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# Severe undernutrition in growing and adult animals

## 6.* Changes in the long bones during the rehabilitation of cockerels

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It is well known that prolonged undernutrition of immature mammals severely restricts growth, although some bone growth goes on even if the weight of the animal is not increasing. Rapid growth may be resumed with the restoration of unlimited food (McCay, Crowell & Maynard, 1935; McCance, 1960). Earlier workers, whose findings were reviewed by Jackson (1925, 1937), found that during recovery full stature was not always attained if the period of undernutrition was long and the animals were very immature. Pratt & McCance (1960) showed that the bones of cockerels continued to grow at a very reduced rate in spite of profound undernutrition but that an atypical bone matrix was formed. McCance & Widdowson (1955) showed that cockerels maintained at 200 g for 8 weeks were able to make a good recovery if given adequate food, and McCance (1960) found that this was so even after 6 months. Similar results were obtained in the present investigation, although the femur did not attain its proper length. The period of 6 months is significant, as it has been shown that the growth cartilages of the normal femur have disappeared well before this date (Pratt, 1961), so that in fact the normal femur should be incapable of further elongation by this time.

This investigation is concerned principally with two problems. First, how does the bone from the stunted bird recover and, second, can a bone from a fully rehabilitated bird, even if it has failed to attain its proper length, be distinguished histologically from a normal bone?

### METHODS

The experiments were carried out on Rhode Island Red chickens which were 15 days of age at the beginning of the period of undernutrition and weighed approximately 100 g. Table 1 gives the ages at death, the duration of undernutrition and rehabilitation, the body-weights and the lengths of the femurs of the birds. Six of the birds (nos. 1–3 and 7–9 in Table 1) were derived from the group of undernourished cockerels described by Pratt & McCance (1960); during undernutrition they were held at a body-weight of 110–150 g. Three cockerels (nos. 4–6 in Table 1) came from another undernourished group which had been maintained at a slightly higher weight

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(150–200 g). A pullet accidentally included in the experimental group is shown in Table 1 as bird no. 9. The details of the rehabilitation methods have been described by McCance (1960).

The long bones were removed and X-rayed. The femurs (and in bird no. 3 the humerus and tibiotarsus also) were fixed, decalcified, embedded, sectioned and stained as described by Pratt & McCance (1960). In addition, the lengths of the femurs were measured at frequent intervals during rehabilitation. These measurements were obtained from the subcutaneous palpation of the extremities of the greater trochanter and the lateral condyle.

Table 1. *Age at death, duration of rehabilitation, body-weights and femoral lengths of the cockerels*

| Bird no. | Age at death (days) | Duration of under-nutrition (days) | Duration of re-habilitation (days) | Body-weight at commencement of re-habilitation (g) | Length of femur at commencement of rehabilitation (mm) | Body-weight at death (g) | Length of femur at death (mm) |
|---|---|---|---|---|---|---|---|
| 1 | 212 | 182 | 15 | 137 | 52 | 227 | 56 |
| 2 | 244 | 182 | 47 | 113 | 47 | 400 | 62 |
| 3 | 261 | 182 | 64 | 144 | 54 | 940 | 85 |
| 4 | 252 | 175 | 62 | 160 | — | 1074 | 88·5 |
| 5 | 252 | 175 | 62 | 175 | — | 814 | 80 |
| 6 | 392 | 175 | 202 | 160 | — | 2750 | 100 |
| 7 | 463 | 182 | 266 | 127 | 52 | 2930 | 99 |
| 8 | 470 | 182 | 273 | 115 | 47 | 2880 | 102 |
| 9* | 470 | 182 | 273 | 136 | 48 | 1970 | 86 |

* Pullet.

## RESULTS

### *Growth in length*

The growth in length of the femur during the recovery of bird no. 8 from the effects of undernutrition is shown in Fig. 1. There was little or no elongation by the end of the 1st week of rehabilitation in any of the birds though they were already gaining weight. After the 1st week the rate of elongation became very rapid and seemed to be nearly equal to that of the controls. Growth in length had ceased in the three surviving cockerels and the one pullet by 300 days, that is, after 4 months of recovery. The bones, however, were considerably shorter than those of fully grown controls and corresponded more closely to the bones of the adults of a lighter breed, such as White Leghorns (Latimer, 1927).

McCance & Widdowson (1955) showed that rehabilitated birds increased in weight at a rate similar to that of normal growing birds, but later McCance (1960) pointed out that such birds did not attain the full weight of normal ones. All the birds included in the present series, however, were killed before their weight had reached its plateau and therefore the rehabilitated birds would probably have increased in weight after the femur ceased to grow. This failure to attain full bony stature after a period of undernutrition is in line with previous work on mammals (Aron, 1911; Jackson, 1925, 1937; Clarke & Smith, 1938) and has been overcome in rats by the administration of growth hormone during rehabilitation (Fábry & Hrůza, 1956; Hrůza & Fábry, 1957).

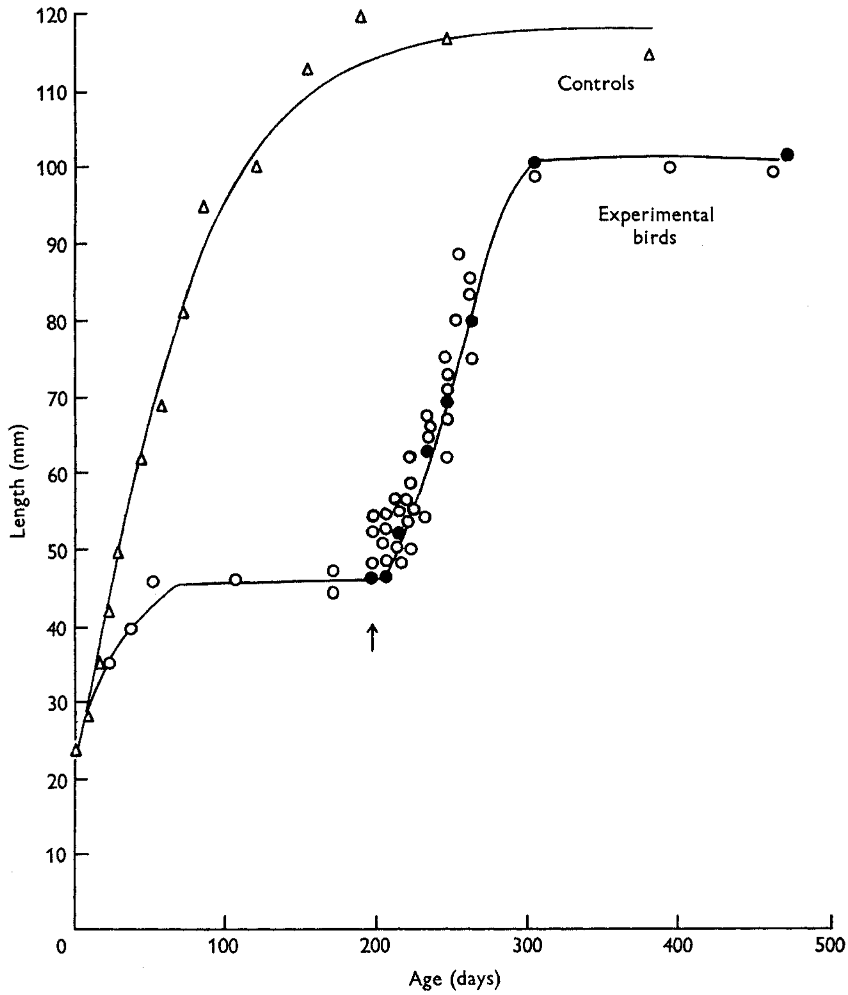


Fig. 1. Growth in length of the femur in undernourished and rehabilitated cockerels. ●, cockerel during rehabilitation; ○, undernourished and other rehabilitated birds before and after they were killed; ↑, beginning of rehabilitation; △, values for well-nourished Rhode Island Red controls, based on the lengths of the femur of thirteen birds of known age from the same stock.

### *Radiological appearances*

The metaphyses of the long bones of bird no. 1 showed changes after 15 days of rehabilitation. The dense narrow band of calcified tissue found in undernourished birds (Pratt & McCance, 1960, Pl. 1 *b*) was separated from the extremity in the femur by a zone (1–2 mm deep) of less dense tissue. It will be shown later that this appearance was due to the formation of a wide zone of hypertrophic cartilage and the associated deposition of endochondral bone. In bird no. 2, which had been rehabilitated for 47 days, this moderately dense zone in the metaphysis was much deeper and reached 5 mm at both extremities of the femur and more than that in other bones such as the tarsometatarsus. The cortex of the femur appeared to be considerably thickened in its posterior part.

In most of the long bones of birds nos. 3, 4 and 5, double contours of the shaft wall gave the appearance of a vestigial shaft within the medullary cavity (Pl. 1 *a–c*). It will be shown subsequently that this was in fact a partially separated vestige of the shaft which was formed during the period of undernutrition (Pl. 4*b*). Double contours of the shaft wall can be seen in the normal bones of birds aged 71 days (Pl. 2*d*) and over (Pl. 2*f*), but they are much thinner, inconstant, and rarely complete. They represent structures formed during the enlargement of the medullary cavity, when portions of the shaft become undermined and persist as intramedullary structures (Pratt, 1961). The position of the vestigial shaft in rehabilitated birds indicates the sites of growth, both in length and in width, of the particular long bone. The vestigial shaft in the femur was fused with the lateral wall of the parent bone and lay almost in the middle of its length (Pl. 2*c*). The vestigial shaft also lay midway between the ends of the humerus (Pl. 1 *a*, *b*), radius, and ulna and phalanges, but in the distal third of the shaft of the tibiotarsus (Pl. 2*a*, *b*) and the tarsometatarsus.

The long bones of birds nos. 6, 7, 8 and 9 showed no features which distinguished them from normal bones (Pl. 2*e*).

### *Histological observations*

*The early stages of recovery (birds nos.* 1–5)

*Subperiosteal tissue.* After 15 days of rehabilitation there were dramatic changes in the narrow, avascular and almost fibreless zone of closely packed elongated cells found in the undernourished birds (Pratt & McCance, 1960, Pl. 2*b*). In the rehabilitated birds large numbers of capillaries could be seen, and coarse fibre bundles passed from the dense underlying bone to the fibrous periosteum (Pl. 3*a*). The subperiosteal cells had become much more numerous and formed a wider zone. Mitotic activity, which was absent during the starvation period, had reappeared. Some of the cells differentiated into osteoblasts, and the zone lost its previously uniform appearance. This tissue remained in an active state throughout the early stages of recovery.

*Subperiosteal surface of the shaft.* Subperiosteal deposition of bone was greatly reduced during the period of undernutrition. Such bone as was formed was atypical. It was compact and contained only irregularly woven, tightly packed, fine fibre bundles (Pratt & McCance, 1960, Pl. 2*f*). By the 15th day of rehabilitation a wide zone of trabecular bone had been formed under the periosteum (Pl. 3*a*). As in a normal young cockerel, the deposition was eccentric in relation to the longitudinal axis of the bone. The new part of the bone, which was eosinophilic, was separated from the underlying basophilic part by a smooth cement line. This line and the basophilic zone had been formed during the period of undernutrition. The bone formed during recovery was further distinguished by its fibrous structure from the bone formed during undernutrition (Pl. 3*b*). The fibrous structure of the bone formed during the period of undernutrition is described above, and the newly deposited bone consisted of a loose irregular network of fine fibre bundles, interspersed with coarser bundles, whose extra-osseous course has already been commented upon, and a dense collection of fibre bundles around the vascular spaces. This new subperiosteal bone therefore was structurally identical with that found in normal growing birds (Pratt, 1961), and should be regarded as the continuation of the periosteal osteogenesis which was interrupted by the onset of undernutrition.

*Medullary surface of the shaft.* Intense intramedullary osteoclastic activity and resorption are conspicuous in the bones of normal young cockerels. Osteoclasts were rarely found in the medullary surface of the shaft during the later stages of undernutrition, and as a consequence there was no internal resorption of bone.

Osteoclasts formed a sheet lining the medullary surface of the shaft of the femur taken from the bird which had been rehabilitated for 15 days (Pl. 3*a*), and sometimes these cells were present in the vascular spaces of the basophilic part of the bone. In spite of the numerous osteoclasts, however, the older basophilic shaft was still *in situ*, and 32 days later, when there had been a considerable subperiosteal deposition of bone, the old basophilic shaft still persisted intact and little thinned (Pl. 4*a*). At this stage there was osteoclastic activity within the vascular spaces of the deepest part of the newly formed eosinophilic bone, and isolated pockets of marrow tissue lay between the old bone and the new. These pockets enlarged and became continuous, so that in the bird killed 64 days after the commencement of rehabilitation there were two marrow cavities, one within the vestige of the old shaft and the other between the vestige and the new cortex (Pl. 4*b*). The vestige was anchored to the new shaft where there had been no separation: elsewhere it was attached by unresorbed trabeculae of new periosteal bone. These were consolidated by the deposition of endosteal lictor-bundle bone, which appears in the medullary cavity of the bones of normal growing cockerels by 71 days (Pratt, 1961). A vestige, similar in site and structure, was found in the femur of two birds killed 62 days after the commencement of rehabilitation. In the tibia and humerus the vestige was more centrally placed in transverse sections. In the humerus the extension of the cervical air sac into the diaphysis did not extend beyond the vestige at this stage.

*Epiphysial cartilage.* Undernutrition had little effect upon the structure of the epiphysial cartilage, except to stop all mitotic activity and lead to the disappearance of cell nests, i.e. clusters of chondrocytes (Pratt & McCance, 1960, Pl. 3*h*).

Fifteen days after the commencement of rehabilitation the mitotic activity had returned. The zone of spindle-shaped cells adjacent to the growth cartilage which had disappeared early in undernutrition reappeared immediately on rehabilitation (Pl. 5*a*). This zone is probably the area where epiphysial chondrocytes arise from the proliferative zone of the growth cartilage. Cell nests were numerous 47 days after refeeding.

*Growth cartilage.* The growth cartilage had regained an almost normal appearance within 15 days of rehabilitation (Pl. 5*a*), by which time the proliferative and hypertrophic zones had already greatly widened. The latter zone had become deeply eroded, giving a serrated appearance, and endochondral bone had begun to be deposited on any persisting trabeculae of hypertrophic cartilage. Remains of the hypertrophic cartilage laid down during the period of undernutrition (Pratt & McCance, 1960, Pl. 3*e*) could be recognized by their striated appearance, but they had disappeared by the 47th day of rehabilitation.

*Metaphysis*. Prolonged undernutrition resulted in a club-shaped metaphysis (Pratt & McCance, 1960, Pl. 5*b*). Subperiosteal osteoclasis reappeared with rehabilitation and the normal contours were restored, and with them the metaphysial subperiosteal osteogenesis which consolidated the more peripheral endochondral trabeculae. Thus the vestige of the old shaft, formed during undernutrition, was remodelled at its extremities, and did not persist as in the diaphysis (Pl. 1*a*).

*The late stages of recovery* (*birds nos*. 6–9)

*Subperiosteal tissues*. The cells of the subperiosteal zone were sparse and flattened. The extra-osseous fibre bundles were no longer obvious and there were few capillaries. This is the normal state of affairs in adults (Pratt, 1961).

*Diaphysial bone*. The diaphysis consisted of the normal three zones, an outer one of compact bone containing longitudinally arranged lictor-bundles and elastic fibres, a middle one with a fibrous structure similar to that seen in the early stages of recovery, and an inner one of lamellar bone, formed by endosteal osteogenesis, and separated from the middle zone by a cement line. Secondary osteones were occasionally found and were similar in structure to those found in bones of normal adult cockerels (Pratt, 1961).

*Medullary cavity*. Some, usually incomplete, vestiges of the old shaft, which were basophilic and atypical in fibrous structure and which had been formed during the period of undernutrition, persisted within the medullary cavity. The normal sheets of bone formed by the partial erosion of the medullary surface of the diaphysis (Pratt, 1961), which were eosinophilic and had a fibrous structure similar to adjacent cortical bone, were always to be seen, and lamellar bone was deposited on their surfaces in the normal way. The pullet, which had been included accidentally in the experiment, showed the extensive and normal deposition of bone within the medullary cavity which is a characteristic of well-nourished hens during the egg-laying cycle. The distribution and fibrous structure of this bone were identical to those in bones from normal laying hens (Pratt, 1961).

*Growth cartilage*. In normally nourished birds, the proliferative and hypertrophic zones of the growth cartilage had disappeared by 155 days (Pratt, 1961). In the last four birds undergoing rehabilitation, they had also gone by the time of killing (Pl. 5*b*).

*Epiphysial cartilage*. With normal ageing the hyaline zone of the epiphysial cartilage, containing cell nests, persisted for a short time after the disappearance of the growth cartilage and was gradually replaced by a terminal plate of bone (Pratt, 1961). The fibrous articular zone remained, but occasional narrow bands of the hyaline one also persisted. This was true also of the rehabilitated birds nos. 6, 7, 8 and 9 (Pl. 5*b*) and, if it was present, the hyaline zone was very narrow and confined to certain situations such as the head of the femur. The articular zone was structurally normal and contained scattered chondrocytes lying between dense fibre bundles.

## DISCUSSION

This experiment demonstrates that a bone which appears grossly abnormal can recover, continue its natural history and almost complete its full development as though it had never been disturbed. This power of recovery is possible because even in the most severely undernourished birds the progenitors of osteoblasts and chondrocytes persist in fair numbers as the spindle-shaped cells below the periosteum and in the reduced proliferative zone of the growth cartilage (Pratt & McCance, 1960). With rehabilitation these cells divide, differentiate, and form their appropriate matrices, which are indistinguishable from the normal. Furthermore, they take about the same time to do it. The femurs, for example, from birds nos. 3, 4 and 5 which had been rehabilitated for 62–64 days and had had 15 days of normal growth before underfeeding, showed for the first time some of the endosteal lictor-bundle bone, which appears in the femur of normal birds by 71 days (Pratt, 1961).

Although rehabilitation brought about full structural recovery, the femur only reached about 85 % of its expected length. No further growth could have taken place, for the growth cartilage and almost all the hyaline zone of the epiphysial cartilage had disappeared in birds nos. 6, 7, 8 and 9. It is possible that this inability of the bone to attain its normal length may be due to disturbances in the hypertrophic zone of the growth cartilage during the later stages of undernutrition (Pratt & McCance, 1960). The ultimate length of the bone must be determined by the chondrocytes in the growth cartilage, for if some of these cells fail to hypertrophy (as happens in the later stages of undernutrition) and consequently do not produce any longitudinal growth, then the final length of the bone will suffer.

The persistent vestige of the shaft formed during the period of undernutrition does not appear to have been reported before. Landauer (1935) may have been observing the early stages of this phenomenon when, in young chickens, he described micromelia, which he believed to be due to prenatal nutritional deficiencies. He noted abnormalities in the periosteal bone of the shafts of the long bones. Some of the birds described by Landauer recovered, and at 3 months remains of the abnormal periosteal bone were visible on the medullary surface of the shaft.

As already stated, the position of the vestigial shaft clearly indicates the sites of greatest growth, which previously were little known in avian bones. The findings suggest that avian bones differ from mammalian bones in that there is little inequality in the contribution of the two growth cartilages to the longitudinal growth of most avian long bones. In the tibiotarsus, however, the proximal growth cartilage, as in the mammalian tibia, accounted for most of the growth in length.

Though the histological examination of a bone during the period of rehabilitation should indicate its previous history if any vestige of the old shaft can be found, the radiological evidence may be misleading, as double contours of the shaft wall may be seen in the long bones of birds which have always been well nourished (Pl. 2*d*, *f*, and Pratt, 1961). The high degree of mineralization of the bone formed during undernutrition (Dickerson, 1959) may account for the persistence of the vestige during rehabilitation, in spite of the proximity of large numbers of osteoclasts.

SUMMARY

1. Eight cockerels and one pullet which had been undernourished for 6 months were given unlimited food and the anatomical structure of the long bones was investigated during recovery.

2. Measurements of the femurs during rehabilitation showed that after an initial delay they increased in length as fast as those of normal birds, but did not attain the normal dimensions.

3. Radiological changes in the metaphysis of the long bones during the early weeks of recovery were conspicuous, and during the 3rd month double contours of the walls of the shaft were visible, which were due to the persistence of the vestigial shaft.

4. The histological changes in the femur which occurred during the early stages of rehabilitation consisted of a return of mitotic activity and osteoblastic differentiation in the subperiosteal tissues, the deposition of cancellous bone on the old shaft, the reappearance of mitotic activity and cell nests in the hyaline zone of the epiphysial cartilage, the reappearance of mitotic activity and widening of the proliferative zone of the growth cartilage, and the widening of the hypertrophic zone of the growth cartilage with the redeposition of endochondral bone.

5. The structure of the vestigial shaft confirmed that it was formed during the period of undernutrition, and its position located which end of the bone was responsible for most of the growth in length.

6. The femurs of birds which had been rehabilitated for over 4 months were indistinguishable structurally from those of normal adult birds, except that a vestigial shaft was frequently present.

7. It is suggested that the failure of the bones to reach their optimal length is due to disturbances in the growth cartilage during undernutrition.

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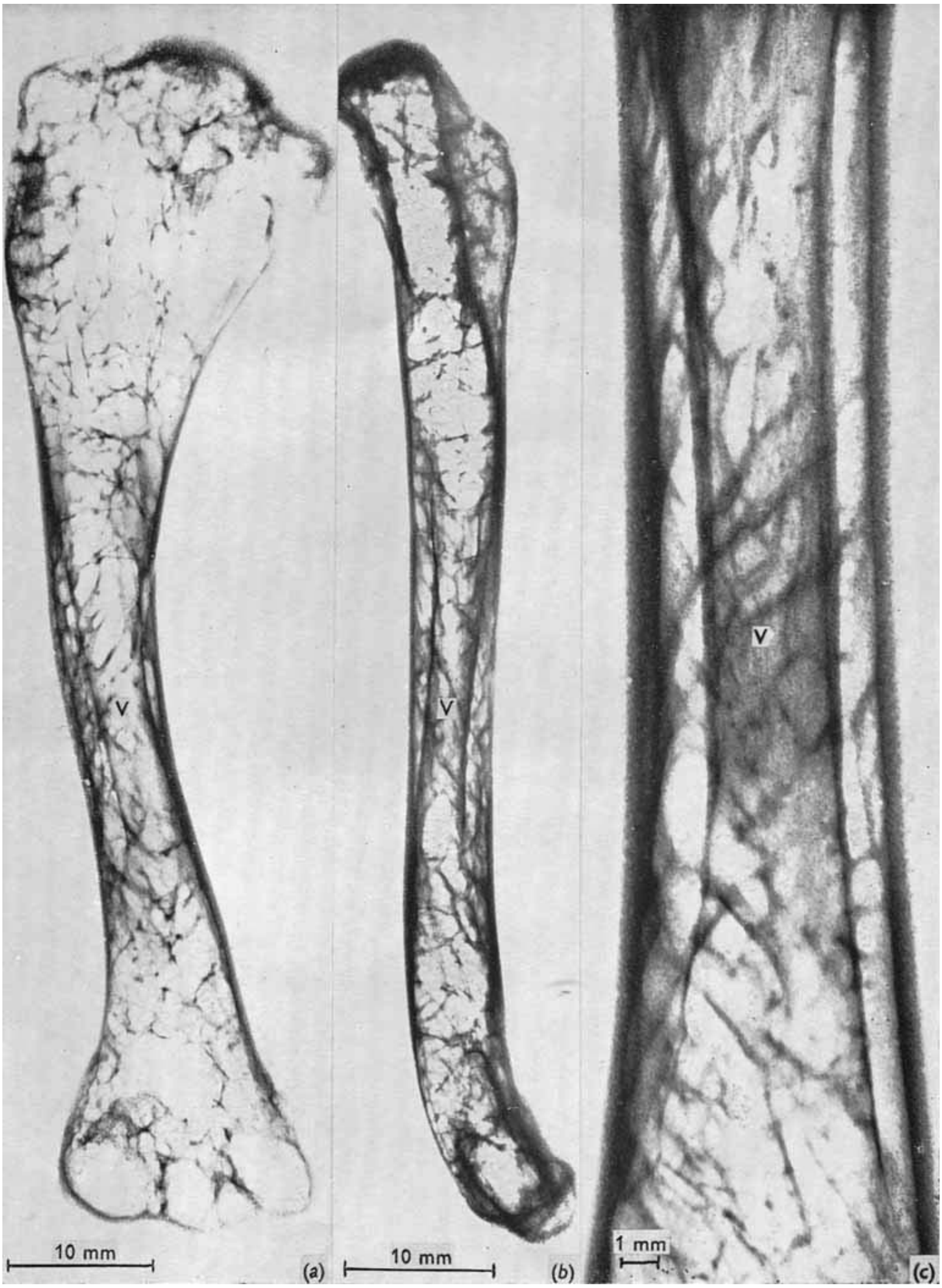
REFERENCES

Aron, H. (1911). *Philipp. J. Sci.* (B, *Med.*), **6**, 1.
Clarke, M. F. & Smith, A. M. (1938). *J. Nutr.* **15**, 245.
Dickerson, J. W. T. (1959). The effect of development on the composition of the organs and tissues. Ph.D. thesis, University of Cambridge.
Fábry, P. & Hrůza, Z. (1956). *Physiol. Bohemoslov.* **5**, Suppl. 11.
Hrůza, Z. & Fábry, P. (1957). *Gerontologia*, **1**, 279.
Jackson, C. M. (1925). *The Effects of Inanition and Malnutrition upon Growth and Structure.* London: J. and A. Churchill.
Jackson, C. M. (1937). *Anat. Rec.* **68**, 371.
Landauer, W. (1935). *Anat. Rec.* **4**, 267.
Latimer, H. B. (1927). *Amer. J. Anat.* **40**, 1.
McCance, R. A. (1960). *Brit. J. Nutr.* **14**, 59.
McCance, R. A. & Widdowson, E. M. (1955). *Z. Vitaminforsch.* **26**, 79.
McCay, C. M., Crowell, M. F. & Maynard, L. A. (1935). *J. Nutr.* **10**, 63.
Pratt, C. W. M. (1961). *J. Anat., Lond.* (In the Press.)
Pratt, C. W. M. & McCance, R. A. (1960). *Brit. J. Nutr.* **14**, 75.

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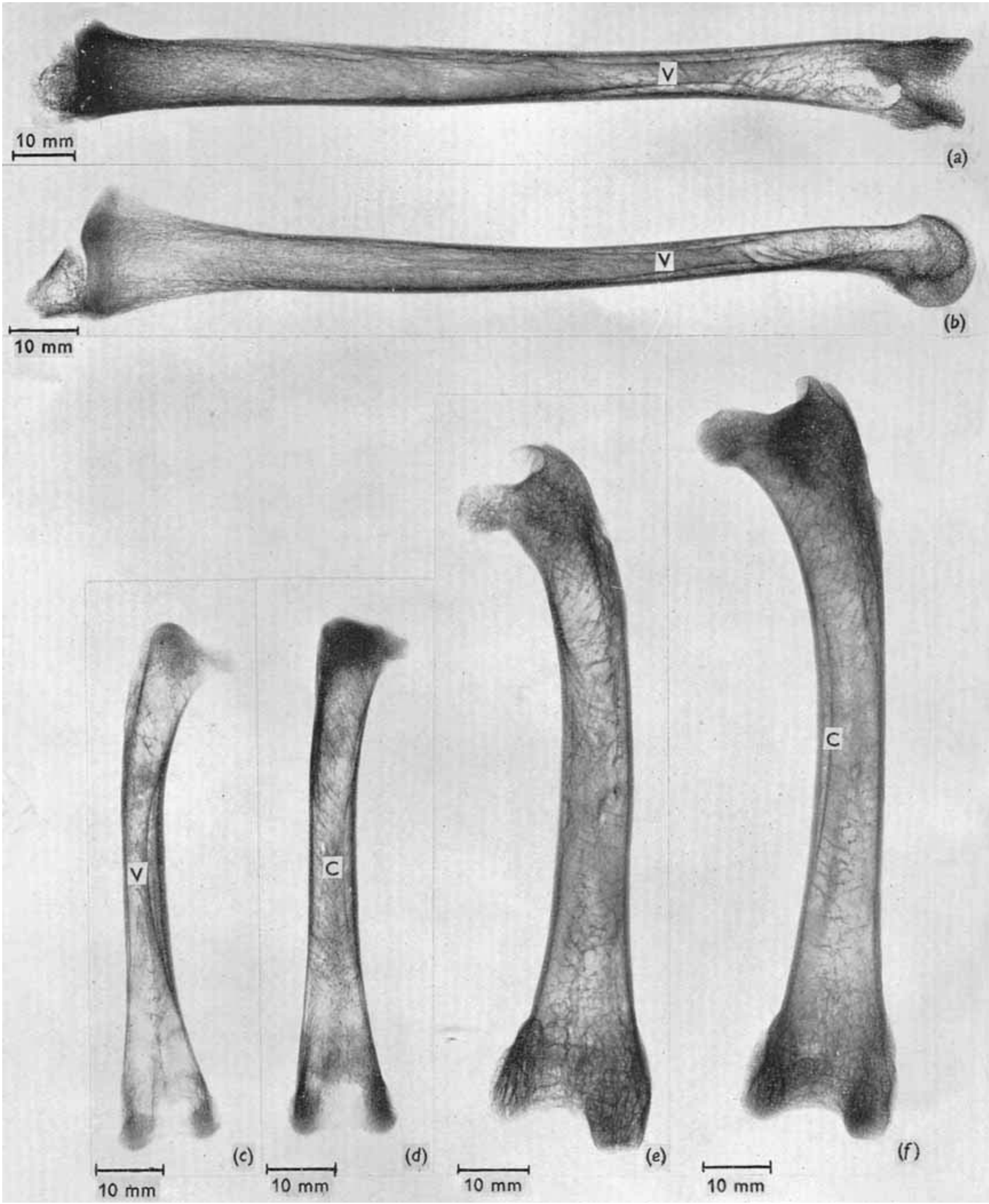


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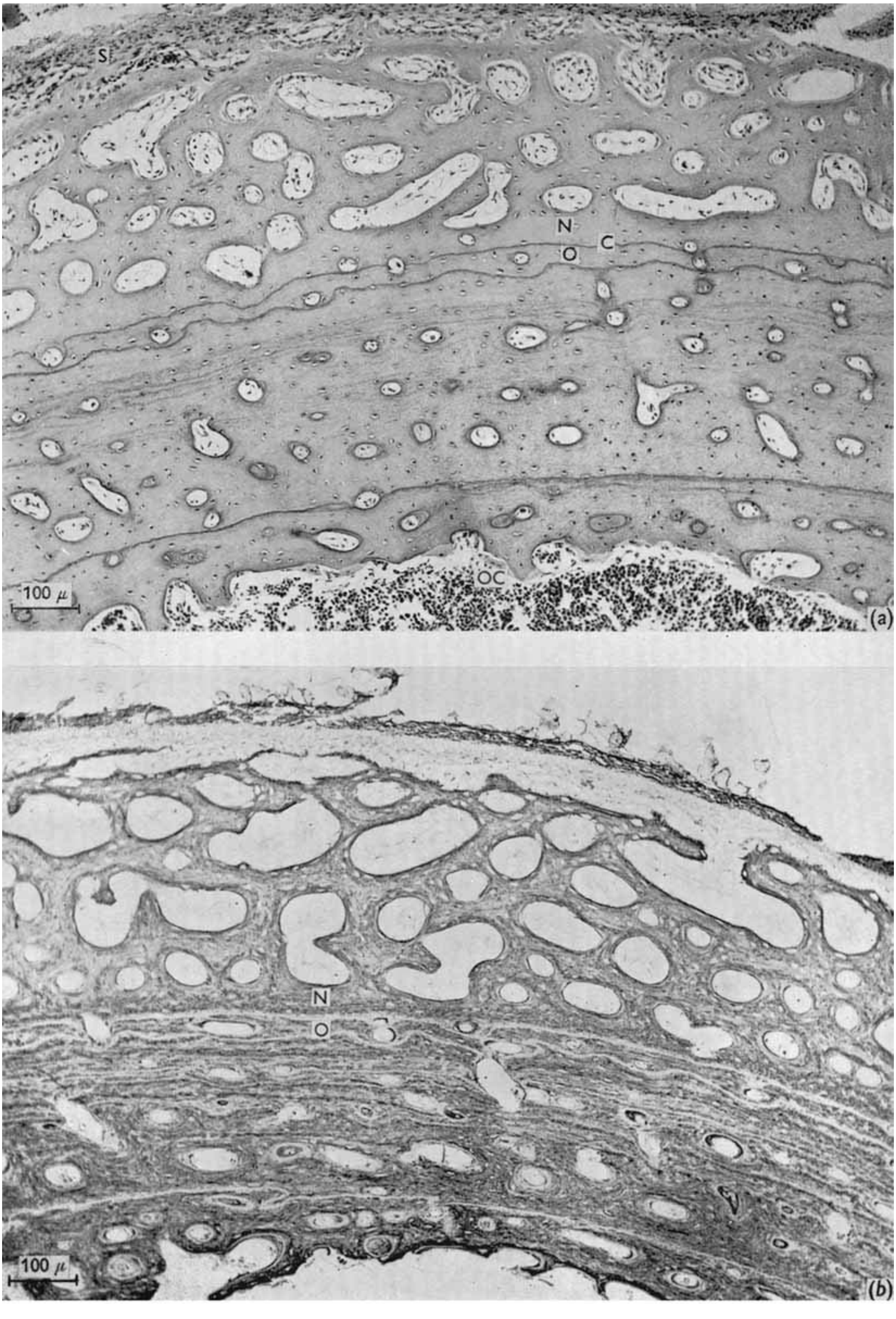


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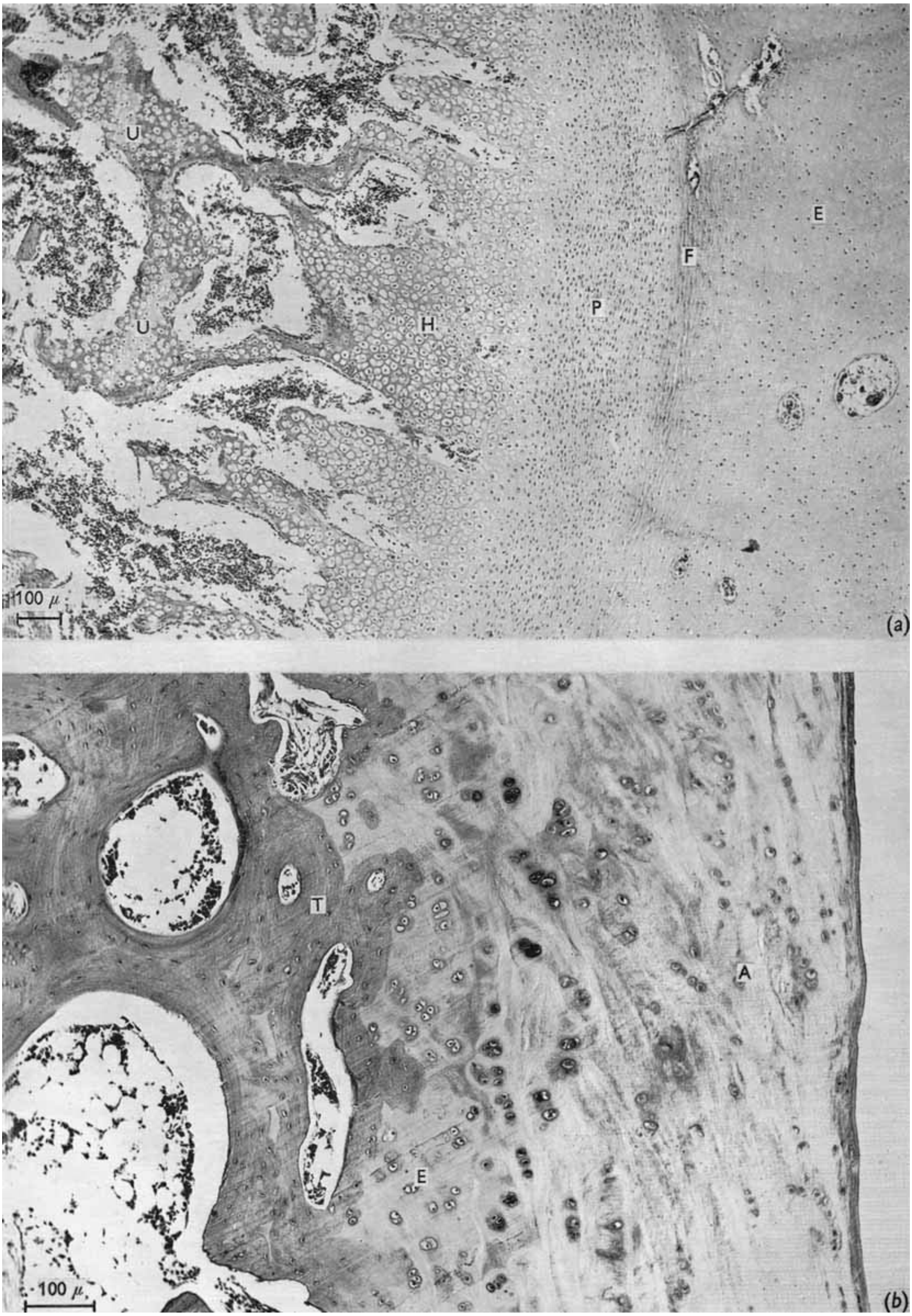


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## EXPLANATION OF PLATES

### Plate 1

*a.* Antero-posterior radiograph of the humerus of cockerel no. 3. Note the vestigial shaft (V) in the middle portion of the bone.

*b.* Lateral view of the same bone. The vestigial shaft (V) occupies a central position in relation both to the anterior and posterior surfaces and to the extremities.

*c.* Antero-posterior radiograph of the distal third of the shaft of the tibiotarsus of the same bird. Note the position and appearance of the vestigial shaft (V).

### Plate 2

*a.* Radiograph of the tibiotarsus from cockerel no. 3 (lateral view). The vestigial shaft (V) is at the distal end of the bone but lies approximately in the longitudinal axis.

*b.* Antero-posterior radiograph of the tibiotarsus from the same bird. Note the vestigial shaft (V). Compare with Pl. 1 (*c*).

*c.* Lateral radiograph of the femur from bird no. 3. The vestigial shaft (V) is in the middle third of the bone but in contact with the lateral wall.

*d.* Lateral radiograph of the femur from a normal cockerel aged 71 days. Note the presence of a double contour (C) to the medial wall of the shaft. Compare with (*c*).

*e.* Radiograph of the femur from bird no. 8 (lateral view). Compare with (*f*).

*f.* Radiograph of the femur from a normal cockerel aged 379 days. Note the double contour (C) of the medial wall of the shaft.

### Plate 3

*a.* Photomicrograph of a transverse section of the shaft of the femur from a cockerel rehabilitated for 15 days. Note the well-populated subperiosteal zone (S), the trabecular bone (N) laid down during the last 15 days, the most external cement line (C) separating this new bone from the old, more compact, bone (O) which was laid down during the period of undernutrition. Osteoclasts (OC) are present on the medullary surface of the shaft. Haematoxylin and eosin.

*b.* Photomicrograph of an adjacent area to the one illustrated in (*a*) but silver-impregnated by a modified Long's method. It shows the distinction between the fibrous structure of the old bone (O) and of new bone (N).

### Plate 4

*a.* Photomicrograph of a transverse section of the shaft of the femur from a cockerel rehabilitated for 47 days. Note the old bone (O) and the new bone (N) and the large resorption spaces (R) which have appeared between them. Haematoxylin and eosin.

*b.* Photomicrograph of a transverse section of the shaft of the femur from a cockerel rehabilitated for 64 days. The old bone (O) has now become separated as a medullary vestige. Note the eccentric periosteal deposition of new bone, small in amount where the vestige is anchored, but greater at the opposite pole. Haematoxylin and eosin.

### Plate 5

*a.* Photomicrograph of a longitudinal section of the distal end of the femur from a cockerel rehabilitated for 15 days. Note from right to left the hyaline zone of the epiphysial cartilage (E) with its deepest zone of flattened cells (F), the wide proliferative zone of the growth cartilage (P), the hypertrophic zone of the growth cartilage (H) which is extensively invaded and the persistence of the striated cartilage (U) which was formed during the period of undernutrition. Haematoxylin and eosin.

*b.* Photomicrograph of a longitudinal section of the distal end of the femur of a cockerel rehabilitated for 273 days. The articular zone of the epiphysial cartilage (A) persists, lined on its deep surface by the 'terminal plate' of bone (T). There are isolated areas of the hyaline zone of the epiphysial cartilage (E). Haematoxylin and eosin.

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