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The blood supply of the growth cartilage in young rats

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INTRODUCTION

There has been a renewal of interest in recent years in both the distribution and functional importance of blood vessels in bone. Numerous workers have reinvestigated the histology and distribution of these vessels, and their results have led to a re-appraisal of previously established concepts of the osseous circulation and its relation to osteogenesis (Brookes, Elkin, Harrison & Heald, 1961; Trueta & Morgan, 1960; Crock, 1962; Trueta, 1963).

It is usually held that the blood supply in the region of the growth cartilage (syn. epiphysial cartilage) arises from the epiphysial and metaphysial vessels, although Morgan (1959), and McLean & Urist (1961) described in addition an anastomotic ring of blood vessels around the periphery of this cartilage.

It is also generally accepted that the metaphysial surface of the growth cartilage is supplied by vessels from both the principal nutrient artery and perforating metaphysial arteries, the former supplying the central part of the cartilage whilst the latter vascularize its peripheral part (Grégoire & Carrière, 1921; Reichel, 1947; de Marneffe, 1950). Lexer, Kuliga & Turk (1904) differ from this viewpoint and claim that the growth cartilage is supplied only by metaphysial arteries, whilst Hinkel (1943) attributes the supply to the periosteal arteries alone.

Additional to these morphological investigations Stump (1925), Dahl (1937), and Lewis (1956) have commented upon the relation between the metaphysial vessels and osteogenesis. More recently Trueta & Morgan (1960) conclude that the epiphysial vessels are primarily responsible for the nutrition of the growth cartilage, whilst the vessels on the metaphysial surfaces are concerned with endochondral ossification. Their views have been substantiated by later studies (Trueta & Little, 1960; Trueta & Amato, 1960; Trueta & Trias, 1961) and confirmed by Briant, Dale & Harris (1961) and Dale & Harris (1958).

Investigations on the vascularity of the growth cartilage itself have resulted in conflicting reports. Harris (1929), Nussbaum (1923) and Lewis (1956) consider that the cartilage is avascular and that there are no vascular communications between the vessels of the epiphysis and the metaphysis through the substance of the cartilage. Lexer *et al.* (1904), Bidder (1906) and Gill (1940), however, state that the epiphysial and metaphysial circulations are connected by vessels traversing the growth cartilage. Bidder (1906) believes that they are important in the nutrition of the growth cartilage and for the ossification of epiphyses. Tilling (1958) has also demonstrated comparable vessels in both human and bovine material.

The present investigation on the blood supply in the region of the growth

cartilage of the young rat has been carried out prior to investigating the circulatory changes which take place in the region of the growth cartilage in experimentally induced rickets.

MATERIAL AND METHODS

The investigations were carried out on 25 genetically heterogeneous albino rats of both sexes during the first 2 months of life. The growth cartilage principally investigated was that situated at the proximal end of the tibia. This cartilage was almost flat and of optimum size for investigating vascular arrangements in both the epiphysis and metaphysis.

The vessels were examined by injecting either India ink, for later study by clearing techniques and histology, or Micropaque (Damancy and Co.) and Thorotrast, followed by radiography and microradiography.

The media were injected into the left ventricle of the anaesthetized animal, adequate filling of the vessels being assessed by blackening of the paws and external nares when India ink was used, and by satisfactory filling of the vessels on the gut wall when Thorotrast and Micropaque were employed.

Material removed after injection was decalcified in 5% formol nitric acid and embedded in either paraffin wax or low-viscosity nitrocellulose (Chesterman & Leach, 1949). Specimens injected with India ink and embedded in low-viscosity nitrocellulose were sectioned at 250 μ and cleared by a modification of the Spalteholz technique. Other specimens similarly embedded but injected with Thorotrast and Micropaque were sectioned at 600 μ and subsequently examined by microradiographic techniques. Some paraffin sections were also cut at 12 μ and stained with haematoxylin and eosin.

In order to clarify the description of the results which follows, the terminology suggested by Ham & Leeson (1961) is used throughout in this paper. The growth cartilage is divided into four zones, the zones of resting, proliferating, maturing and calcifying cartilage, in order, from the epiphysial to the metaphysial surfaces (Pl. 1, fig. 1).

RESULTS

The vascular arrangements of the metaphysis

When observed under low-power magnification the metaphysis contains a dense vascular network simulating an inverted cone, and termed the metaphysial cone (Pl. 1, fig. 2). The base of this cone is in apposition with the metaphysial surface of the growth cartilage, and has a brush-like border.

The arteries of the metaphysis are derived from two sources, first from the principal nutrient artery, and secondly from large periosteal arteries. The latter pierce the cortex in the metaphysial region and form U-loops in the cortex before reaching the medullary cavity where they accompany branches of the principal nutrient artery (Pl. 1, fig. 3). The perforating metaphysial arteries are distributed principally to the peripheral parts of the growth cartilage in contrast to the principal nutrient artery which supplies the central part.

The venous component of the vascular network consists of numerous dilated and tortuous veins which anastomose irregularly. The venous plexus receives tributaries

from the vessels on the metaphysial surface of the growth cartilage and terminates in the central venous sinus as well as communicating with the extra-osseous circulation through emissary veins which traverse the cortex of the metaphysis.

The blood supply of the growth cartilage

Since the growth cartilage is an undulating structure the vessels related to it follow its contours. Three groups of vessels contribute to its blood supply.

(1) On the epiphysial surface of the growth cartilage branches of the epiphysial arteries pierce the bone plate of the epiphysis, which lies on the epiphysial surface of the growth cartilage, and divide to form a network beneath it. The terminal branches of these arteries come into relationship with the cells in the zone of resting cartilage. The veins which drain this network pass back through the bone plate of the epiphysis, either through the same foramina as the arteries entered, or through separate openings (Pl. 1, fig. 5).

(2) On the metaphysial surface the arteries form a brush-like border where they are related to the growth cartilage (Pl. 1, fig. 4). These arteries, which do not anastomose, are disposed vertically between the longitudinal intercolumnar bars of calcified cartilage that persist to form a framework in the metaphysis (Pl. 2, fig. 6), and terminate as inverted U-loops related to the lowest transverse intercellular partition of the growth cartilage (Pl. 2, fig. 7). The vascular loops are not uniform in diameter but exhibit a sinusoidal dilatation at their termination from which the descending limb, itself wider than the ascending limb, returns to the metaphysial cone.

A detailed examination of these vessels is necessary in order to understand their relationship to endochondral ossification. It is observed that each metaphysial vessel is usually related to a column of chondrocytes in the growth cartilage, though more than one column and the intervening matrix may be eroded by a single vessel.

The transverse intercellular partitions are eroded whilst the longitudinal intercolumnar matrix is left as a scaffolding upon which the osteoblasts deposit the first trabecular bone. The advancing vessels are separated from the last chondrocyte nucleus in the zone of calcifying cartilage by the clear cell which contains neither nucleus nor cytoplasm (Pl. 2, fig. 7). When the transverse bar of this cell is eroded by the metaphysial vessels, the nucleus and cytoplasm of the chondrocyte next above shrink and disappear.

An accurate interpretation of the processes which result in the erosion of the transverse intercellular bar is difficult, since conclusions have to be drawn about an active process occurring in the living animal from histological preparations showing only one particular phase. In any one preparation, however, not all the cells are at the same stage of penetration, and tentative observations may, therefore, be made on the various stages in this process.

In many sections, where the transverse intercellular bar is still intact, an ill-defined slightly eosinophilic material may be observed linking the bar to the advancing blood vessel. This is followed by the thinning of the bar and its subsequent perforation and complete erosion, the vessel entering into the empty cell (Pl. 2, fig. 9). Immediately after penetration has occurred the type of cell seen in the empty chondrocyte varies. The space is sometimes filled with erythrocytes whose arrangement suggests that they are enclosed in a limiting membrane, although such is not

apparent on microscopical examination (Pl. 2, fig. 10; Pl. 3, fig. 11). However, in specimens showing injected U-loops, it is sometimes possible to see buds arising from the dilated part of the loop and passing into the empty cell (Pl. 2, fig. 8), an appearance that strengthens the impression that the erythrocytes within the empty cell are membrane-enclosed. Occasionally the erythrocytes are scattered throughout the empty cell, and this may indicate that physiological rupture of the endothelial protrusion has occurred, thereby establishing an open circulation at this point. However, the most common finding is either a marked reduction or absence of erythrocytes in the clear cell, and their replacement by cells of various shapes and staining reactions, though they are usually spindle-shaped and have basophilic nuclei and eosinophilic cytoplasm. These cells are in close apposition and grow into the clear cell as a group in the centre of which lies the blood vessel. These cells are identical with those which surround the vessel in the metaphysis and are considered to produce the osteoblasts which lay down bone on the trabeculae.

Osteoclasts are not observed in the region of the advancing metaphysial vessels related to the lowest calcified transverse intercellular partition of the growth cartilage although their presence in the lower regions of the metaphysis is undisputed.

(3) The peripheral vascular ring of the growth cartilage is an anastomosing network of blood vessels of capillary size around the periphery of the growth cartilage, most clearly visible at the costochondral junction. The vessels of this ring are derived from the periosteal network and run slightly medially at the level of the metaphysial border of the growth cartilage, to anastomose and form a network closely applied to the periphery of this cartilage (Pl. 3, figs. 12–14).

In the present investigation no vessels have been observed to pierce the growth cartilage and connect the epiphysial and metaphysial circulations. The growth cartilage of the normal rat (up to the age of 8 weeks) can, therefore, be said to constitute a complete barrier between the epiphysial and metaphysial vessels.

DISCUSSION

It has been shown that the growth cartilage derives its blood supply from three sources, the vessels of which vary in their termination. Those on the epiphysial side of the growth cartilage having pierced the epiphysial bone plate end in relation to the cells situated in the zone of resting cartilage. The present findings are in agreement with those of Trueta & Morgan (1960) regarding the comparatively rich blood supply to this area. The second group of vessels consists of a network of capillaries derived from the periosteal vessels which form an anastomosis around the periphery of the growth cartilage.

The third group lies on the metaphysial side of the growth cartilage and arises both from the perforating metaphysial and also from the principal nutrient arteries; after repeated division the arteries approach the columns of chondrocytes as straight end-arteries. Trueta & Morgan (1960) said that they resembled the vasa recta spurea of the kidney though differing from the latter in that no anastomoses occurred between them.

The termination of the metaphysial arteries as inverted U-loops in relation to the clear cells of the chondrocyte columns has been previously observed by Ranvier (1875), Doan (1922), Morgan (1959) and Brookes & Lloyd (1961).

Trueta & Morgan (1960) consider that histologically it is not possible to distinguish the ascending from the descending limb of the loop; however, microscopical examination of many specimens shows that frequently one limb of the U-loop is narrower than the other, and in those specimens in which isolated loops have been injected the narrow limb is continuous with the metaphysial arteries. This suggests that the descending limb has the wider calibre.

A similar sequence of events to propagation of a growth cartilage is seen in the costal cartilage of the young rat where actively dividing and growing chondrocytes are present at the periphery of the cartilage lying deep to the rich perichondrial vascular plexus. In the centre of the cartilage are the larger more mature cells which degenerate with advancing age, the intercellular matrix undergoing calcification. A comparison between these sites suggests that the function of the vessels on the epiphysial surface of the growth cartilage is to nourish the chondrocytes in this structure, particularly those which are undergoing active division and growth.

Trueta & Amato (1960) have shown that interruption of the vessels on the epiphysial side of the growth cartilage leads to necrosis of its cells, and Dale & Harris (1958) observed that when separation of the epiphysis occurs the growth cartilage remains viable if the epiphysial vessels are intact. By contrast, when these vessels are divided the growth cartilage undergoes necrosis; these observations have been confirmed by Ham & Leeson (1961).

Brodin (1955), on the basis of experiments in which he administered fluorescent substances, claims that the metaphysial vessels are primarily responsible for the nourishment of the growth cartilage because these substances diffuse into the cartilage mainly from the metaphysial surface. On the other hand, Dale & Harris (1958) and Ham & Leeson (1961) have shown that the growth cartilage remains viable even when it is separated from the metaphysial vessels.

Foster, Kelly & Watts (1951), who have performed experiments in which the periosteum is stripped from the bone, and the principal nutrient artery interrupted, state that when infarction involves the metaphysial surface of the growth cartilage, the latter does not necrose but, on the contrary, increases in thickness four to five times. More recently Trueta & Amato (1960) have similarly demonstrated that interruption of the metaphysial vessels does not cause destruction of the chondrocytes in the growth cartilage but predisposes to an accumulation of these cells because erosion can no longer occur. There is an absence of calcification of the matrix associated with this cellular accumulation which indicates that interference with the metaphysial circulation is responsible.

The considerable diffusion that Brodin (1955) has shown to occur into the growth cartilage from the metaphysial vessels is understandable because of the greater vascularity of the metaphysial surface. The fact that diffusion occurs to a greater extent at the metaphysial surface of the growth cartilage does not necessarily imply that the chondrocytes are normally nourished in this manner, though the calcium salts and vitamin D necessary for calcification undoubtedly are gained from metaphysial diffusion. The maturing chondrocytes may simply be biochemically incapable of metabolizing the other constituents of the metaphysial diffusate.

It would be irrational to suggest that the metaphysial vessels are not concerned primarily with the nourishment of the growth cartilage by arguing that they fail

to allow diffusion of the metabolites necessary for the nutrition of the chondrocytes, for they are able to nourish the mesenchymal cells that surround them, in the metaphysis.

The present results agree with those of other investigators in supporting the view that the metaphysial vessels are not concerned primarily with the nourishment of the growth cartilage, which is nourished from the epiphysial surface. But as indicated above the observations made by Brodin (1955) are capable of a different interpretation from that put upon them by that author.

A consideration of the fine capillaries that form a ring around the periphery of the growth cartilage seems relatively unimportant on first reflexion. These small vessels are difficult to demonstrate with injection techniques, and in the past the majority of workers have not described them; Morgan (1959) and McLean & Urist (1961), although referring to these capillaries, do not illustrate them.

Trueta & Amato (1960) observed that when all the epiphysial blood supply is eliminated, a rapid necrosis of the central parts of the growth cartilage ensues. The only area not so affected is at the periphery of the growth cartilage, where the blood supply comes from a 'vascular anastomosis around its border'. These vessels are not mentioned later in their paper when they refer only to the epiphysial and metaphysial vessels as supplying the growth cartilage.

Brodin (1955) noted some diffusion of fluorescent injection medium into the growth cartilage from its periphery. With the exception of these experiments, no work appears to have been performed on the contribution of this peripheral vascular ring to the nutrition of the growth cartilage.

Some appreciation of its importance can be gained by examination of the growth cartilage of a rib, which is the most rapidly growing growth cartilage in the rat, although it has no vessels or cartilage canals on its epiphysial surface in the young rat. The explanation probably lies in the fact that in the young rat diffusion distances are small, and sufficient nourishment can be afforded by the perichondrial plexus and the peripheral vascular ring.

The process of erosion of the transverse intercellular partitions of the calcified chondrocytes is of interest. In the normal growth cartilage the inverted U-loop in which the metaphysial vessels terminate is a dilated structure consisting only of endothelium with no surrounding muscular or adventitial coat; it is regarded as a sinusoidal advancing edge of the metaphysial vessel. Although a large number of histological preparations of normal bone have been examined, no osteoclasts have ever been observed between the blood vessel and the calcified transverse bar. It must be admitted that the amorphous eosinophilic material which is often present between the advancing blood vessel and the transverse intercellular bar may be the cytoplasm of such cells, the nuclei of which are not shown because of the plane of the section, although this is unlikely, in view of the many sections examined. Such cells are seen quite clearly in a similar position in histological preparations of rachitic bone (Irving, 1964).

These observations support the contention of Cameron (1961) that absorption of calcified material can take place around capillary walls in the absence of osteoclasts. Storey (1955) has arrived at similar conclusions in his work on the absorption of bone under pressure.

It is suggested that the vascular endothelium itself is responsible for the erosion of the calcified transverse intercellular partition, a concept that is supported by the work of Macklin & Macklin (1920), Sabin (1920) and Cunningham (1922), all of whom demonstrated the phagocytic potentiality of vascular endothelium. There is no evidence that these endothelial cells are converted into osteoclasts as a preliminary to erosion as suggested by Trueta (1963).

SUMMARY

1. The growth cartilage is surrounded by three groups of blood vessels, all of which have different terminations in relation to the chondrocytes.

2. The metaphysial vessels end in relation to the clear cell of the growth cartilage as dilated sinusoids, shaped like an inverted U. Evidence is presented that outpouchings from these endothelial structures are responsible for the erosion of the calcified transverse intercellular partitions of the clear cells of the growth cartilage, without the intervention of osteoclasts. It is considered that the circulation at the cartilage-metaphysis junction remains closed during the ingrowth of the metaphysial vessels.

3. The nutrition of the growth cartilage is primarily a function of the epiphysial arteries and the vessels of the peripheral vascular ring. Emphasis is laid upon the latter as a source of nourishment to the growth cartilage. In the case of the growth cartilage of a costal element it appears to be the main source of nourishment.

4. The principal function of the metaphysial vessels is in relation to osteogenesis, and they are of less importance as a source of nourishment to the chondrocytes. It is postulated that maturing and calcifying chondrocytes cannot metabolize the nutritive products which diffuse from the metaphysial vessels.

5. The normal growth cartilage in the rat up to the age of 8 weeks is completely avascular and constitutes a barrier between the epiphysial and metaphysial circulations.

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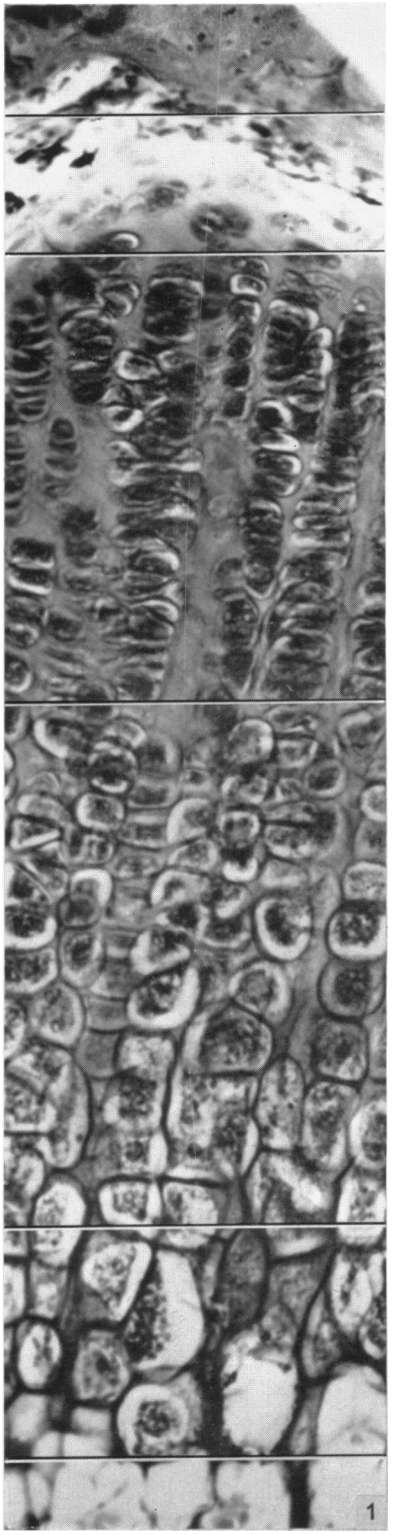

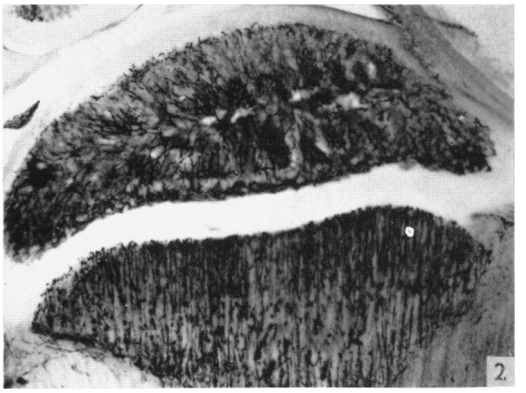

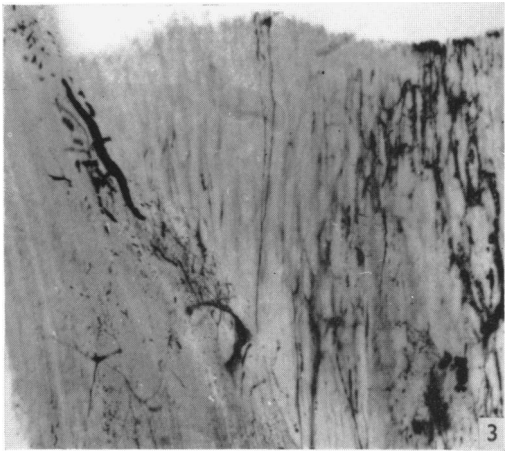

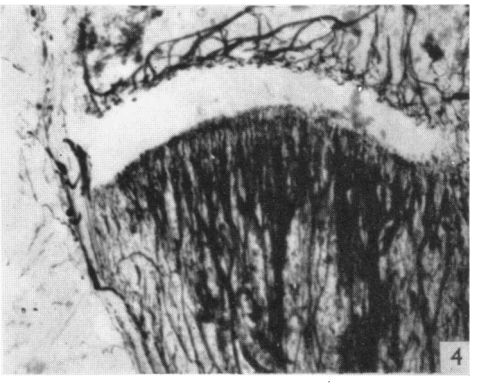

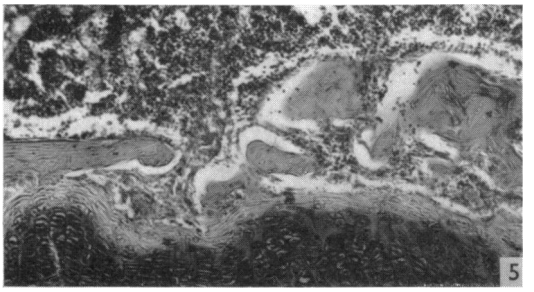

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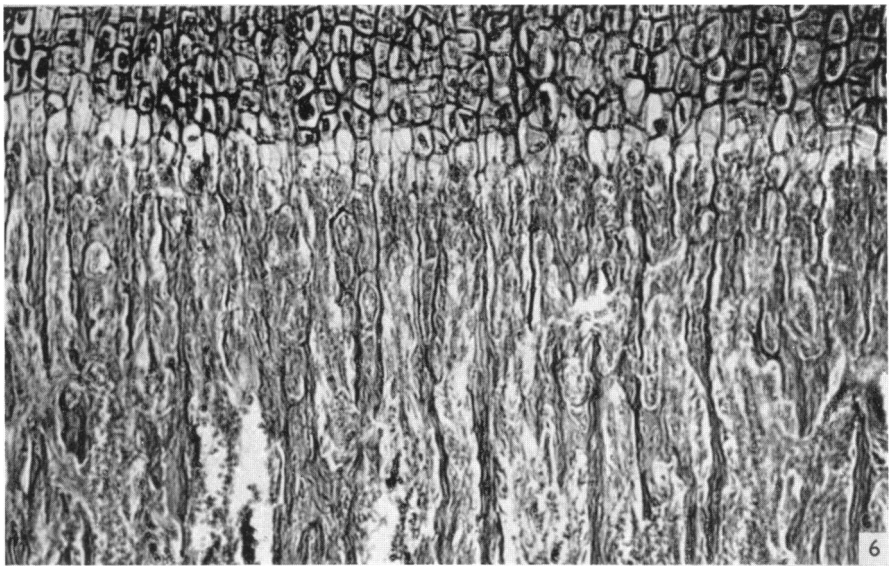
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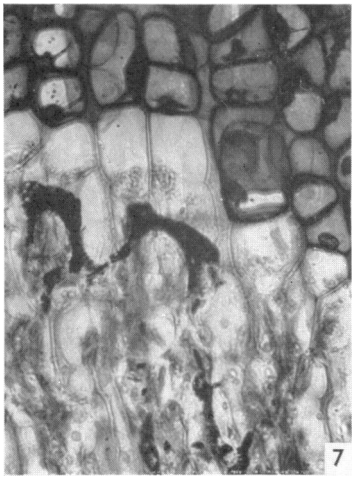
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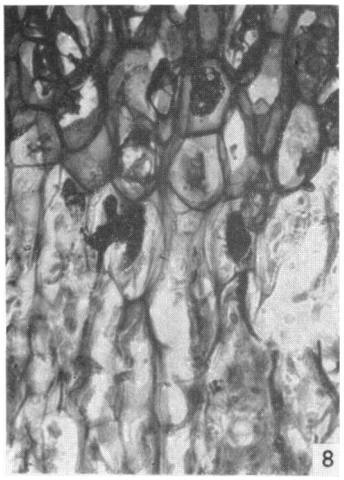
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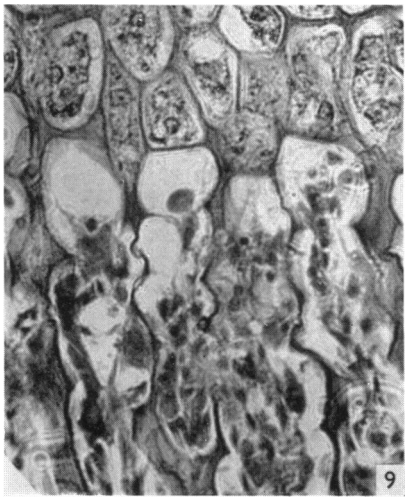
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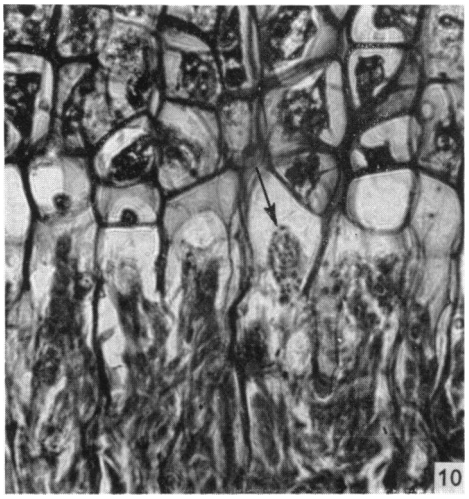
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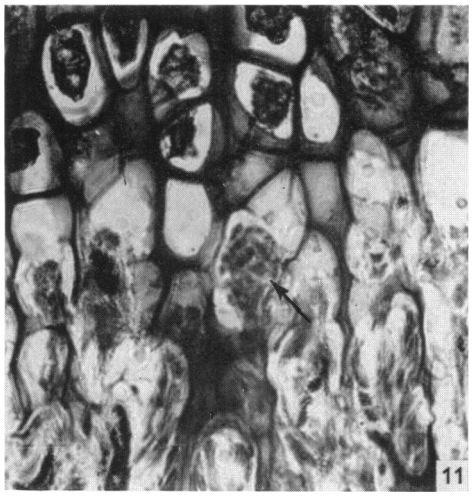
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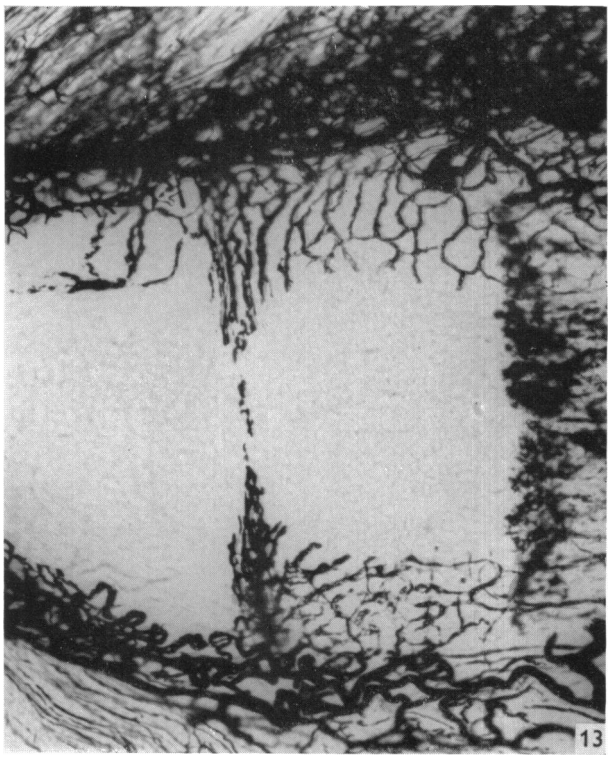
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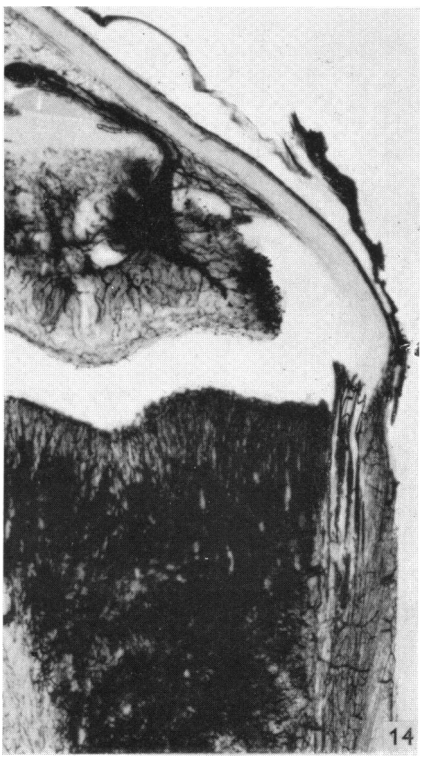
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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Microphotograph of a longitudinal section of the normal proximal tibial growth cartilage in a 4-week-old rat demonstrating the different zones. Haematoxylin and eosin, × 300.

Fig. 2. Spalteholz preparation demonstrating metaphysial cone, and epiphysial network separated by avascular growth cartilage. Four-week-old rat vessels injected with India ink, × 14.

Fig. 3. Spalteholz preparation of an isolated perforating metaphysial vessel terminating in a dilatation at the metaphysial surface of the growth cartilage. Four-week-old rat vessels injected with India ink, × 24.

Fig. 4. Microradiograph demonstrating differing manner of termination of epiphysial vessels in relation to growth cartilage compared with metaphysial vessels. Three-week-old rat vessels injected with Thorotrast and Micropaque, × 20.

Fig. 5. Microphotograph showing vessels penetrating bone plate of epiphysis to lie in relation to the zone of resting cartilage cells. Four-week-old rat. Haematoxylin and eosin, × 80.

PLATE 2

Fig. 6. Microphotograph of the metaphysis in a 5-week-old rat showing the metaphysial vessels arranged longitudinally between the trabeculae. Haematoxylin and eosin, × 156.

Fig. 7. Microphotograph demonstrating the inverted U-loop terminations of the metaphysial arteries in relation to the clear cell of the growth cartilage. Six-week-old rat. Haematoxylin and eosin, × 300.

Fig. 8. Microphotograph showing a saccular extension arising from the upper surface of a U-loop termination. Six-week-old rat. Haematoxylin and eosin, × 300.

Fig. 9. Microphotograph of the moment of breakthrough of a metaphysial vessel into the clear cell. Six-week-old rat. Haematoxylin and eosin, × 300.

Fig. 10. Microphotograph at the cartilage-metaphysis junction of a 6-week-old rat, showing formation of erythrocytes (indicated by arrow) that suggests they are enclosed in a limiting membrane. Haematoxylin and eosin, × 300.

PLATE 3

Fig. 11. Microphotograph at the cartilage-metaphysis junction of an 8-week-old rat, showing formation of erythrocytes (indicated by arrow) that suggests they are enclosed in a limiting membrane. Haematoxylin and eosin, × 300.

Fig. 12. Spalteholz preparation demonstrating the peripheral vascular ring of the growth cartilage in the rib of an 8-week-old rat. Vessels injected with India ink, × 8.

Fig. 13. Microphotograph of an incomplete peripheral vascular ring of the growth cartilage of an 8-week-old rat, showing in more detail the vessels of which it is composed. Vessels injected with India ink, × 33.

Fig. 14. Spalteholz preparation showing in longitudinal section the vessels of the peripheral vascular ring of the growth cartilage of the tibia in a 5-week-old rat. Vessels injected with India ink, × 15.