerels			
	'Weight' control	Under-nourished	'Age' control	At beginning of under-nutrition	'Weight' control	Under-nourished	'Age' control
No. of bones	6	7	3	12	12	11	12
No. of analyses	6	7	3	3	4	11	12
Composition (g/100 g dry fat-free solids)							
Total nitrogen	4·24	3·33††*	3·70	4·91	4·36	3·50*†	3·72
Collagen (N × 5·55)	21·8	16·5††*	18·6	17·4	17·4	14·9**†	19·3
Calcium	25·0	27·5*	27·1	22·6	22·8	25·4*	26·0
Phosphorus	11·1	12·4*	12·5	10·6	10·9	11·2	11·5
Ratios of major constituents							
Collagen N (as percentage of total N)	92·5	89·2	90·5	63·8	71·9	77·0**	93·8
Ca:total N	5·90	8·20††*	7·32	4·62	5·27	7·25*	7·01
Ca:collagen	1·15	1·63††*	1·46	1·30	1·32	1·71*†	1·35
Ca:P	2·25	2·21	2·17	2·13	2·09	2·27	2·26

Difference between undernourished and 'weight' control statistically significant: * $P < 0.01$, ** $0.01 < P < 0.02$.

Difference between undernourished and 'age' control statistically significant: † $P < 0.01$, †† $0.02 < P < 0.05$.

Table 4. *Effect of undernourishing cockerels from 27 to 42 weeks of age on the composition of cortical bone*

(Values are means for the number of bones shown)

		g/100 g dry fat-free solids				Ratios of major constituents			
	No. of bones	Total nitrogen	Collagen (N × 5·55)	Calcium	Phos-phorus	Collagen N (as percent-age of total N)	Ca:N	Ca:Col-lagen	Ca:P
Undernourished	8	3·86	19·2	27·0*	12·2	92·0	7·21	1·41	2·23
Control	12	3·72	19·3	26·0	11·5	93·8	7·01	1·35	2·26

* Significantly higher than control ($0.01 < P < 0.02$).

Table 4 shows that underfeeding cockerels from 27 to 42 weeks of age caused no change in the percentages of total N and collagen in the cortical bone but resulted in a small but significant ($0.01 < P < 0.02$) increase in the percentage of Ca. The small increase in the percentage of P was not statistically significant. As a consequence of these changes the Ca:N and the Ca:collagen ratios were slightly, but not significantly, increased.

Table 5 shows the effect on the composition of the bone cortex of rehabilitating undernourished pigs and cockerels. In both species rehabilitation significantly increased ($P < 0.01$, or $0.02 < P < 0.05$) the percentages of total N and collagen to values similar to those in animals of the same weight well nourished for the whole of their lives. In the pigs the percentage of Ca fell during rehabilitation ($P < 0.01$) but in the cockerels it did not. In both species, the percentages of Ca and P after rehabilitation were almost the same as in the 'weight' controls.

Table 5. *Effect of rehabilitating undernourished pigs and cockerels on the composition of cortical bone*

(Values are means for the number of bones shown)

	Pigs					Cockerels			
		Stage 1		Stage 2			Rehabilitated		
	Undernourished	Rehabilitated	'Weight' control	Rehabilitated	'Weight' control	Undernourished	Stage 1	Stage 2	Controls
No. of bones	7	5	5	2	3	11	7	4	12
	Composition (g/100 g dry fat-free solids)								
Total nitrogen	3·33	3·88*	4·00	3·75	3·70	3·50	4·02*	4·01	3·72
Collagen (N × 5·55)	16·5	18·8**	19·6	19·0	18·6	14·9	20·2*	19·9	19·3
Calcium	27·5	25·9*	26·1	26·4	27·1	25·4	25·4	25·2	26·0
Phosphorus	12·4	11·9	11·9	12·2	12·5	11·2	11·7	11·4	11·5
	Ratios of major constituents								
Collagen N (as percentage of total N)	89·2	87·1	88·5	91·2	90·5	77·0	88·0*	89·5	93·8
Ca:total N	8·2	6·69*	6·58	7·04	7·32	7·25	6·26*	6·31†	7·01
Ca:collagen	1·63	1·38†*	1·23	1·39	1·46	1·71	1·25*	1·27	1·35
Ca:P	2·21	2·18	2·19	2·16	2·17	2·27	2·18	2·21	2·26

Difference between undernourished and rehabilitated at stage 1 statistically significant: * $P < 0.01$, ** $0.02 < P < 0.05$.

Difference between rehabilitated at stage 2 and controls statistically significant: † $0.02 < P < 0.05$.

In the pigs rehabilitation did not change the percentage of the total N present as collagen or the Ca:P ratio but decreased the Ca:N and Ca:collagen ratios. At both stages of rehabilitation the Ca:N ratio was the same as in bone from animals of the same size but much younger. The Ca:collagen ratio in the bone of the pigs rehabilitated to stage 1 was lower ($P < 0.01$) than it was when rehabilitation began, but was still somewhat higher ($0.02 < P < 0.05$) than it was in bone from animals of the same body-weight which had never been undernourished. In pigs rehabilitated to stage 2, the Ca:collagen ratio was the same as it was in the weight controls.

In cockerels the values for the percentage of the total N present as collagen show that the process of replacement of non-collagen N by collagen which had been slowed down when the animals were undernourished had progressed during rehabilitation to values approaching those for the controls. There was an initial fall ($P < 0.01$) in the Ca:N and Ca:collagen ratios during rehabilitation. The changes in the Ca:P ratio were not statistically significant.

Table 6 shows the composition of the pig's humerus and the cockerel's femur expressed per 100 g of bone. The composition of the humeri of the two kinds of controls shows that during normal growth the percentage of water in the bone fell. Some of this fall in the amount of water was due to the large increase in the amount of fat, but even on a fat-free basis the proportion of water in the bone fell by 24% over the age range

Table 6. *Effect of undernutrition on the composition of the pig's humerus and the cockerel's femur*

(Values are means for the number of analyses shown)

	Pigs			Cockerels			
	'Weight' control	Under-nourished	'Age' control	At beginning of under-nutrition	'Weight' control	Under-nourished	'Age' control
No. of bones	6	7	3	12	9	12	10
No. of analyses	6	7	3	3	3	12	10
Water (g/100 g)	59·4	66·1***	22·0	52·4	53·7	49·8	28·9
Fat (g/100 g)	1·7	0·4	39·7	6·2	1·5	0·8	15·0
Composition of fat-free bone (g/100 g)							
Water	60·4	66·4**	36·2	55·8	54·6	51·2	34·6
Total nitrogen	2·69	2·08*	3·53	2·88	3·04	3·14	3·52
Collagen (N × 5·55)	9·57	8·87	14·9	9·37	9·91	10·2	13·7
Calcium	7·18	6·18	14·8	6·04	6·90	9·35***	12·8
Phosphorus	3·33	3·04	6·75	2·92	3·33	4·52***	5·56

Difference between undernourished and 'weight' control statistically significant:
* $0·02 < P < 0·05$, ** $0·01 < P < 0·02$, *** $P < 0·01$.

during which the experimental animals were undernourished. The changes in the other constituents show that the water was replaced by collagen and minerals of which Ca and P formed the bulk. Undernourishing pigs apparently reversed this process, for the percentage of water was significantly higher on a fat-free basis ($0·01 < P < 0·02$) in these bones than in the 'weight' controls, and the percentage of total N was lower ($0·02 < P < 0·05$). The percentages of collagen, Ca and P were also lower but the differences were not individually statistically significant.

Underfeeding the cockerels prevented the fall in the percentage of water which normally takes place with increasing age. The fall in the percentage of fat which appeared to take place in well-nourished birds between 2½ and 4 weeks of age also took place in the undernourished birds, but whereas the fat content increased again during later life in well-nourished animals, it remained at a lower level in the undernourished animals. The percentage of water in the fat-free bones of the undernourished animals was rather variable, and the mean value was not significantly different from that in the 'weight' controls. The bones of the undernourished animals contained percentages of total N and collagen similar to those of the 'weight' controls, but the percentages of Ca and P were higher ($P < 0·01$). The percentages of the latter were, however, lower than those in the 'age' controls.

Table 7 shows the absolute amounts of the various constituents in the bones of the undernourished animals and in their respective 'weight' controls. The amount of

N in the undernourished pig bones was almost the same as in those of their 'weight' controls. Apart from this, the bones of the undernourished animals contained more of all the measured constituents.

Table 8 shows the composition of the femur of cockerels undernourished from 27 to 42 weeks of age and those of their well-nourished controls. The marrow cavity of these undernourished bones was filled with dark-red gelatinous material quite unlike

Table 7. *Effect of undernutrition on the absolute amounts of various constituents in the humerus of the pig and the femur of the cockerel*

(Values are expressed as g/bone and calculated from the mean values for the bone weights shown in Tables 1 and 2 and the mean composition shown in Table 6)

	Pigs		Cockerels	
	'Weight' control	Undernourished	'Weight' control	Undernourished
Water	15·4	20·9	0·63	0·73
Total nitrogen	0·68	0·66	0·035	0·046
Collagen (N × 5·55)	2·44	2·80	0·115	0·148
Calcium	1·83	1·95	0·080	0·131
Phosphorus	0·85	0·96	0·039	0·066

Table 8. *Effect of undernourishing cockerels from 27 to 42 weeks of age on the composition of the femur*

(Values are means for the number of bones shown)

	No. of bones	Water (g/100 g fresh bone)	Fat (g/100 g fresh bone)	Composition of fat-free bone (g/100 g)				
				Water	Total nitrogen	Collagen (N × 5·55)	Calcium	Phosphorus
Undernourished	8	53·7***	0·8***	54·1***	2·57***	9·44***	10·1**	4·56*
Controls	10	28·9	15·0	34·6	3·52	13·7	12·8	5·56

Difference between undernourished and controls statistically significant: * $0·02 < P < 0·05$, ** $0·01 < P < 0·02$, *** $P < 0·01$.

Table 9. *Effect of rehabilitating undernourished pigs on the composition of the humerus and of rehabilitating undernourished cockerels on the composition of the femur*

(Values are means for the number of bones shown)

	Pigs					Cockerels			
		Stage 1		Stage 2			Rehabilitated		
	Undernourished	Rehabilitated	'Weight' control	Rehabilitated	'Weight' control	Undernourished	Stage 1	Stage 2	Controls
No. of bones	7	5	5	2	3	12	7	4	10
Water (g/100 g fresh bone)	66·1	44·6	45·6	19·2	22·0	49·8	37·3	31·2	28·9
Fat (g/100 g fresh bone)	0·4	15·5	15·2	31·4	39·7	0·8	20·8	12·2	15·0
	Composition of fat-free bone (g/100 g)								
Water	66·4	52·7	52·4	27·9	36·2	51·2	47·2	35·5	34·6
Total nitrogen	2·08	3·06	2·64	3·51	3·53	3·14	3·22	3·74	3·52
Collagen (N × 5·55)	8·87	10·9	10·9	17·6	14·9	10·2	12·7	14·8	13·7
Calcium	6·18	9·75	10·1	16·3	14·8	9·05	10·0	12·6	12·8
Phosphorus	3·04	4·50	4·60	7·49	6·75	4·52	4·41	5·89	5·56

the fatty marrow normally present in birds of this age. Chemically, the bones contained a higher percentage of water and less fat. The difference in water content was not, however, all due to fat, for on a fat-free basis the bones still contained more water and less of the other constituents than the controls.

Table 9 shows that the rehabilitation of undernourished pigs restored the composition of the humerus to that in animals of the same body-weight well nourished for the whole of their lives.

Table 9 also shows that the rehabilitation of undernourished cockerels changed the composition of the femur towards that in the control animals of 27–42 weeks of age. The percentage of fat was, however, higher after rehabilitation for 15 weeks than it was after rehabilitation for a further 12 weeks ($0{\cdot}02 < P < 0{\cdot}05$), but not significantly higher than in the control animals.

DISCUSSION

As pigs grow and age the humerus becomes a more robust bone, i.e. the shaft of the bone grows in thickness relatively more than in length; the same changes took place during undernutrition. In normal cockerels the femur also becomes more robust as the animals grow older. In this species, however, undernutrition interfered with growth in thickness of the shaft more than with growth in length and the femur became abnormally thin.

In some experiments on the effects of mild undernutrition McMeekan (1940) found that growth in 'thickness' of the femur measured as the weight:length ratio (Hammond, 1932; Pálsson, 1939) was retarded relatively more than growth in length. A similar result has been obtained in our experiments. The fall in the weight:length ratio of the femurs of cockerels undernourished after reaching maturity was probably due to the resorption of osseous tissue and its replacement by water.

Bones are complex structures and changes in the composition of parts of a bone may be completely masked if the bone is analysed as a whole. Thus, in the bones of the undernourished animals the cortex was hypercalcified and the Ca:collagen ratio was abnormally high even for bone of the same chronological age. In whole bones, however, the Ca:collagen ratio in undernourished animals was lower than in well-nourished ones of the same age, although the difference was less in cockerels than in pigs. In the cockerels the changes in composition associated with age continued to take place and underfeeding simply retarded this normal process. In the pig the percentage of water rose and the percentage of total N fell a little as compared with those in normal animals of the same weight. The explanation of the difference in the effect of undernutrition on the composition of the bones of the two species may be that in the pig humerus the resorption of bone from the endosteal surface had resulted in a relatively greater marrow cavity than in the cockerel femur. There was no evidence in any of these animals of Ca deficiency, and the bones of the undernourished animals contained larger amounts of Ca, P and collagen than those of healthy animals of the same weight.

The abnormally high Ca:collagen ratio in the cortex of the undernourished bones may have been due to the number of cement lines present (Pratt & McCance, 1960). They surrounded spaces in the undernourished bone, and there were also straight or resting 'cement lines' in the subperiosteal region. These narrow bands of bone matrix contained no fibres and their calcification may be a degenerative process. Cohen & Lacroix (1953) showed that they contain much Ca and little organic matter. In the cortex of the pig femur Pratt & McCance (unpublished observations) have also found that some of the vascular spaces in the bone when underfeeding began were filled up with sparsely fibred material during the period of underfeeding. Other parts of the cortex formed during undernutrition in cockerels have also been shown to be more finely fibred than normal bone (Pratt & McCance, 1960) and, since fine collagen fibres might accommodate more crystals per unit weight of fibre than coarser fibres, this change might also favour overcalcification of the collagen. Microradiographs of these bones are being made to determine the distribution of the mineral.

The bones of undernourished pigs and cockerels were abnormal in chemical composition and structure when compared with those of normal animals of either the same body-weight or the same chronological age. When the animals were restored to ample food the bones grew and their composition returned to normal owing to the resorption of the bone formed during undernutrition and its replacement by a bone of normal structure. Bones differ in this respect, however, from teeth, for teeth are not resorbed and the abnormalities in tooth deposition and structure resulting from undernutrition persist throughout rehabilitation (McCance, Ford & Brown, 1961).

SUMMARY

1. Bones of undernourished pigs and cockerels were heavier and larger than those of normal animals of equal weight.

2. Undernutrition did not prevent the normal increase in the thickness:length ratio of the shaft of the pig's humerus. It did prevent it in the cockerel's femur.

3. In the cortex of the pig humerus the collagen nitrogen:total N ratio did not change either during normal growth or underfeeding, but in the cockerels underfeeding retarded the normal increase.

4. The calcium:collagen ratio of the undernourished cortical bone was abnormally high.

5. Undernutrition from an early age increased the percentage of water and decreased the percentage of total N in the fat-free bone. The percentages of collagen, calcium and phosphorus also fell a little. In the cockerel's femur the percentages of Ca and P rose.

6. Underfeeding adult cockerels greatly increased the percentage of water and decreased that of fat in the femur. On a fat-free basis the percentages of total N, collagen, Ca and P fell.

7. The composition of the bones returned to normal during rehabilitation.

8. The composition of the cortical bone is discussed in relation to its anatomical structure.

The authors are grateful to Messrs Stan and Terry Cowan for their care of the animals. The technical assistance of Mr Peter John in the cleaning of the bones and in their analysis has been much appreciated.

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Printed in Great Britain