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Severe undernutrition in growing and adult animals

2.* Changes in the long bones of growing cockerels held at fixed weights by undernutrition

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Drummond (1916) described the dwarfing that was produced by the prolonged underfeeding of growing chickens. This dwarfing must have been due to the retardation of bone growth. Undernutrition has repeatedly been shown to retard growth in mammals, but it has also been shown that bones continue to grow very slowly even though the body-weight is maintained at a constant level by underfeeding (Waters, 1908, 1909; Aron, 1911; Jackson, 1915). This latter finding was not remarked upon by Drummond or by Pernice & Scagliosi (1895) who had also used chickens in underfeeding experiments. There does not appear to have been any careful investigation of the structure of such bones, either in chickens or mammals. Pernice & Scagliosi (1895) reported no changes in the bones of their chicks after they had arrested growth by underfeeding, and more recently Wolbach & Hegsted (1952*b*, *c*), who studied the effects of a short-term restriction of diet on immature chicks, only reported a 'balanced' retardation of all the growth sequences. The object of the present paper is to present the morphological changes which followed the almost total arrest of growth for 6 months in cockerels weighing about 100 g.

METHODS

The birds used in the experiment were reared on an adequate diet for 14 days after hatching, and were then given the same food but in reduced amounts. Sufficient food was given for survival only, so that there was little increase in body-weight. Further details of the method have been given by McCance (1960). Table 1 gives the ages, weights, periods of undernutrition and the lengths of the femurs of the birds used for this investigation.

In all birds the skeleton was X-rayed, the femur (and in most the humerus and tibia) was removed and fixed in 5% formol saline, decalcified with the disodium salt of ethylenediaminetetra-acetic acid, embedded, and sections cut to a thickness of 4μ. Sections were stained with haematoxylin and eosin, iron haematoxylin, and by a modification of Long's silver impregnation designed to show the fibrous structure of the bone.

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RESULTS

General observations

Table 1 shows that undernutrition produced a considerable dwarfing effect. The oldest of the undernourished birds (197 days) weighed only 120 g, whereas a normal bird of approximately the same age (190 days) weighed 3334 g. There was, however, some bone growth during the first part of the period of undernutrition, and the histological findings confirm that this elongation took place during the early weeks. Dickerson (1959), in an experiment similar to this one in design, has shown that bones increased in all dimensions during undernutrition. Thus in chickens as well as in various species of mammal (for review see Jackson, 1925) bone growth is slowed down but not stopped at once by underfeeding.

Table 1. *Age at death, duration of undernutrition, weights and femur lengths of the birds used in this study*

Age at death (days)	Duration of undernutrition (days)	Body-weight: Initial (g)	Body-weight: At death (g)	Length of femur (mm)
		Control birds		
1	—	—	45	24·0
8	—	—	80	28·0
15	—	—	95	35·0
22	—	—	150	42·0
28	—	—	250	49·5
43	—	—	415	62·0
57	—	—	710	69·0
71	—	—	1080	81·0
85	—	—	1500	95·0
120	—	—	2240	100·0
155	—	—	2950	113·0
190	—	—	3334	120·0
246	—	—	3780	117·0
379	—	—	3700	115·0
		Experimental birds		
22	7	86	90	35·0
36	21	82	100	39·5
64	49	80	120	46·0
120	105	110	110	46·0
169	154 (died)	91	110	47·0
169	154 (died)	80	107	44·0
197	182	91	120	48·0

Radiological findings

The most interesting radiological findings were in the metaphysis. In all of the long bones from the undernourished birds, except those of the bird undernourished for only 7 days, there was a narrow dense band of calcified tissue, lying between the spongiosa of the metaphysis and the cartilaginous extremity (Pl. 1*b*). Histological studies showed it to be calcified cartilage matrix. Such bands have never been found in normal birds (Pl. 1*d*). Other transverse bands of calcified tissue were found throughout the metaphysis of undernourished birds (Pl. 1*b*). These multiple bands were more numerous in the bones of older birds, and also were not found in normal birds (Pl. 1*d*). They resemble the lines of arrested growth which have frequently been observed in mammals but do not appear to have been described in birds.

The other radiological findings were for the most part negative ones. There was no evidence of skeletal deformity (Pl. 1*a*, *c*), such as bending of the tibia, or enlargement of the extremities of the bones. No fractures or local pathological changes were found in any of the long bones.

Histological findings

The subperiosteal tissue of the diaphysis. The subperiosteal tissue lies between the closely packed longitudinally directed fibres of the periosteum and the underlying bone. In the bones of a bird growing under normal conditions it forms a deep layer (Pratt, unpublished observations). The cells lying immediately under the periosteum are flattened, undifferentiated connective-tissue cells, many of which are undergoing mitotic division. These cells may be traced through intermediate forms into osteoblasts which lie in the deeper part of the zone and also extend between the trabeculae of the bone. These latter cells are larger than the undifferentiated cells, and are intensely basophilic on staining and polyhedral in shape (Pl. 2*a*). Large numbers of argyrophilic fibres are present between the cells of this zone and are either irregularly arranged or collected into thick bundles. These fibre bundles are obliquely directed and pass from the fibrous periosteum into the substance of the bone trabeculae.

The subperiosteal zone is an area of intense activity during normal growth and it was profoundly affected by undernutrition. Mitosis ceased abruptly in the undifferentiated cells as soon as the food was restricted, and active osteoblasts became so reduced in numbers that they had completely disappeared by 120 days. In birds of 120, 169 and 197 days, the zone was considerably narrowed and contained only flattened cells with elongated and densely staining nuclei (Pl. 2*b*). It is difficult to say whether these cells were all the undifferentiated cells of the normal zone, or whether they also included resting osteoblasts. The subperiosteal fibres were greatly decreased in number and stained badly, and the few thick fibre bundles present were so oblique that they were almost longitudinal.

The subperiosteal tissues after prolonged undernutrition closely resembled the same region of normal birds of the same age, that is in bones that had ceased to grow, In the latter, however, the cellular exhaustion was more complete and only occasional subperiosteal cells were found.

Changes in the bone matrix of the diaphysis. A transverse section through the diaphysis of a femur from a cockerel, of similar size to an undernourished one, but growing normally, had a cancellous structure; and there were numerous vascular spaces in the bone, increasing in size as they approached the outer surface (Pl. 2*c*). The form of the vascular spaces was closely related to the fibrous structure of the bone matrix (Pratt, 1958). The fundamental structure was one of fine irregularly arranged fibres, intermingled with which in the peripheral parts there were bundles of coarse fibres, which were the intra-osseous extensions of the coarse fibre bundles of the subperiosteal zone. The deeper vascular spaces were lined by closely packed fibres which ran in the plane of the vascular channel; this arrangement had the effect of reducing the size of these vascular spaces and giving them a distinct lining (Pl. 2*e*).

In the early stages of undernutrition the medullary erosion, which normally maintains the proportions of the walls of the shaft, occurred at a greater rate than the subperiosteal deposition. During this period large numbers of osteoclasts were present on the medullary surface of the shaft, though few were seen in these situations in undernourished birds older than 68 days. The transverse section of the femur of an undernourished cockerel presented a very striking contrast to that of a well-nourished bird, for the wall of the diaphysis was so much narrower (Pl. 2*c*, *d*). At the beginning of undernutrition, moreover, the walls were of unequal thickness, but this inequality did not persist and the walls became uniformly narrow.

The bone matrix formed during undernutrition had a different fibrous structure from that already described in the normal bone, and no normal periosteal bone remained after the long period of undernutrition. It was presumably removed by medullary erosion (Fig. 1). The fibres found in the bones of undernourished birds were in fine bundles, closely packed, and irregularly arranged (Pl. 2*f*). This structure gave a homogeneous appearance to the fibrous matrix and contrasted strongly with the appearance in well-nourished immature and adult birds, which will be described in greater detail elsewhere.

In those birds that had undergone undernutrition for 120 days or more, the subperiosteal bone was compact and contained eccentric cement lines, these being narrow bands of bone matrix apparently devoid of fibres (Pl. 2*f*). The cement lines increased in number with age and were not confined to the periphery for they were also found singly about vascular spaces (Pl. 2*f*). This finding indicates that the vascular spaces may have become reduced in diameter by the deposition of bone.

Changes occurring in the epiphysial cartilage. The epiphysial cartilage at the proximal end of the tibia of chickens between the ages of 18 and 32 days was described by Wolbach & Hegsted (1952*a*). The authors divided it into two parts, which we have been able to confirm, a thin outer layer of flattened cells lying in an eosinophilic matrix, which they called the articular cartilage, and a deeper portion which formed the major part of the epiphysial cartilage and was referred to as the hyaline zone. This zone was characterized by a basophilic matrix and actively dividing rounded cells which were usually collected into small groups (Pl. 3*g*). By the time growth had ceased in the normal birds this hyaline zone had disappeared and the articular cartilage had the appearance of fibro-cartilage.

The epiphysial cartilage continued to expand for some time during the period of undernutrition, for it was as large at death as that found in a bone of the same length taken from a normal bird aged 28 days. It did not, however, show the changes characteristic of a bone from a normal bird of the same age. The articular cartilage persisted unchanged, but all mitosis ceased in the hyaline zone, the cells became smaller, and were all discrete and there were no cell nests (Pl. 3*h*).

Changes in the growth cartilage. The growth cartilage of normal birds was divided by Wolbach & Hegsted (1952*a*) into two parts—the proliferative zone and the

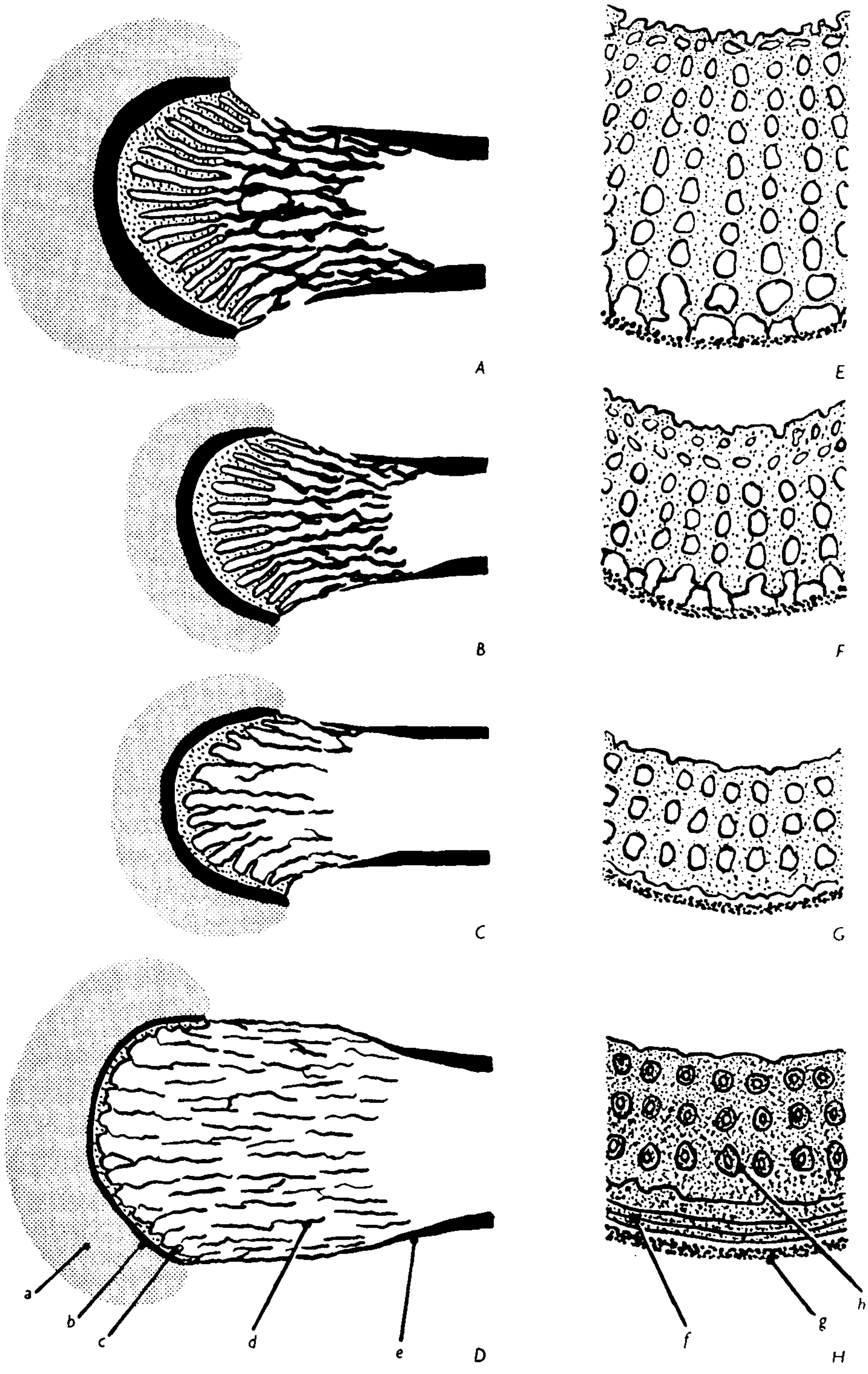

Fig. 1. A semi-diagrammatic illustration of the changes which occur in an avian long bone during restricted growth due to undernutrition. *A–D* represent longitudinal sections through the extremity of a long bone, and *E–H* represent transverse sections through its shaft. Normal growth over 14 days is illustrated by the changes shown in *B* and *F* and *A* and *E*. The early effects of undernutrition are seen when *C* and *G* are compared with *B* and *F* (beginning of undernutrition). The late effects of undernutrition are seen in *D* and *H*. *a*, epiphysial cartilage; *b*, proliferative zone; *c*, hypertrophic zone; *d*, spongiosa; *e*, periosteal bone; *f*, subperiosteal cement line; *g*, fibrous periosteum; *h*, perivascular cement line.

hypertrophic zone (Pl. 3*a*). The proliferative zone in the control birds consisted of longitudinal rows of closely packed, actively dividing, flattened cells. The matrix was deeply basophilic. This zone was sharply marked off from the adjacent epiphysial cartilage and the hypertrophic zone. The cells of the latter zone arose from those of the proliferative zone and there was a simultaneous increase in the basophilia of the matrix. The cells of the hypertrophic zone enlarged and became spherical and vacuolated but no longer remained in rows. As a result of the activities of the invading tissues of the marrow, the hypertrophic zone became irregularly tunnelled, and endochondral bone was deposited on the walls of these tunnels. Thus it was not possible to make any distinction between primary and secondary spongiosa as in mammals. The tunnel walls were later remodelled and the hypertrophic cartilage replaced by trabeculae of endochondral bone.

The growth cartilage and its adjacent tissues were profoundly affected by undernutrition (Fig. 1). The changes can be conveniently classified into 'early' and 'late'.

Within a few days of the beginning of undernutrition, mitotic division effectively ceased in the proliferative zone and did not recur during the whole period of undernutrition. Extensive invasion of the hypertrophic cartilage and increased remodelling of the cartilage remains in the metaphysis (Pl. 3*b*) also began very soon and had the effect, within 2 weeks, of producing a narrow hypertrophic zone, a smooth invasion front, and a reduced amount of endochondral bone (Pl. 3*c*). The narrow hypertrophic zone with an almost smooth medullary surface explains the radiological appearance of a narrow dense band in this position (Pl. 1 *b*).

Further changes appeared within the growth cartilages of those birds that had been undernourished for longer than 68 days. The proliferative zone became narrow (Pl. 5*b*, *d*), its cells were widely separated by matrix and lost their columnar arrangements (Pl. 3*e*). The hypertrophic zone showed transverse striations (Pl. 3*e*), consisting of bands of closely packed and flattened cells in a pale staining matrix, alternating with bands of more widely separated, larger, spherical cells which had an eosinophilic capsule and lay in a deeply basophilic matrix (Pl. 3*f*). The dark-staining bands closely resembled normal hypertrophic cartilage and the pale-staining areas suggest periodic failure of the maturation process which should have resulted in hypertrophic cartilage. Osteoblasts were rarely found at the medullary surface of the hypertrophic cartilage, as in normal bones (Pl. 4*b*), though osteoclasts were present in large numbers (Pl. 4*d*). This scarcity of osteoblasts is in keeping with the greatly reduced endochondral bone formation during prolonged undernutrition. The normal metaphysis contained trabeculae of cartilage which were covered and gradually replaced by endochondral bone (as shown in Pl. 4*a*). The metaphysis of an undernourished bird, however, consisted of slender trabeculae of calcified cartilage matrix with little endochondral bone (as in Pl. 4*c*). These trabeculae were not remodelled in the usual way, and replaced by bone, and consequently their disposition often reflected the plane of the growth cartilage and accounted for the transverse striations seen on X-ray (Pl. 1*b*).

The metaphysis. The metaphysis from an immature well-nourished bird contained trabeculae of endochondral bone which packed the diverging extremity of the shaft

(Pl. 5*a*, *c*). The most peripheral of the trabeculae were removed in some parts almost as soon as they were formed, which gave a concave outline to longitudinal sections of the metaphysis. The external surface of the metaphysis was thereafter consolidated with bone formed by both periosteal and endosteal osteoblasts (Pratt, unpublished observations).

Prolonged undernutrition prevented these remodelling processes, and the result was a clubbed metaphysis (Pl. 5*b*). There was in places, however, some consolidation of the metaphysial wall by osteoblasts derived from the subperiosteal tissue.

Pneumatization of the humerus. It is well known that the marrow cavity in the humerus of the fowl is invaded by an extension of the cervical air sac, which is itself an outgrowth of the lung (King, 1957). Bremer (1940) described the mode of this invasion which began at 2 weeks after hatching, though at this stage there was only a penetration of the cortical bone. An examination of control material in stages up to 6 weeks of age showed no extensive invasion of the marrow cavity (Pl. 5*c*).

This invasion process had not involved even penetration of the bone cortex at the beginning of undernutrition, but the marrow cavity was completely invaded 3 weeks later (Pl. 5*d*) in birds which were still only 5 weeks old. Thus it would seem that this process went on at an accelerated rate during undernutrition.

Ossification centres in the distal tibial cartilage. There are normally two ossification centres in the distal tibial cartilage. These are believed to represent tarsal elements that have fused with the tibia. In the control femurs equal in length to those of the undernourished birds but taken from cockerels aged 29 days these centres were large, contained trabeculae of endochondral bone (Pl. 4*a*) and closely resembled the secondary centres of ossification found in mammals. These centres later fused with the main mass of the tibia and thus lost their separate identity.

These centres were visible during the whole period of undernutrition but little or no endochondral bone was laid down on the scattered trabeculae of calcified cartilage matrix (Pl. 4*c*).

DISCUSSION

It has been shown by Jackson (1925) that the bones of young mammals continue to grow even in severe undernutrition, and it is well known that bones have a high growth priority when food supplies are suboptimal (Hammond, 1952). Birds have been less investigated and no studies of the present type have hitherto been made, but something of the same kind is evidently true of them. Normal bone growth, however, is a most complicated process, involving the division and differentiation of cells, the deposition and absorption of highly differentiated protein structures and mineral salts, resulting in reorganization of the whole structure. The present work shows that severe undernutrition interferes with each of these processes to a different extent. Cell division ceased in all situations in the bone as did the differentiation of osteoblasts but the activities of these cells only gradually came to an end, occurring first in the marrow cavity. Maturation of the cells of the growth cartilage continued. Osteoclastic reabsorption was much less subdued, and the reabsorption associated with the formation of the humeral air sacs appeared to be chronologically accelerated.

It is quite unknown at present what disorganized these normal features of bone growth. The birds were not receiving enough protein, calcium, phosphorus, or indeed enough of any of their dietary ingredients to allow normal growth, but the deficiency was a 'balanced' one and it is suggested that the sequence of events portrayed in the bones of these undernourished birds was just one aspect of the development of one part in preference to the rest, so well recognized as taking place when any young animal is subjected to undernutrition (McMeekan, 1940; Hammond, 1952). When the undernutrition is not severe the competition between the parts is less intense and the bones may develop slowly but with an almost normal structure. It is only when the undernutrition is severe that the differential changes within the bone become obvious. Differential changes may be discoverable in other tissues with less growth 'priority' than bone when the undernutrition is relatively mild.

SUMMARY

1. The femur, humerus and tibia were studied in cockerels that had been severely undernourished for periods of up to 182 days, and compared with bones from well-nourished growing birds.

2. The bones increased in size (for upwards of 50 days) although there was little increase in body-weight.

3. There was radiological evidence of disturbed endochondral ossification.

4. The undifferentiated cells in the subperiosteal zone ceased to divide and to differentiate, and osteoblasts disappeared.

5. The periosteal bone formed before the period of undernutrition was gradually removed and the bone formed during the experiment was more finely fibred than normal bone and contained cement lines.

6. The epiphysial cartilage showed little change other than cessation of mitosis.

7. Mitotic figures gradually disappeared from the proliferative zone of the growth cartilage, but maturation continued at a very reduced rate with, consequently, partial exhaustion of the proliferative zone. In later stages there was a periodic failure of maturation and transverse bands of uncalcified matrix appeared in the hypertrophic zone.

8. Medullary osteoblasts became scarce and endochondral bone ceased to form. This change resulted in a metaphysis composed largely of trabeculae of calcified cartilage, and as a consequence, remodelling was disturbed.

9. Osteoclasts were numerous in all situations throughout the period of undernutrition, and considerable activity was apparent in the early weeks.

10. The invasion of the humerus by the cervical air sac took place before the normal age.

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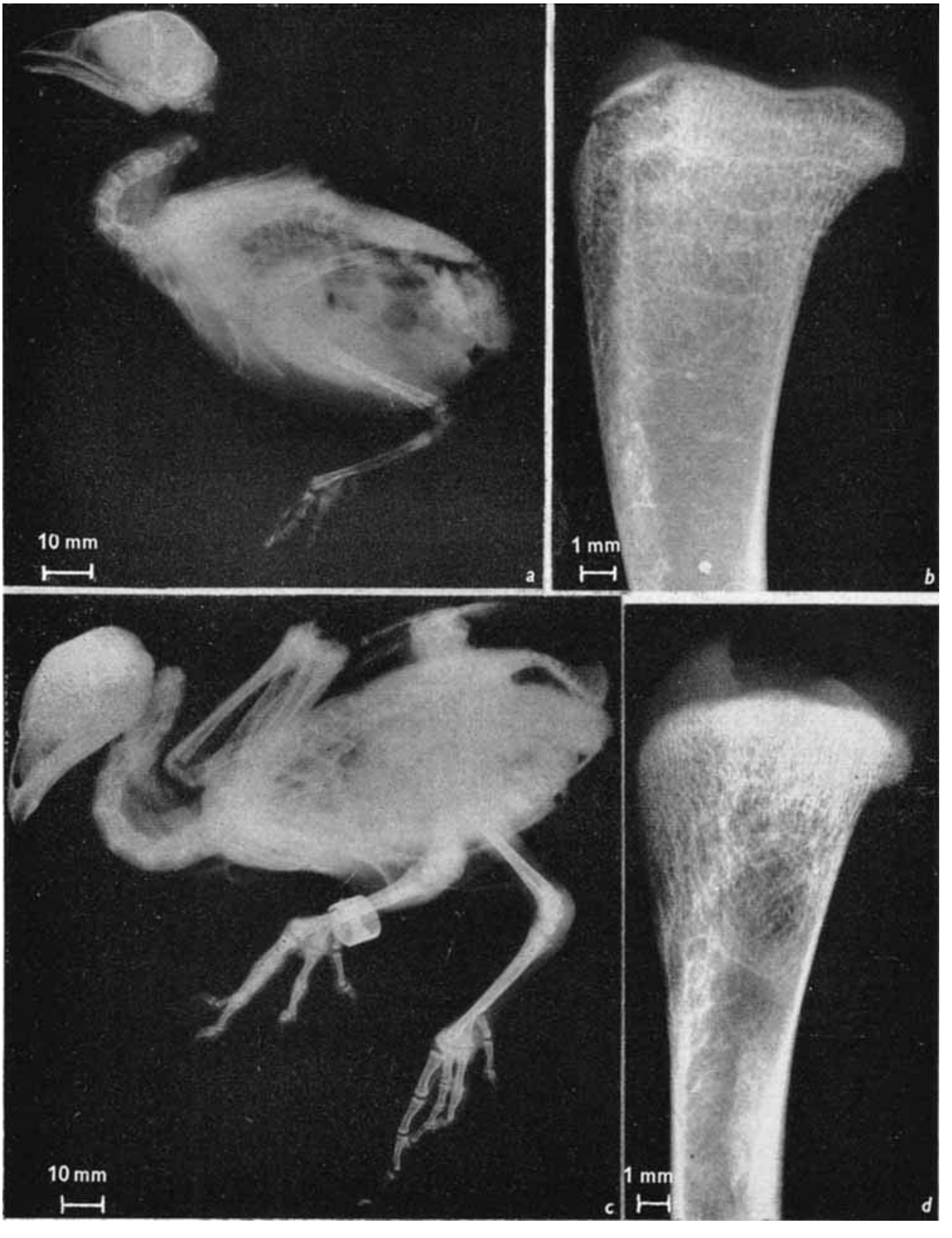

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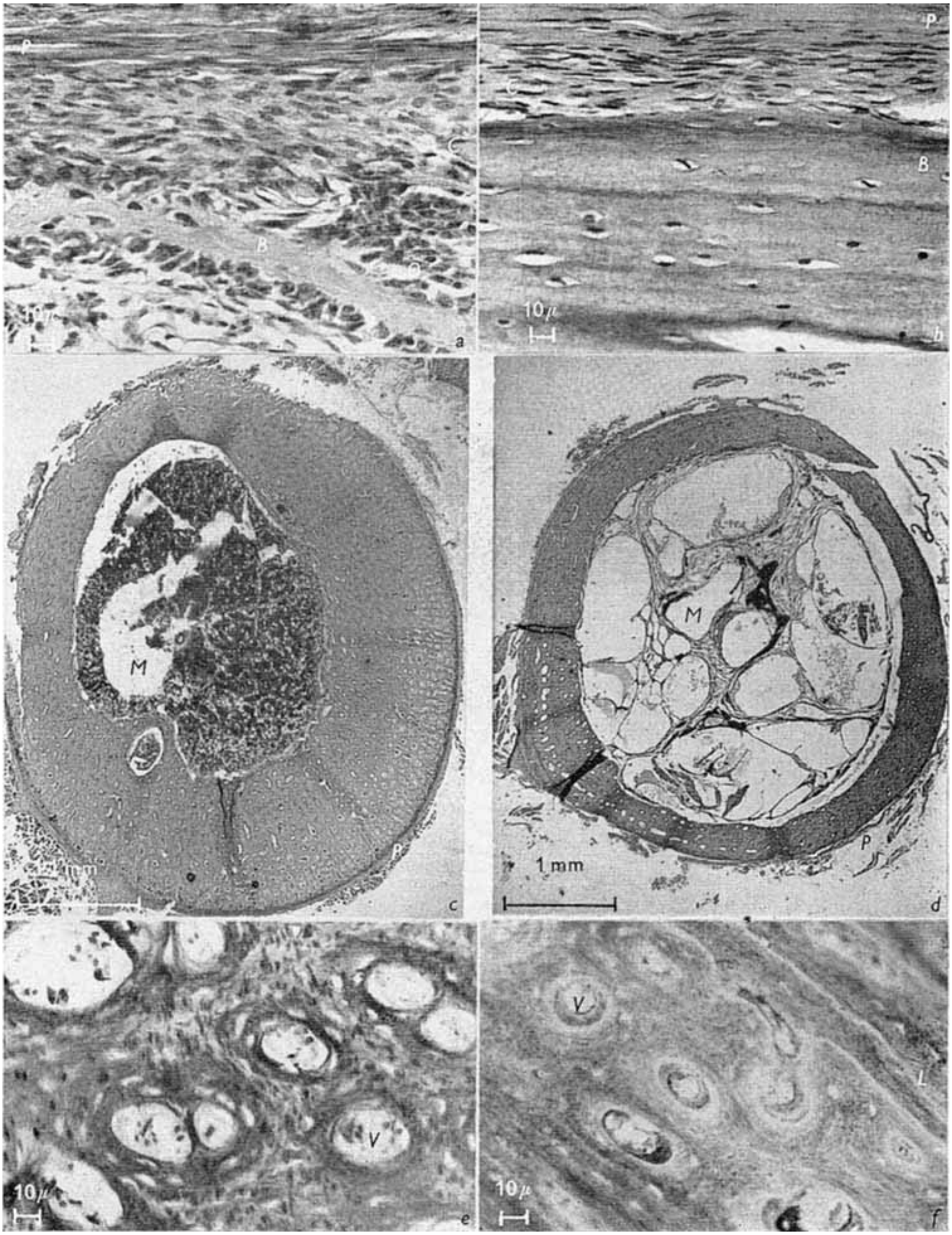

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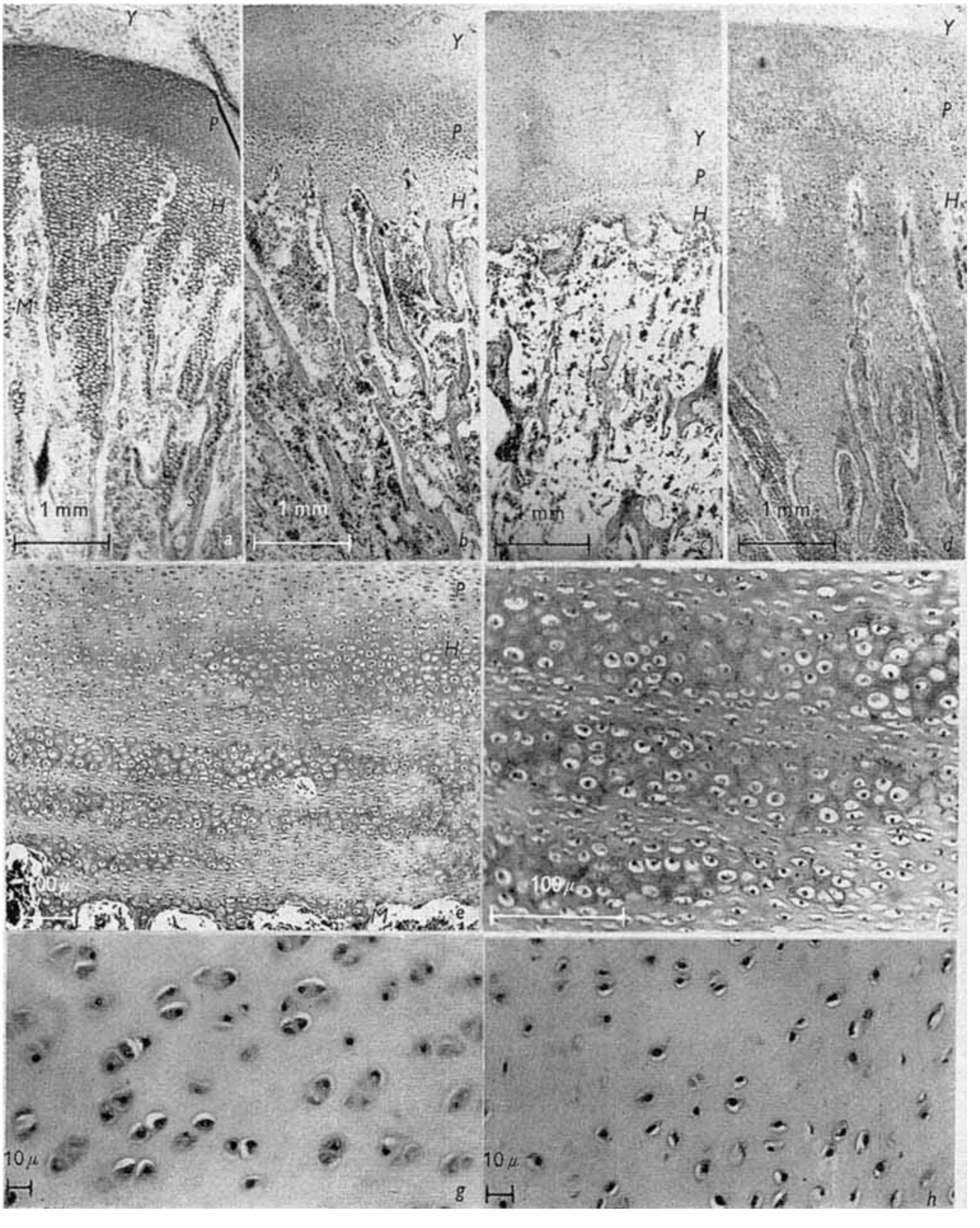

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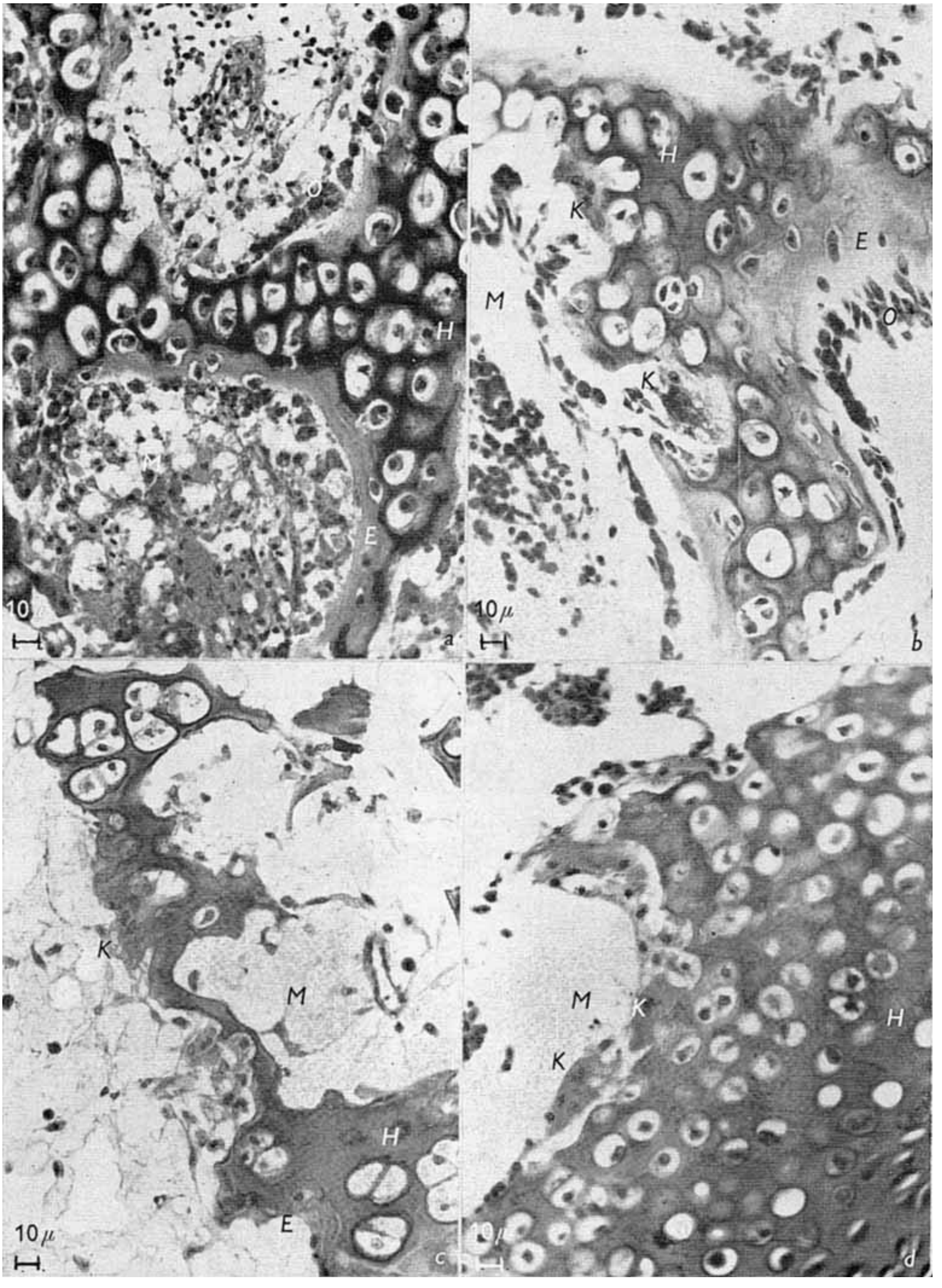

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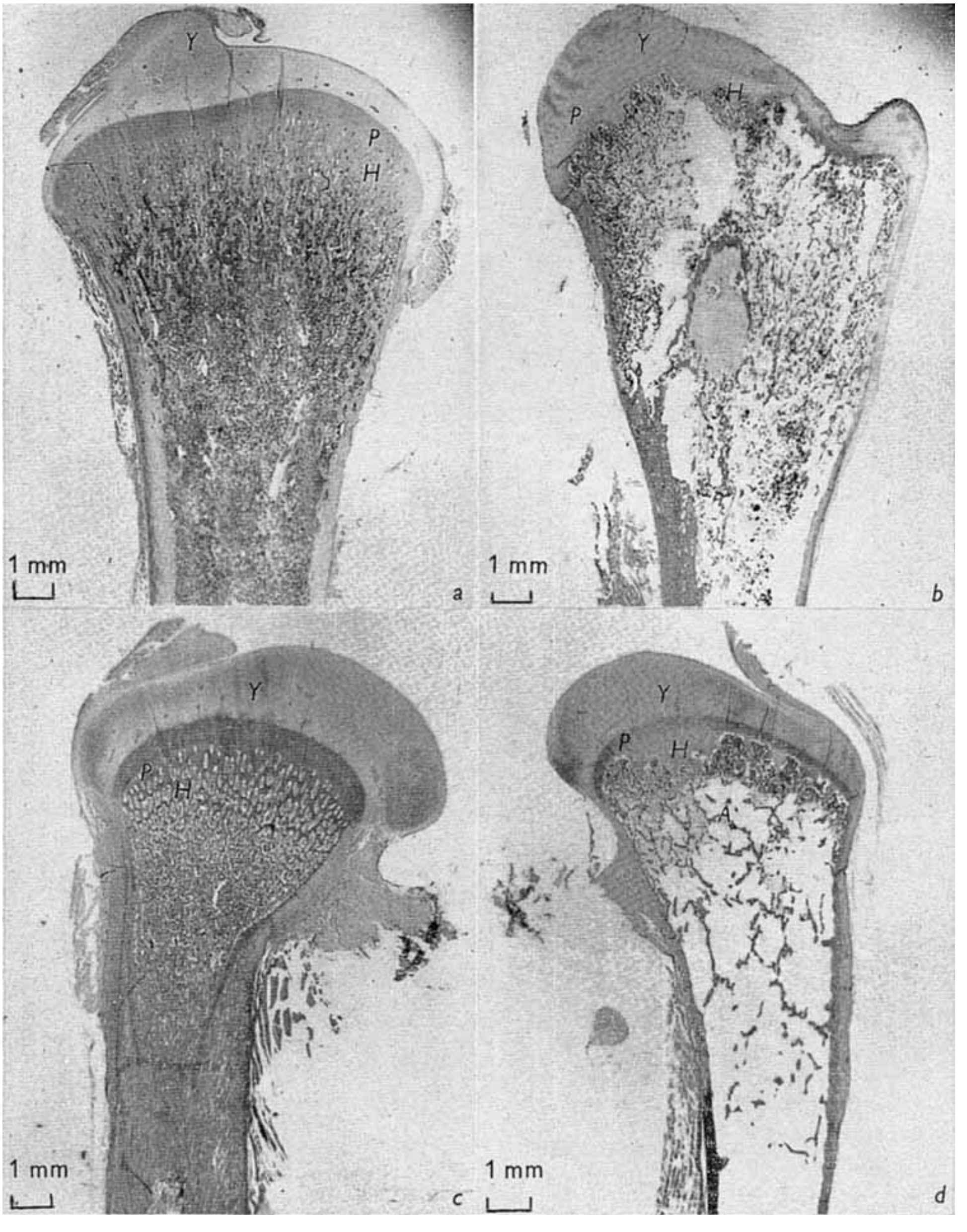

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EXPLANATION OF PLATES

PLATE 1

Radiographs of undernourished cockerels and of the femur from an undernourished and from a control bird.

(*a*)* Bird aged 22 days, after 7 days of underfeeding.

(*b*) The proximal end of the femur of bird illustrated in (*c*). Note the band of calcified tissue lying between the cartilage and the metaphysis, and also the transverse striations in the metaphysis.

(*c*)* Bird aged 197 days after 182 days of underfeeding. Note the lack of deformities and pathological lesions of the bones.

(*d*) The proximal end of the femur from normal bird aged 29 days. Note the absence of any of the features seen in (*b*).

PLATE 2

Photomicrographs of sections of the shafts of long bones from an undernourished and a normal cockerel. *B*, bone; *C*, subperiosteal cells; *L*, cement line; *M*, marrow cavity; *O*, osteoblast; *P*, periosteum; *V*, vascular spaces.

(*a*) Control, aged 29 days. Longitudinal section of humerus showing subperiosteal region. Note periosteum, underlying undifferentiated connective-tissue cells, bone and adjacent osteoblasts. Haematoxylin and eosin.

(*b*) Undernourished, aged 197 days. Longitudinal section of humerus showing subperiosteal region. Compare with (*a*). Note the changed appearance of the subperiosteal cells, the lack of osteoblasts and the compact subperiosteal bone. Haematoxylin and eosin.

(*c*) Control, aged 29 days. Transverse section through the middle of the shaft of the femur. Note the cancellous nature of the subperiosteal bone. Haematoxylin and eosin.

(*d*) Undernourished, aged 169 days. Transverse section through the middle of the shaft of the femur. Compare with (*c*). Note the decreased thickness of the walls and the more compact structure of the bone. Haematoxylin and eosin.

(*e*) Control aged 29 days. Transverse section through the shaft of the femur showing the structure of the fibrous matrix. Note the background of fine fibres, the coarse bundles which have been cut transversely and the dense collection of fibres about the vascular spaces. Long's silver impregnation.

(*f*) Undernourished, aged 197 days. Transverse section through the shaft of the femur showing the structure of the fibrous matrix. Compare with (*e*). Notice the dense collection of fine fibres, the annular cement lines surrounding the vascular spaces, and the subperiosteal cement lines. Long's silver impregnation.

* The neck has been severed and the femora removed.

PLATE 3

Photomicrographs of longitudinal sections of the epiphysial and growth cartilages of long bones from normal and undernourished cockerels. *H*, hypertrophic zone; *M*, marrow cavity; *P*, proliferative zone; *S*, spongiosa; *Y*, hyaline zone.

(*a*) Control, aged 15 days. Distal growth cartilage of femur. Haematoxylin and eosin.

(*b*) Undernourished, aged 22 days. Distal growth cartilage of femur showing the earliest effects of undernutrition. Compare with (*a*) and note the narrowing of the hypertrophic zone. Haematoxylin and eosin.

(*c*) Undernourished, aged 36 days. Distal growth cartilage of femur showing early effects of undernutrition. Compare with (*a*) and (*b*) and note the narrow proliferative zone and decreased amount of spongiosa. Haematoxylin and eosin.

(*d*) Control, aged 29 days. Distal growth cartilage of femur. Compare with (*c*) and note extensive proliferative and hypertrophic zones. Haematoxylin and eosin.

(*e*) Undernourished, aged 197 days. Proximal growth cartilage of tibia showing late effects of undernutrition. Note the striated appearance of the hypertrophic zone. Haematoxylin and eosin.

(*f*) As (*e*), hypertrophic zone at higher magnification. Note the zones of spherical cells with the deeply staining matrix separated by zones of flattened cells with paler staining matrix.

(*g*) Control, aged 29 days. Hyaline zone of distal epiphysial cartilage of humerus. Note the cell nests. Haematoxylin and eosin.

(*h*) Undernourished, aged 197 days. Hyaline zone of distal epiphysial cartilage of humerus. Compare with (*g*) and note the absence of cell nests and the decrease in size of the cells. Haematoxylin and eosin.

PLATE 4

Photomicrographs of longitudinal sections of the long bones from a normal and an undernourished cockerel, showing the effects of undernutrition on endochondral osteogenesis. *E*, endochondral bone; *H*, remains of hypertrophic cartilage; *K*, osteoclast; *M*, marrow cavity; *O*, osteoblast.

(*a*) Control aged 29 days. Ossification centre in the distal tibial epiphysial cartilage. Haematoxylin and eosin.

(*b*) Control aged 29 days. Medullary surface of hypertrophic zone of distal growth cartilage of humerus. Haematoxylin and eosin.

(*c*) Undernourished aged 197 days. Ossification centre in the distal tibial epiphysial cartilage. Compare with (*a*). Note the lack of osteoblasts and scarcity of endochondral bone. Haematoxylin and eosin.

(*d*) Undernourished aged 197 days. Medullary surface of hypertrophic zone of distal growth cartilage of humerus. Compare with (*b*). Note the absence of both osteoblasts and endochondral bone. Haematoxylin and eosin.

PLATE 5

Photomicrographs of longitudinal sections of the metaphyses of long bones from a normal and an undernourished cockerel. *A*, humeral air sac; *H*, hypertrophic zone; *M*, marrow cavity, *P*, proliferative zone; *S*, spongiosa; *Y*, hyaline zone.

(*a*) Control, aged 29 days. The proximal end of the femur. Haematoxylin and eosin.

(*b*) Undernourished, aged 197 days. The proximal end of the femur. Compare with (*a*) and Fig. 1. Note the changes in the growth cartilage, the decreased amount of spongiosa and the clubbed outline of the metaphysis. Haematoxylin and eosin.

(*c*) Control aged 29 days. The proximal end of the humerus. Haematoxylin and eosin.

(*d*) Undernourished, aged 197 days. The proximal end of the humerus. Compare with (*c*). Note the invasion of the humeral air sac, the decreased amount of spongiosa, and changes in the growth cartilage similar to those found in (*b*). Haematoxylin and eosin.

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