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Severe undernutrition in growing and adult animals

12.* The extremities of the long bones in pigs

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In an earlier paper (Pratt & McCance, 1960) the effects of severe undernutrition on growing avian bone were described. Radiological and histological evidence showed that the initial rate of elongation was not maintained but that some growth in length and width continued throughout the period of the experiment.

There are few records of the effects of severe and prolonged undernutrition on the anatomy of the bones of growing mammals. Aron (1911) starved puppies and noted that the bones continued to grow, but he did not comment upon the structure of these bones. Jackson (1932) restricted the suckling of newborn rats and reported that though there might have been no increase in weight from birth the skeleton continued to grow and develop. In an earlier paper Jackson (1925) reviewed the literature in this field and, when considering the histological structure of such bone, noted an unpublished account of Silvernale who had found an apparently normal process of ossification.

Jackson & Stewart (1920) and Jackson (1925, 1937) showed that severe and prolonged undernutrition in rats, particularly early in life and even if followed by return to a full diet, led to permanent reduction of stature. These results were discussed by Clarke & Smith (1938) and have been confirmed and extended by Widdowson & McCance (1960, 1963) and McCance & Widdowson (1962). The contrary results obtained by Hatai (1907), Osborne & Mendel (1915), and others, may be attributed in the light of this recent work to the timing and duration of the period of undernutrition. Conflicting results have been obtained in other species. Pratt & McCance (1961) found that the stature of cockerels was reduced after prolonged undernutrition, although rehabilitation restored the structure of the bones to normal. Aron (1911) obtained permanent stunting in dogs, and so did Bonnier & Hansson (1945–6) in cattle, but Waters (1908, 1909) and Crichton, Aitken & Boyne (1960) did not. Wilson & Osbourn (1960) reviewed the whole subject of compensatory growth in animals and concluded that the ability to recover completely depended upon the species, the nature of the preceding undernutrition, its time of onset, duration and severity, and finally upon the pattern of realimentation. Nevertheless, the evidence about mammals other than the rat is still conflicting and difficult to interpret, partly because there are

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not enough precise facts about the various species and partly because so little is known about the effect of undernutrition and of its relief on the histological structure of the bones.

EXPERIMENTAL

The present paper deals with the effects of prolonged and severe undernutrition and of its relief on the structure of the metaphysis, growth cartilage and epiphysis of the pig. The effects on the structure of the diaphysis will be considered later.

Forty-two pigs were used in these experiments of which sixteen were healthy normal animals used for controls. They were derived originally from a Large White × Essex cross but subsequently always bred from Large White boars. The undernourished animals were weaned at 7–14 days of age and were fed and housed as described by McCance (1960). The food, had they been given enough of it, would have produced excellent rates of growth. Two of the animals were given about 3·6 g $CaCO_3$ per day. This rather more than doubled their calcium intakes. Some undernourished animals were killed at intervals up to 59 weeks by which time they weighed from 4 to 8 kg. Others were rehabilitated in the way described by McCance (1960) and by Mount, Lister & McCance (1963), and killed at stages up to full recovery. The control pigs came from the same stock and often from the same litters as the undernourished animals, and were measured, X-rayed or killed when they corresponded in size or in age to one of the undernourished or rehabilitating animals.

The limb bones were removed and X-rayed and the femur was sectioned and stained as previously described (Pratt & McCance, 1960). In some animals the length of the femur was roughly measured at intervals during undernutrition and early rehabilitation. The measurements were made from the tip of the greater trochanter to the lateral condyle, both points being located by subcutaneous palpation. Rehabilitation was also followed by a series of radiological examinations under Halothane (BP) anaesthesia. The measurements of femurs after removal from the body were made from the tip of the greater trochanter to the most distal point on the articular surface of the lateral condyle.

RESULTS

General observations

The femurs of the undernourished animals were longer than those of normal animals of the same weight, but very much shorter than those of normal animals of the same age. This observation is similar to that reported on the humerus of the same or similar animals (Dickerson & McCance, 1961). Serial measurements in living animals showed that, although the weight continued to increase very slowly, as did the height, there was little if any appreciable increase in the length of the femur from week to week after the restricted regime had been fully established. On rehabilitation there was always an immediate and considerable increase in weight (Mount *et al.* 1963) but the femur did not begin to resume active growth in length for about 4 weeks. Measurements are given in Table 1. Thereafter, lengthening went on at the normal rate for the general growth of the animal and the length of the bone corresponded as it should with the animal's weight (see Tables 1 and 2).

Table 1. *Gain in weight and femur length of animals during rehabilitation*

Pig no.	Stage of rehabilitation (weeks)	Weight of body: At the beginning (kg)	Weight of body: At the end (kg)	Length of femur: At the beginning (cm)	Length of femur: At the end (cm)
1	1–4	4·31	7·05	8·0	8·0
2	1–4	2·95	6·25	7·5	7·5
3	1–4	12·95	20·0	12·0	12·0
4	10–11	17·3	18·4	11·5	12·0
5	10–14	12·7	25·5	10·5	12·0
5	14–19	25·5	36·4	12·0	15·0

Table 2. *A comparison of the weights and femur lengths of rehabilitating and normal animals*

Rehabilitated pig: Weight (kg)	Rehabilitated pig: Length of femur (cm)	Normal pig: Weight (kg)	Normal pig: Length of femur (cm)
18·4	12·0	20·0	12·0
36·4	15·0	39·0	15·0
55·0	17·0	51·0	17·0
159·0	25·0	150·0	22·0

Radiological appearances

The radiological profiles of the extremities did not differ from those of the normal. Pl. 1 shows the lateral radiographs of the femurs of (*a*) a normal pig 3 weeks old weighing 3·7 kg, (*b*) a pig, 3·7 kg in weight, that had been undernourished for 6 weeks, (*c*) a pig weighing 3·64 kg that had been undernourished for 40 weeks, and (*d*) a normal pig 4 weeks old weighing 7·23 kg. In undernourished animals (Pl. 1 *b*, *c*) the epiphysial centres were larger than those in the bones of the weight controls (Pl. 1 *a*), but the same size as those in normal bones of the same length (Pl. 1 *d*). The uncalcified plate of tissue which consisted largely of the growth cartilage was narrower in the undernourished animals than in the normal controls and was sharply delineated on both its metaphysial and epiphysial aspects. These boundaries would, in the opinion of Harris (1931 *a*, *b*), have been 'lines of arrested growth'. The whole of the epiphysis was also enclosed by this boundary and the articular aspect had a crenated appearance in most of the undernourished animals (Pl. 1 *c* and particularly 2 *a*). Separated 'lines of arrested growth' were rarely found in the metaphyses of the undernourished animals. Well-defined lines were, however, found in all the limb bones of one pig that had been undernourished for only 6 weeks (Pl. 1 *b*), where there were one or two lines in the metaphysis lying a short distance away from the opaque boundary layer. Two pigs that had been given diets with added calcium had multiple lines in the metaphyses (Pl. 2 *a*). Multiple well-defined 'lines of arrested growth' were, however, frequently found in the bones that were not so much concerned with weight bearing, such as in the scapula adjacent to its vertebral border and the mandible near its angle. The spongiosa of the metaphysis of the long bones which formed a fine lattice-like structure in normal bones (Pl. 1 *d*) was less dense in those of the undernourished animals

(Pl. 1*c*) and sometimes appeared almost to be absent. However, the metaphysial trabeculae always tended to be arranged in a linear fashion similar to those in the bones of normal animals older than the size controls. The spongiosa within the epiphysis and within the short bones of the tarsus and carpus of the undernourished animals was always as well developed as in their weight controls.

During rehabilitation there were considerable changes in the metaphysial spongiosa of the long bones. Pl. 2*b* shows the femur of a pig X-rayed after 4 weeks of rehabilitation. There was widening of the gap between the epiphysis and metaphysis and a band 1 mm wide had appeared immediately adjacent to the growth cartilage which was still limited on its medullary aspect by the dense narrow boundary that had formed during undernutrition. The femurs of two other pigs X-rayed after 5 weeks of rehabilitation were in precisely the same state, and in all three similar bands were also visible over the articular aspects of some of the epiphysial centres. In three animals X-rayed between the 9th and 14th weeks of rehabilitation, this band was more extensive (Pl. 2*c*), particularly at the growing end of the bones, that is at the proximal end of the humerus and at the distal end of the femur (Payton, 1934). The band was still limited at this time by a fine dense line. In three of the animals X-rayed between the 14th and 19th weeks of rehabilitation this distinctive band, together with its limiting line, had disappeared from the metaphysis, and the spongiosa was reorganized into linear trabeculae. Reorganization was not yet complete at this time in the epiphysial centres where the vestiges of the limiting line could still be made out. These centres ultimately became reorganized in the fully rehabilitated animals.

There was no radiological evidence of the fusion of the epiphysial centres in the limb bones of any of the undernourished animals. Some of them had reached a chronological age of 1 year, by which time in the normal control pig several epiphysial centres had already fused with the rest of the bone. These changes of maturation in the normal pig include the fusion of the bicipital tuberosity with the rest of the scapula, the fusion of the distal humeral, the proximal radial and the proximal second phalangeal epiphyses with their respective shafts, and also fusion together of the elements of the pelvic girdle (Silver, 1963). In the rehabilitating animals even after a period of undernutrition lasting 2 years, these fusions took place when the bone had reached the size at which such a fusion was to be expected (Silver, 1963). The distal femoral epiphysis and the majority of the long bone epiphyses had not fused in either a 3-year-old control or the 3-year-old animals that had been undernourished for over a year and then rehabilitated.

Histological observations

The growth cartilage. As the period of undernutrition progressed the metaphysis became slightly cup-shaped. The irregularity in the profile of the growth cartilage made it impossible to define its overall thickness with any accuracy. The thickness of the central portion, excluding the adjacent part of the cartilaginous epiphysis, was found to vary between 0·65 mm (being occasionally even less at isolated points) and 1·25 mm. The normal neonatal plate had an average thickness of 1·45 mm, and in animals about a month old it was about 0·8 mm, and had been reduced to about

0·35 mm by the end of a year. Thus the thickness of the plate in an undernourished animal was sometimes the same as that in a normal animal of the same size but more often was rather thinner. The narrowing of the 'growth cartilage' which appeared to be so obvious in X-ray pictures was not entirely due to the reduction of the true growth cartilage but, as will be shown later, was also due to a reduction of the adjacent cartilaginous epiphysis.

Pl. 3*a* and Fig. 1*A* show sections of the growth cartilage of a normal animal of the same size as the undernourished ones. The zones, as defined and described in the rat by Dodds & Cameron (1934), are numbered. Zone 1 (the reserve cell zone) formed rather less than half of the thickness of the growth cartilage (0·35 mm) in such normal young pigs and was the source of the cells of the columns. It decreased with age from 1·05 mm at birth to 0·15 mm at 3 years of age. In undernourished animals (Pl. 3*d* and Fig. 1*C*) it usually tended to form rather more than half of the total thickness, but was the major variant amongst the constituent zones of their growth cartilages, and ranged between 1·15 mm and 0·15 mm and thus was sometimes much wider than in the bones of the size controls. Undernutrition increased the density of the cell population in this zone as compared with the size controls. As in normal animals most of the cells were elongated in the transverse plane, and those lying adjacent to zone 2 formed pairs. In the controls the cells were either isolated in the matrix or in pairs, though there were many transitional stages showing varying degrees of separation. In the undernourished animals the cell groups were larger and frequently contained more than four cells. This, and the general increase in the cell population, was due to mitotic divisions taking place without separation of the daughter cells. These findings indicate that though some mitotic activity occurred during undernutrition it was not followed by separation and matrix formation.

In the normal size controls (Pl. 3*a*) the thickness of zone 2 (the flattened cell zone) was about 0·35 mm and depended upon the balance between the rate of formation and maturation of these orientated cells. In bones taken from experimental animals that had been undernourished for a short time it measured about 0·3 mm, but in those of fully undernourished animals this zone was much narrower and varied little (0·15 mm). In the normal size controls the cells of zone 2 were collected into small groups, which were arranged longitudinally into little piles or columns of variable length. The width of the separating matrix was about a quarter of the width of the column of cells, though in many situations wider gaps suggested that a column was missing in the plane of section. In some of the undernourished animals (Pl. 3*d* and 4*a*) similar piles of cells were found, which in turn formed columns but these were shorter than those found in control animals, though they were of similar transverse diameter and with similar intercolumnar spacing. However, in other undernourished animals the piles of cells were not so orderly or extensive, and could better be described as 'cell nests'. These were usually observed in the more central parts of the plate of growth cartilage, and rarely were there columns in the central part.

In the normal animals of the same size the cartilage cells on the metaphysial side of zone 2 remained in columns, reached a thickness of 0·15 mm and formed zone 3 (the zone of cell growth). These cells, which were flattened, were larger than those in zone

2. They were often wedge-shaped and arranged within the columns as transversely interdigitating pairs. This zone of cell growth in the bones of undernourished animals was from 0·05 mm to 0·15 mm thick and, unlike in the well-nourished size controls, it formed the metaphysial surface of the growth cartilage (see later), and the matrix was

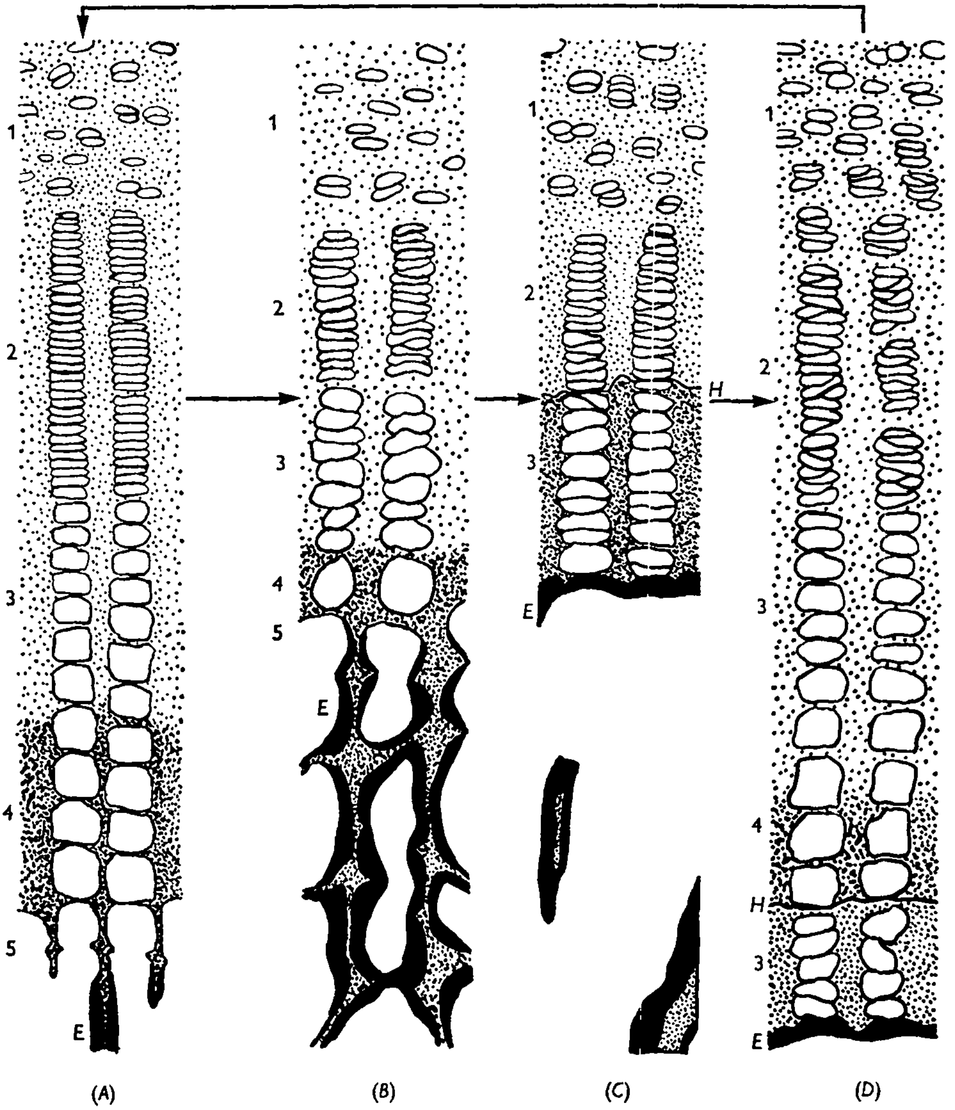

Fig. 1. A semi-diagrammatic illustration of the changes in the growth cartilage of the pig during undernutrition and after rehabilitation. All the illustrations represent longitudinal sections with the metaphysis at the bottom. 1–5 indicate zones of growth cartilage; *E*, endochondral bone; *H*, hair-line. Sparse stippling indicates cartilage matrix, dense stippling indicates calcified cartilage matrix. *A*. A normal immature pig aged 4 weeks. *B*. A pig undernourished for only 6 weeks. The narrowing of zones 2–4 and the persistence of the thick trabeculae of endochondral bone should be noted. *C*. An undernourished pig aged 52 weeks. The absence of zones 4 and 5 and the calcification of the matrix of zone 3 should be noted. *D*. A pig which had been rehabilitated for 4 weeks after being undernourished for 1 year. Note that the growth cartilage has returned to its original state as in *A*, while the calcified zone 3 still persists though shortly it will become a vestige in the metaphysis.

calcified except in one animal that had been undernourished for only 6 weeks (Pl. 3*b* and Fig. 1*B*). The matrix of this zone in the undernourished animals was deeply basophilic and contrasted in this respect with the matrix elsewhere in the growth cartilage. It was usually demarcated from the uncalcified zone 2 by a densely stained undulating 'hair-line' (Pl. 4*a*).

Zone 4, the zone of fully grown cells, was about 0·2 mm wide in the size controls and consisted entirely of intact, fully hypertrophied cells which tended still to be arranged into columns (Pl. 3*a*). The longitudinal trabeculae between the columns were calcified. In undernourished animals, however, this zone was usually absent, but it was found in a pig that had been undernourished for only 6 weeks (Pl. 3*b* and Fig. 1*B*) and in those animals in which the femur was showing localized attempts to elongate (see later).

Zone 5 (the zone of cartilage removal) was about 0·1 mm thick in the size control. It was devoid of chondrocytes, and consisted entirely of primary spongiosa, which were the remains of the longitudinal trabeculae previously separating the columns of cells. In an animal undernourished for only 6 weeks (Pl. 3*b*) the trabeculae were thick and all had persisted, but they had for the most part disappeared in a pig of the same age but undernourished for 7 weeks (Pl. 3*c*), and in fully undernourished animals this zone was represented by a few short cartilagenous processes passing from zone 3 into the marrow cavity and usually ending blindly (Pl. 3*d*). Unlike those in normal animals, many of these processes contained columns of hypertrophied cells that had survived with their adjacent longitudinal septa.

In the bones of normal-size controls, endochondral bone was deposited on the older parts of the cartilaginous trabeculae which penetrated deeply into the metaphysis. In the undernourished animals endochondral bone sometimes covered the whole of the metaphysial surface of the growth cartilage, where it formed a flat bony seal (Pl. 4*b*) of considerable thickness (0·25 mm). Sometimes, however, the seal was thinner and in places absent, and if so the metaphysial surface of the growth cartilage presented a ragged appearance.

Localized activity of the undernourished growth cartilage. In some of the animals the growth cartilage had distinctive appearances after prolonged undernutrition which were due to short periods of some functional activity (Fig. 2 and Pl. 4*b* and *c*). Although the bones did not always show these changes, there was usually evidence that there had in fact been some sporadic growth here and there. In these bones most areas of zone 2 were separated by a 'hair-line' from the calcified zone 3, the metaphysial surface of which was sealed off by endochondral bone as already described. Elsewhere, one or sometimes two additional strata were present between zones 2 and 3. Fig. 2*A* shows the initial stage of the limited growth process with the reappearance of normal, though narrow, zone 3, its matrix not being calcified. The second stratum was not always present, but when it was (Pl. 4*b* and Fig. 2*B*) it lay between the first uncalcified stratum just described and the calcified zone 3, and was separated from each by a 'hair-line'. The matrix of this second stratum was calcified, and it contained fully hypertrophied cells and was thus similar to zone 4 of the normal growth cartilage, which, as has already been pointed out, did not usually exist in the bones of undernourished animals. The second stratum often showed signs of resorption on its metaphysial surface where it was being invaded by marrow tissue which had perforated the original calcified zone 3 and its adjacent seal of endochondral bone (Fig. 2C). This vascular invasion of the growth cartilage resulted in a partial removal

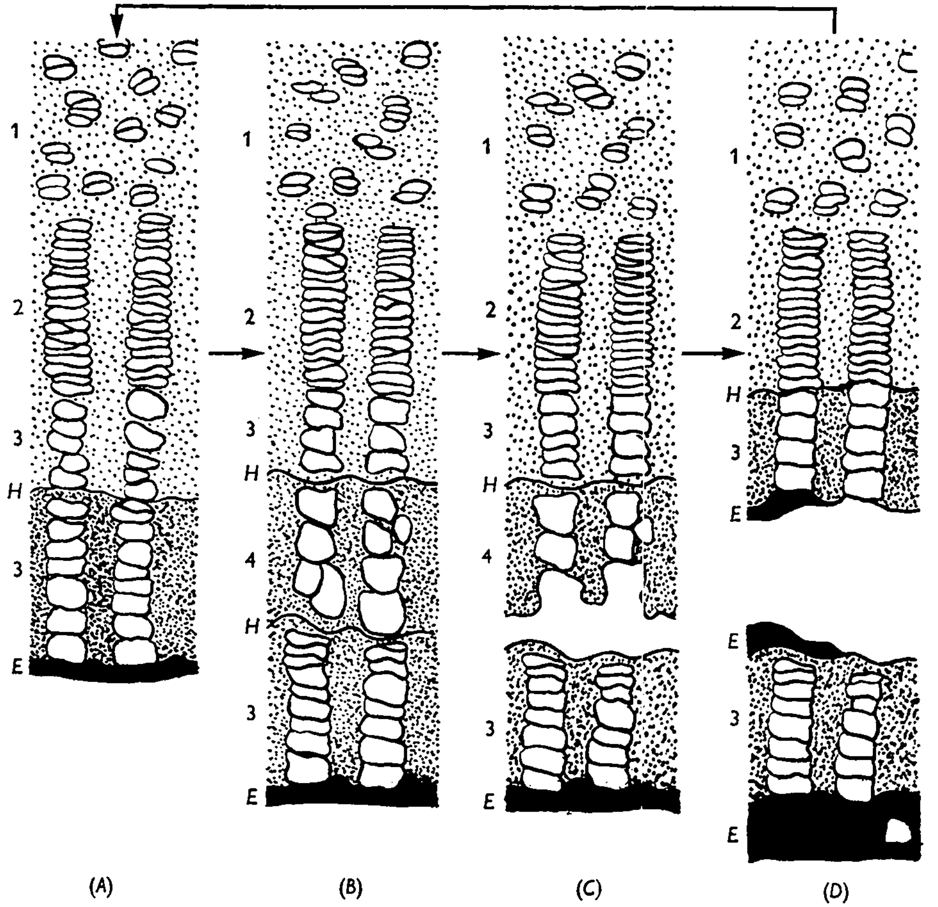

Fig. 2. A semi-diagrammatic illustration of the changes in the growth cartilage of an undernourished pig during an abortive attempt to grow. (Key as in Fig. 1.) *A*. An apparently normal zone 3 has reappeared. *B*. Zone 3 is still present while an apparently normal zone 4 has appeared and the old calcified zone 3 still persists (see Pl. 4*b*). *C*. Resorption of zone 4 has commenced with the resulting separation of the old calcified zone 3 (see Pl. 4*c*). *D*. Zone 4 has disappeared and the newly formed zone 3 has become calcified, thus the growth cartilage has returned to its original undernourished state while the old zone 3 still persists as a vestige in the metaphysis and endochondral bone is being deposited on all surfaces.

of zone 4, leaving only its epiphysial surface undisturbed and the calcified zone 3 which was now isolated from the growth cartilage (Pl. 4*c*). The usual arrangement found in the growth cartilage of the undernourished animals was then restored by calcification of the newly formed zone 3, if this had not already occurred (Pl. 4*b*), and the progressive erosion of the remains of zone 4 (Fig. 2*D*). The summation of several such processes resulted in a certain amount of elongation.

In many of the bones of the undernourished animals there were a number of transverse horizontal plaques of calcified cartilage and endochondral bone lying free in the metaphysis (Pl. 4*c*) which indicated that there had been several attempts at growth during the later periods of undernutrition before the animal was killed for examination. Bones taken from animals that had been undernourished for less than 3 months did not show these features.

The peripheral regions of the growth cartilage of the undernourished pigs (Pl. 5*b*) were more concave towards the metaphysis than the corresponding parts in normal animals of the same size (Pl. 5*a*). If the central regions of the growth cartilage showed that there had been spasmodic attempts to grow, there were always more signs of it at the periphery. In these instances the new zones 3 and 4 (of Fig. 2*B*) formed a hemispherical mass at the periphery of the growth cartilage. When this mass was invaded by marrow tissue and resorbed as in Pl. 5*c*, a mushroom-like cavity appeared below the new metaphysial surface of the growth cartilage, though it remained partially separated from the rest of the metaphysis by the remains of the original transverse plaque of calcified zone 3 and its endochondral bone. Pl. 5*b* shows that in time only this plaque remained to show what had been happening. These changes in the periphery of the growth cartilage of undernourished animals explain why, in longitudinal sections, the extremity of the metaphysis often appeared to form a cup enclosing the epiphysis.

The effect of rehabilitation on the growth cartilage. One pig that had been rehabilitated for 4 weeks doubled its body-weight in this time although the femur did not elongate appreciably. A similar increase in body-weight without resumption of skeletal growth was noticed in the other animals in which the effects of early rehabilitation were studied. During the early period of rehabilitation (Fig. 1*D* and Pl. 4*d*) there was a restoration of the normal proportions of the zones. The overall thickness increased to 1·4 mm, zones 1–3 returned to their original proportions and zone 4 reappeared. The calcified zone 3 together with the seal of endochondral bone persisted for a time, although it had been penetrated and undermined by marrow tissue. Considerable proliferation and extension of the cartilage canals, which lay largely in zone 1, indicated that the blood supply was greater than it ever was in the size controls. The perivascular connective tissue in the canals had also greatly increased and was differentiating into cartilage and contributing to the new tissue formed on rehabilitation. The normal profile of the extremity of the bone had been restored by the extensive growth of the cartilaginous epiphysis. The perichondrial zone (Pratt, 1959) contained large numbers of connective tissue cells, which were differentiating into chondroblasts. Perichondrial bone formation had recommenced. The peripheral part of the growth cartilage had become thicker and showed greater activity than the central portion. These relative activities resembled those in bones in which there had been abortive attempts to grow during undernutrition. Plate 5*d* shows that the metaphysial surface of the peripheral part of the cartilage was still concave and the space below it filled with trabeculae of newly formed endochondral bone. These were still separated from the medullary cavity by the calcified plaque formed from the remains of the original metaphysial surface of the undernourished growth cartilage. The localized nature of these changes confirmed the result of measurements made at the same time, both showing that there had not yet been any appreciable growth in length of the femur.

Thus normal structure was restored when the animals were allowed as much food as they would take. The bones then elongated after the initial delay and ultimately attained proportions approaching, or equal to, those of normal mature animals. There were no abnormalities in the structure of rehabilitated growth cartilages.

The metaphysial spongiosa during undernutrition and rehabilitation. In a pig that had been undernourished for only 6 weeks (Pl. 3*b* and Fig. 1 *B*) the spongiosa adjacent to the growth cartilage was dense and was responsible for the radiological appearance of the line of arrested growth. The spongiosa in the metaphysis of all the other undernourished animals was as extensive in its distribution, though the trabeculae were fewer and thinner (Pl. 3*d*) when compared with their size controls (Pl. 3*a*) and were orientated predominantly in the long axis of the bone. The bone matrix had a normal structure with regard to the arrangement of its collagen fibres.

These trabeculae in normal young animals were covered with a mantle of active osteoblasts, and osteoclasts were plentiful. In animals undernourished for 6 weeks there were still signs of osteogenesis and osteoclasis but soon after this osteoclasts became rare and the trabeculae were sparsely covered with inactive spindle-shaped cells. The persistence of the cartilage matrix within the spongiosa indicated that its reorganization had been limited during the remodelling of the trabeculae which was described in the radiological section.

Larger, active, osteoblasts appeared amongst the spongiosa with rehabilitation, and bone was deposited on both the older and more newly formed spongiosa. In this way the trabeculae became thicker as in the normal ageing process.

The epiphysis during undernutrition and rehabilitation. Undernutrition did not prevent the enlargement of the epiphysial centre of ossification with a concomitant reduction in the cartilaginous epiphysis. Most of this growth took place adjacent to the articular surface, which is where it takes place during normal growth (Payton, 1933). The terminology used by Barnett, Davies & MacConaill (1961) to describe the structure of the articular cartilage has been used to describe the whole of the cartilaginous epiphysis, longitudinal sections of which are shown in Pl. 6 at similar degrees of magnification.

The superficial tangential stratum which was confined to the articular surface appeared unaffected by undernutrition. The underlying intermediate stratum of the cartilaginous epiphysis was not so deep in the undernourished animals as in their size controls. The number of cells in it, however, was greater and they tended to lie in pairs without further orientation. One effect of this reduction of the intermediate stratum was to narrow that part of the cartilaginous epiphysis which lay adjacent to the growth cartilage (Pl. 3*d*) and this made the latter appear radiologically narrower than it really was.

The deeper radiate stratum of the normal cartilaginous epiphysis (Pl. 6*a*) where the cell nests were arranged along radial axes, though not in an orderly manner as in the growth cartilage, was also reduced by undernutrition. In the fully undernourished animals, moreover, this stratum had a characteristic structure which resembled that of their growth cartilage, in that there was a loss of zones 4 and 5 and calcification of the matrix of zone 3 (Pl. 6*b*). In the epiphysis, however, this calcification was not so complete, and islands persisted containing groups of partially hypertrophied cells lying in an imperfectly calcified matrix. There were also changes in some of the under-nourished animals resembling the limited attempts to grow already described in the growth cartilage. These were found both in animals that did and in animals that did not show such changes in the sections of their growth cartilage. In other animals these localized attempts to grow were extensive enough at certain points in the epiphysis to give appearances reminiscent of the mushroom-shaped areas seen at the periphery of some of the growth cartilages (Pl. 6*c*). These localized attempts to grow will explain the crenated appearance of the epiphysial outlines in radiographs (Pl. 1*c* and 2*a*). None of these appearances were found in animals undernourished for 6 weeks, indicating that normal growth continued, though at a reduced rate, in the epiphysis during the early stages of undernutrition.

The epiphysial spongiosa in the bones of the undernourished animals was as extensive as in normal bones of the same length, but the trabeculae were thinner. The plaque of bone normally deposited adjacent to the epiphysial aspect of the growth cartilage as growth slows down was not formed during the period of undernutrition.

After 4 weeks of rehabilitation there was a return to normal structure, and Pl. 6*d* shows the reappearance of fully hypertrophied cartilage cells, their invasion by marrow tissue and the reappearance of large numbers of active osteoblasts. Thus, as in the growth cartilage, a period of reconstruction and restitution preceded the general renewal of growth.

DISCUSSION

The growth of skeletal tissues in very young animals is not immediately suppressed with the onset of adverse conditions such as acute starvation (Jackson, 1932), and Walker, Simpson, Asling & Evans (1950) noted that growth continued at 60% of the normal rate for 3 weeks after hypophysectomy. In this investigation also the shaft continued to elongate and the epiphysial centres to enlarge during the early weeks of undernutrition, and these were the changes responsible for the disproportionate size of the bones when compared with the weight of the body. Similar growth occurred in the skull during this period (McCance, Ford & Brown, 1961). While growth was going on, the growth cartilage and cartilaginous epiphysis retained a more or less normal structure, but the deposition of endochondral bone in the metaphysis and epiphysis was disturbed and transverse trabeculae formed which were visible in radiographs. Similar appearances were called 'lines of arrested growth' by Harris (1931*a*, *b*), and are met with in most disturbances of the growth cartilage where growth is still continuing. Follis & Park (1952) referred to such structures as 'transverse lines'.

Our experiments show that, after the initial elongation, growth in length was almost completely arrested. At this stage the histological findings in the growth cartilage and in the cartilaginous epiphysis were: (1) a reduction in the number of hypertrophic cells and the formation of no new ones; (2) the extension of calcification into the matrix of zones which are not usually calcified, and (3) a somewhat later reduction and change in the pattern of endochondral ossification. Similar findings in the growth cartilage were described by other workers when there had been an arrest of growth after acute starvation (Harris, 1931 *a*, *b*; Acheson, 1959), hypophysectomy (Becks, Simpson & Evans, 1945), calorie deficiency (Follis, 1956), calorie deficiency together with protein deficiency (Platt & Stewart, 1962) and severe illnesses in children (Harris, 1931 *a*, *b*; Follis & Park, 1952). It would seem, therefore, that if an immature bone is unable to grow in length the growth cartilage and cartilaginous epiphysis may take on a resting state, and persist so even if the period of arrest is very prolonged, as in our experiments. The calcified cartilage in the matrix of these pigs resulting from such an arrest is the cause of a radiological phenomenon which was also called a 'line of arrested growth' by Harris (1931 *a*, *b*). Follis & Park (1952), however, called these lines 'zones of increased density' and distinguished them from the 'transverse lines' already discussed which are plates of endochondral bone lying free in the metaphysis and epiphysis.

It has now been found that, if the period of undernutrition is sufficiently severe and prolonged, these 'transverse lines' usually disappear from the long bones, during the reorganization of the metaphysial spongiosa according to the mechanical demands. Nevertheless, 'transverse lines' have persisted in animals on a high calcium intake, in which they may have been too dense to be absorbed and also in which there was less remodelling of the spongiosa such as in the epiphysis of the long bones and in bones such as the scapula and mandible which bear less weight. Harris (1939) described such lines in the latter situations in a 'starveling pig' or runt but he did not comment upon the appearances within its long bones.

Even after prolonged arrest of growth after hypophysectomy there may be some slight increase in length (unpublished account of Frank quoted by Asling & Evans, 1956). Platt & Stewart (1962) noted this also in protein-calorie deficiency, but did not discuss the mechanism. Our experiments show that the arrest of the growth of cartilage during prolonged undernutrition is also never absolutely complete but is interrupted at intervals by localized attempts to elongate, which do not appear to have been described previously. These changes are more noticeable at the periphery of the growth cartilage, which has a more direct blood supply than the rest, the latter being supplied by vessels which first pass through the epiphysis or metaphysis (Morgan, 1959). As the vascularity of the epiphysis and metaphysis is greatly reduced by undernutrition, the periphery of the growth cartilage will have a significantly richer blood supply than the rest, which probably accounts for its greater activity. The transverse trabeculae which result are distinctive histologically, but as they lie very close to the growth cartilage and may not survive long they are not always apparent in the radiographs. These attempts to grow do not individually produce any appreciable elongation of an individual bone, though collectively they will be responsible for the slight and continued increase in height that has been observed even in the severely undernourished animals. They also alter the contour of the growth cartilage, so that the base of the epiphysial centre comes to lie in a cup formed by the elongating periphery of the metaphysis. Two animals were killed when this process was taking place, but all showed some signs of it or similar changes occurring in the epiphysial cartilage, and the contour was also invariably abnormal. Somewhat similar attempts to elongate have been reported during profound undernutrition in cockerels (Pratt & McCance, 1960). These abortive attempts to grow in no way resembled the inception of growth during early rehabilitation. This involved the reconstitution of the growth cartilage, an increase in its vascularization, the multiplication of undifferentiated perivascular cells and their subsequent differentiation into chondroblasts. This process apparently took some time and, although periosteal growth was demonstrable on some surfaces of the bones by the time the animals had doubled their weights (McCance, 1962*a*, *b*), there was no measurable elongation during the first 4 weeks. A shorter stationary period of 1 week was noted in the rehabilitation of underfed cockerels (Pratt & McCance, 1961). Goldstein (1922) reported a similar delay in the inception of growth in the height of children being rehabilitated after the First World War, but this has not been commented upon by others studying the same process (Widdowson & McCance, 1954), and the children were certainly not undernourished to anything like the same extent as the pigs.

Growth took place during rehabilitation at about the normal rate. Skeletal maturation followed at the expected stage of bone development and at the appropriate bone size rather than at the expected chronological age, and was consequently very delayed in time. This is in contrast to the behaviour of the teeth which were less delayed chronologically (McCance *et al.* 1961). The final length of the bones of pigs that had been rehabilitated for 2 years certainly approached the length of fully grown bones in normal pigs. The epiphyses, however, were still unfused in both groups and the possibility of further elongation in both the normal and rehabilitated animals cannot be excluded. No histological reason, therefore, has been found to explain why the bones should not grow to their normal size even after prolonged undernutrition.

SUMMARY

1. Suckling pigs were subjected to profound undernutrition for periods up to 1 year, and they weighed after these times 3–8 kg, usually about 6 kg.

2. Radiographs revealed lines of arrested growth which were modified by time and by the location and function of the bone concerned.

3. The growth cartilage and the adjacent part of the cartilaginous epiphysis became narrow and the profile of the metaphysis changed.

4. After some weeks the growth cartilage and the radiate stratum of the cartilaginous epiphysis ceased to function effectively. The zone of fully grown cells disappeared, endochondral osteogenesis ceased, and the matrix of the zone of cell growth became calcified. Limited and localized attempts to grow, however, were demonstrable, notably near the periphery of the growth cartilage.

5. On rehabilitation, the growth cartilage at once began to recover its normal appearance and function, but measurable increase in length did not begin for about 4 weeks, after which time growth was resumed at the normal rate. Vestiges of the old metaphysis and epiphysis persisted for some time. The secondary centres fused when the bones were approximately the length at which this normally happened.

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EXPLANATION OF PLATES

Plate 1

(*a*) Lateral radiograph of the femur of a normal 3-week-old pig (weight 3·7 kg). The wide uncalcified cartilaginous zone between epiphysis and metaphysis should be noted.

(*b*) Lateral radiograph of the femur of a pig (weight 3·7 kg) that had been undernourished for 6 weeks. The lines of arrested growth (*L*) in the metaphysis should be noted.

(*c*) Lateral radiograph of the femur of a pig (weight 3·64 kg) that had been undernourished for 40 weeks. The reduction in spongiosa in the metaphysis, and the narrowing of the uncalcified cartilaginous zone between the epiphysis and metaphysis should be noted.

(*d*) Lateral radiograph of the femur of a normal pig (weight 7·23 kg) aged 4 weeks. It is the same length as the one shown in (*c*). When compared with (*a*) enlargement of the epiphysis which has taken place by this stage of development should be noted.

Plate 2

(*a*) Lateral radiograph of the femur of a pig (weight 5·68 kg) that had been undernourished for 1 year and had had extra calcium in its diet. The persistence of the lines of arrested growth (*L*) and the crenated outline of the epiphysis should be noted.

(*b*) Lateral radiograph of the femur of a pig (weight 7·05 kg) that had been undernourished 1 year and then rehabilitated for 4 weeks. It should be noted that the changes are small as compared with Pl. 2(*a*) or Pl. 1(*c*) other than the widening of the gap (arrowed) between the epiphysis and metaphysis and the dense band (*D*) limiting the metaphysis.

(*c*) Lateral radiograph of the femur of a previously undernourished pig (weight 18·4 kg) that had been rehabilitated for 11 weeks. This shows the wide zone of new spongiosa (*N*) and the persistence of the old 'line of arrested growth' (*L*).

Plate 3

Photomicrographs of longitudinal sections through the distal growth cartilage of the femur of normal and undernourished pigs. Haematoxylin and eosin. *B*, endochondral bone; *C*, cartilaginous epiphysis; *E*, epiphysis; I, intermediate stratum of *C; M*, metaphysis; *P*, periosteum; *R*, radiate stratum of *C; S*, seal of endochondral bone; *T*, tangential stratum of *C; V*, vascular tissue within cartilage canal; 1–5, zones of growth cartilage or radiate stratum of cartilaginous epiphysis.

(*a*) Control, aged 3 weeks (weight 5·14 kg) showing the structure of the normal immature growth cartilage.

(*b*) Undernourished, aged 8 weeks (weight 3·71 kg). During 6 weeks of undernutrition there has been a reduction in thickness of zones 2–5. Endochondral bone formation has continued but the spongiosa is thick.

(*c*) Undernourished, aged 9 weeks (weight 2·61 kg). When compared with (*b*) it should be noted that zone 4 has disappeared; the matrix of zone 3 is calcified but remains of zone 5 persist.

(*d*) Undernourished, aged 1 year (weight 5·68 kg), showing the reduction in thickness of both growth and epiphysial cartilages and the calcification of their outer zones. No zone 5.

Plate 4

Photomicrographs of longitudinal sections through the distal growth cartilage of the femur of normal, undernourished and rehabilitated pigs. Haematoxylin and eosin. (Key as in Pl. 3.)

(*a*) Undernourished, aged 1 year (weight 5·68 kg). Hair-line which separates the calcified zone 3 from zone 2 indicated by arrows.

(*b*) Undernourished, aged 38 weeks (weight 6·13 kg), showing changes in the growth cartilage which will result in a limited amount of growth. The reappearance of zones 3 and 4 and the multiple hair-lines (indicated by arrows) should be noted. The calcified zone 3 and the seal of endochondral bone on the metaphysial aspect are still present (see Fig. 2*B*).

(*c*) Undernourished, aged 37 weeks (weight 4·96 kg) showing changes in the growth cartilage following a limited attempt to grow. The invasion of zone 4 by marrow tissue (indicated by arrow) which has resulted in the partial separation of the old calcified zone 3 together with the seal of endochondral bone should be noted (see Fig. 2*C*). Note also 'transverse line' (*L*).

(*d*) Rehabilitated for 4 weeks, aged 55 weeks (weight 7·05 kg), showing the reconstitution of the zones of the growth cartilage. The seal of endochondral bone and the old calcified zone 3, which is still persisting, should be noted.

PLATE 5

Photomicrographs of longitudinal sections through the periphery of the distal femoral growth cartilage of normal, undernourished and rehabilitated pigs. Haematoxylin and eosin. (Key as in Pl. 3.)

(*a*) Control aged 4 weeks (weight 7·23 kg), showing the normal profile and structure of the peripheral region of the immature growth cartilage.

(*b*) Undernourished, aged 38 weeks (weight 6·13 kg). The growth cartilage had recently undergone the changes seen in (*c*) but is now inactive and similar to that seen in Pl. 3*d*. The remains of the calcified zone 3 of the earlier state of growth cartilage (indicated by arrow) should be noted.

(*c*) Undernourished, aged 37 weeks (weight 4·96 kg). The growth cartilage is showing evidence of localized attempts to grow. The extensive mushroom-shaped area showing hypertrophic changes which is being invaded by marrow tissues and limited on the metaphysial aspect by the remains of the calcified zone 3 (indicated by arrow) should be noted.

(*d*) Rehabilitated for 4 weeks, aged 55 weeks (weight 7·05 kg). The mushroom-shaped mass of endochondral bone limited on the metaphysial aspect by the remains of the calcified parts of the growth cartilage together with its bony seal (indicated by arrow) should be noted and also the very active growth cartilage.

PLATE 6

Photomicrographs of longitudinal sections through the distal articular part of the femoral cartilaginous epiphysis of normal, undernourished and rehabilitated pigs. Haematoxylin and eosin. (Key as in Pl. 3).

(*a*) Control, aged 3 weeks (weight 5·14 kg), showing the structure of the immature articular cartilage.

(*b*) Undernourished, aged 38 weeks (weight 6·13 kg). Some narrowing of the intermediate stratum, the loss of zones 4 and 5 and the calcification of zone 3 of the radiate stratum should be noted.

(*c*) The epiphysial cartilage of the bone shown in Pl. 4*c* showing similar invasive changes by marrow tissue (indicated by arrows).

(*d*) Rehabilitated for 4 weeks, aged 55 weeks (weight 7·05 kg.) The radiate and intermediate strata have returned to their original state.

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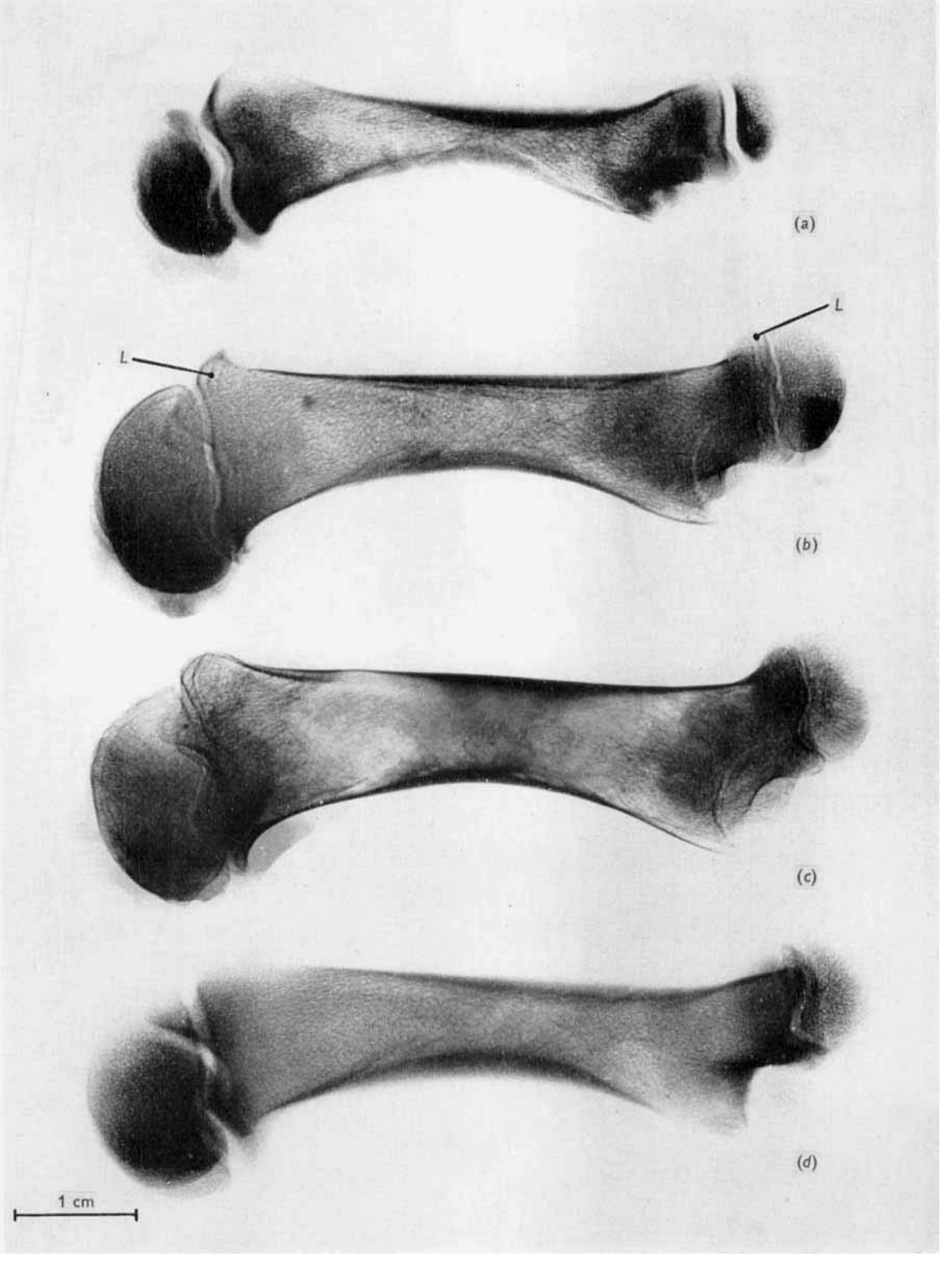
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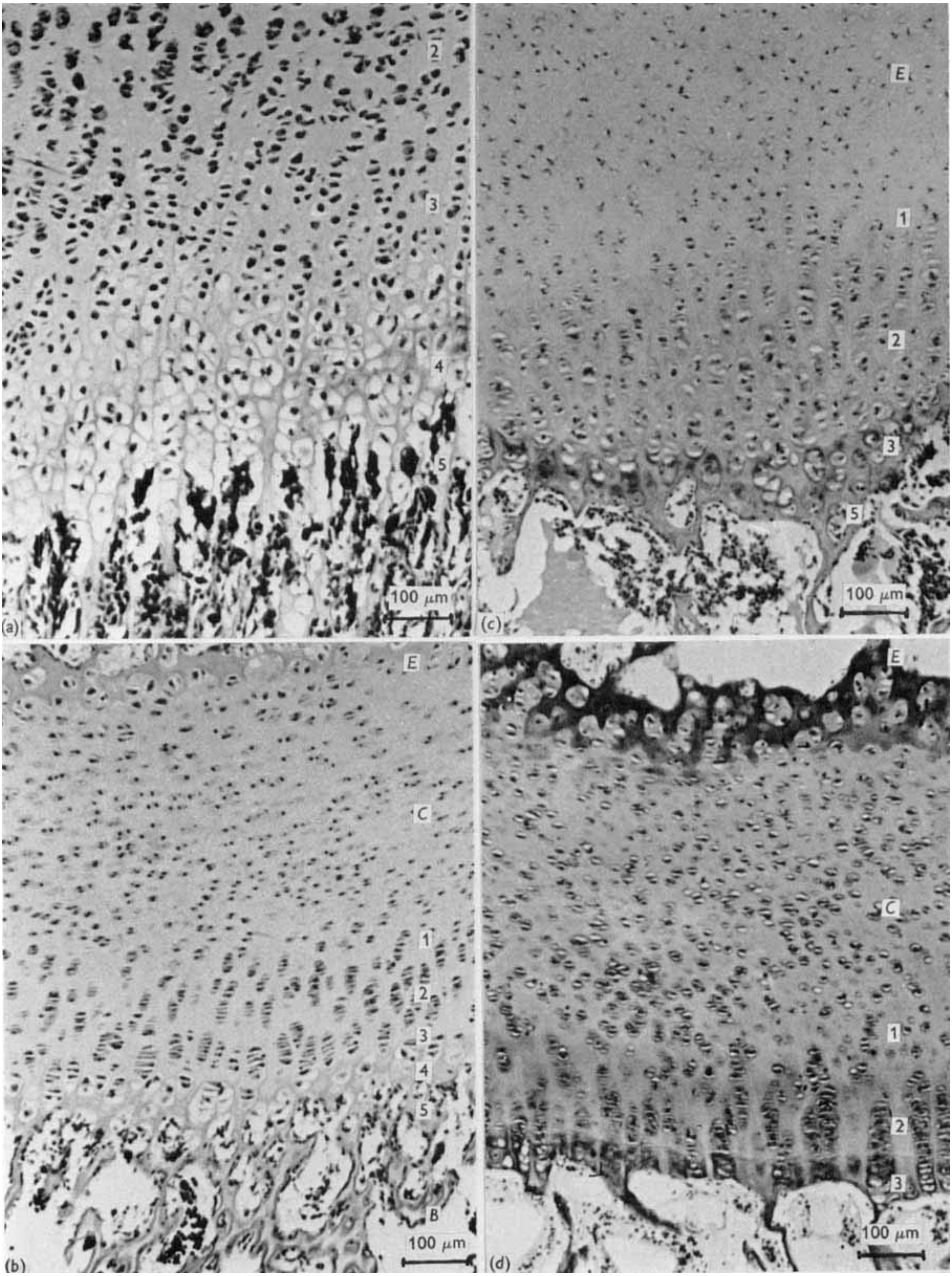

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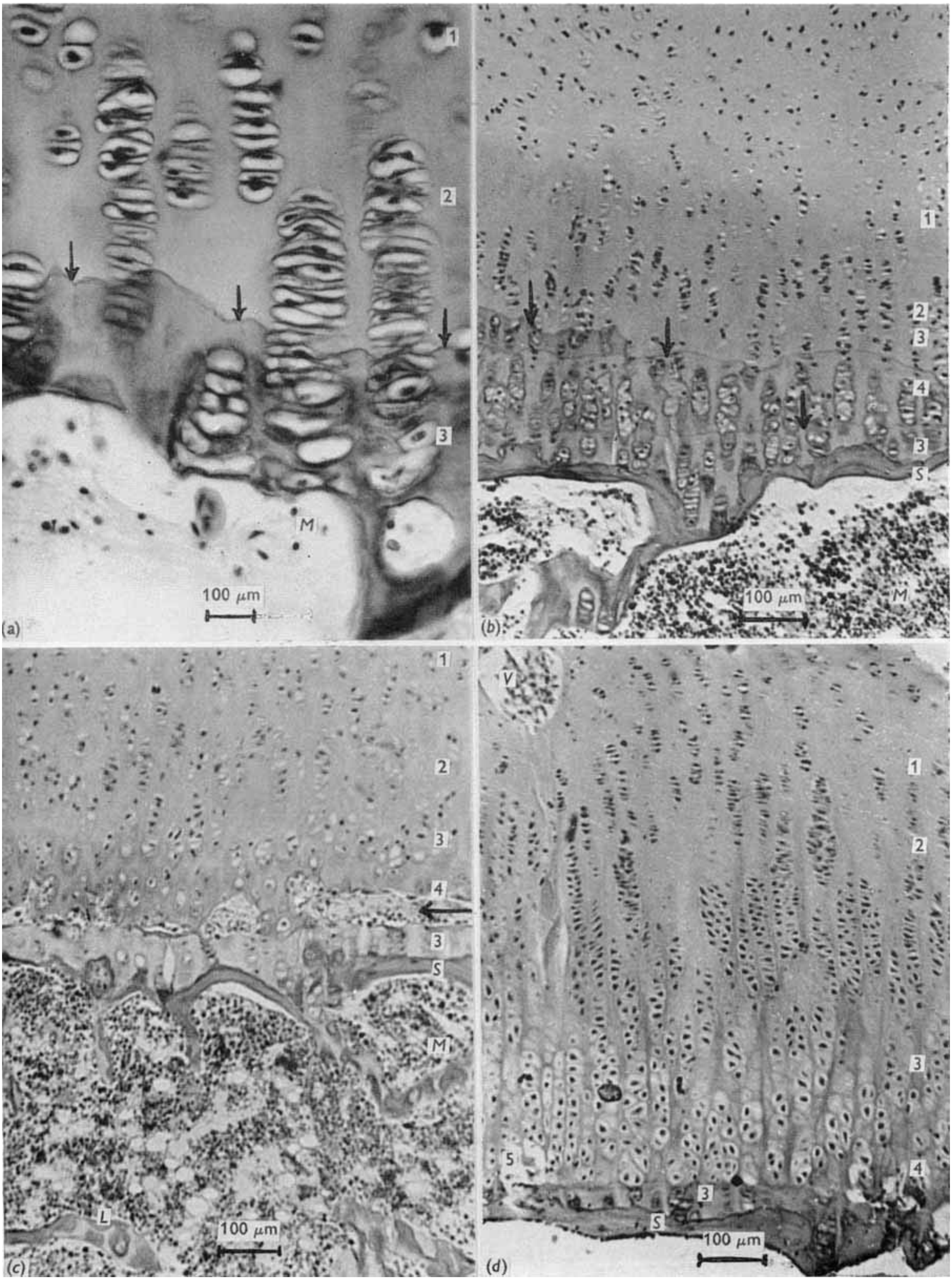

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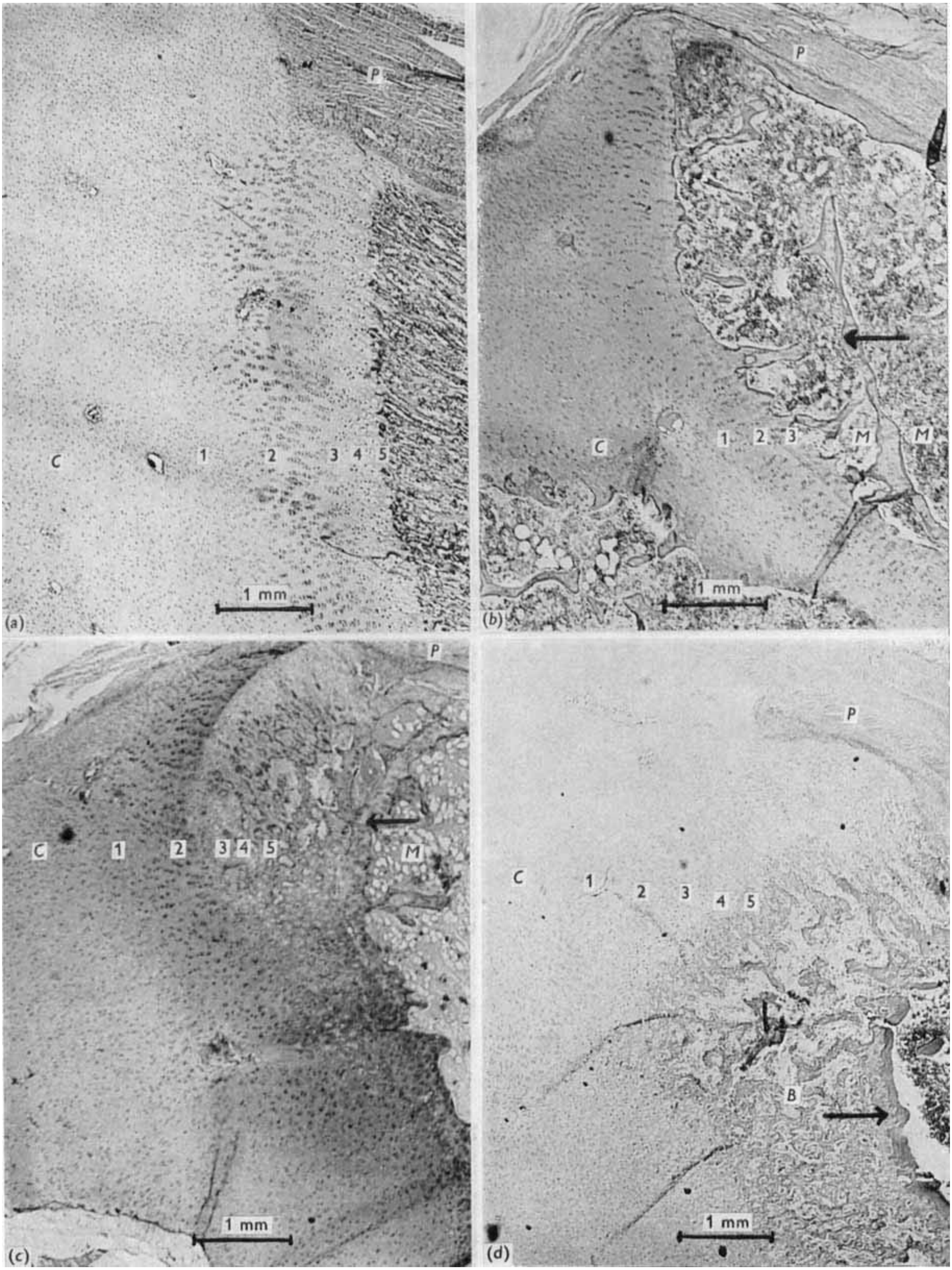

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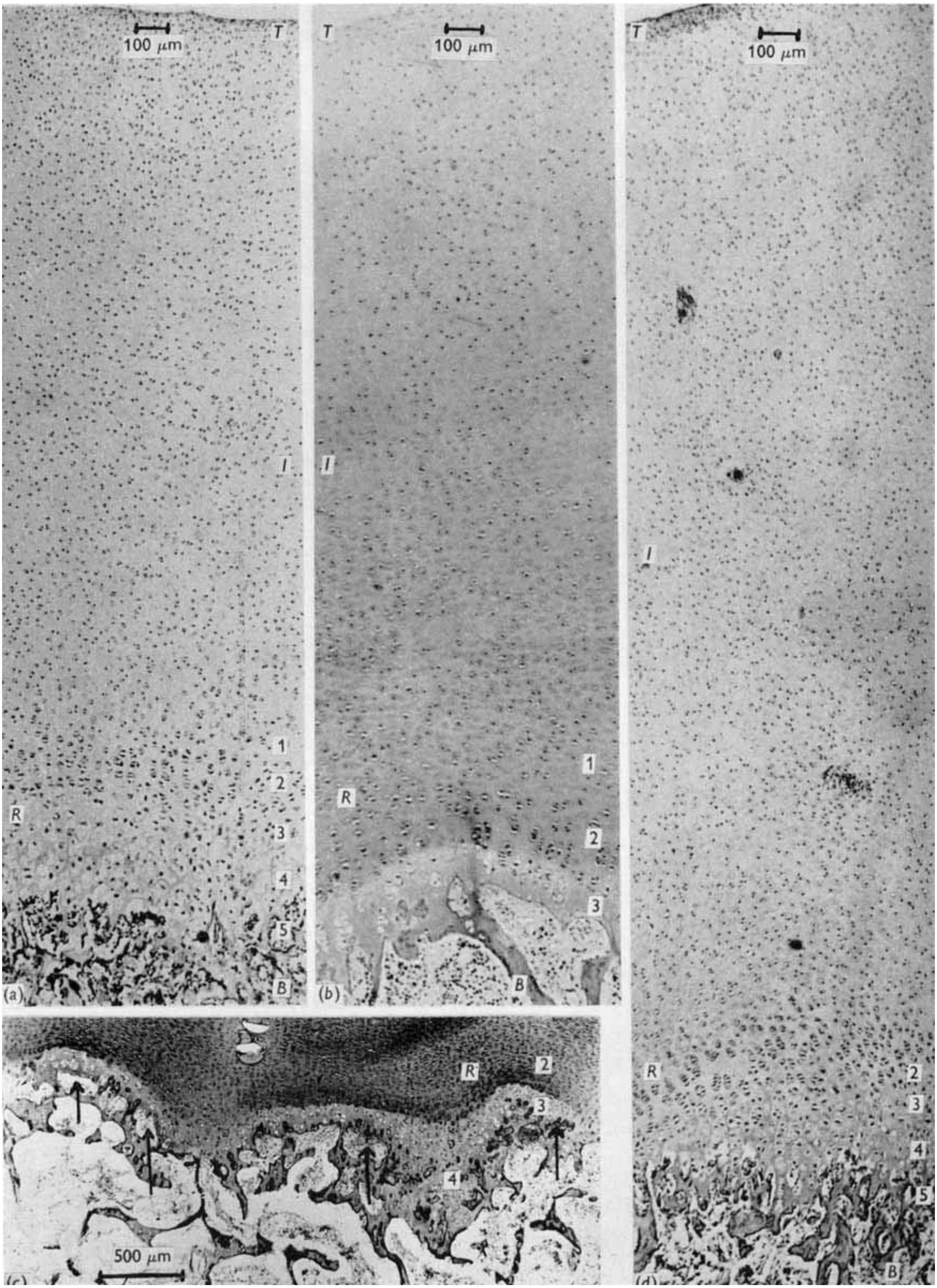

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