SEQUELAE OF EXPERIMENTAL PARTIAL ISCHAEMIA IN LONG BONES OF THE RABBIT

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Previous studies on experimental ischaemia of bone have tended to be restricted to an examination of ensuing cellular changes principally in the bone marrow, the question of the direction of blood flow in ischaemic bone being neglected. Differences of opinion exist as to the nature and degree of these cellular changes (Houang, 1934; Kistler, 1934; Higgins & Wiege, 1939; Bragdon, Foster & Sosman, 1949), and in some investigations the procedures employed go beyond the production of simple ischaemia, introducing uncertainties in the interpretation of the results (charcoal embolism, Kistler, 1934; marrow destruction, Marneffe, 1951; periosteal stripping, Foster, Kelly & Watts, 1951). For these reasons, it was decided that the effects of uncomplicated ischaemia on bone should be studied anew, with special reference to the circulatory pathway in the compactum as well as to the cellular reactions in the cortex and marrow.

MATERIALS AND METHODS

Sixteen adult rabbits were used in the course of this investigation. In each the arterial supply to the right femur and tibiofibula was partially suppressed by ligation and section of the principal nutrient artery. To lay a heavier ischaemic stress on the tibiofibula, the popliteal artery was sectioned between ligatures in the adductor canal below the origins of the A. suprema genu and the saphenous artery. The former provides a large anastomotic pathway to the leg and the latter, by means of its medial and lateral plantar divisions, the bulk of the blood supply to the rabbit foot. Hence the ensuing ischaemia predominantly affected the two bones in question and peripheral gangrene did not occur. The left limbs were used as controls.

Three rabbits were killed at weekly post-operative intervals for 1 month and thereafter one rabbit at monthly intervals of 5 months. Before killing, under ether anaesthesia, the arterial system was filled with a suspension of barium sulphate (Micropaque, Damancy and Co.) injected through a polythene cannula tied into the abdominal aorta. Micropaque was diluted to half strength with normal saline and injected at a manometrically-controlled pressure of 70–100 mm. Hg until complete filling of the arterial system of the posterior extremities was judged to have taken place, utilizing the coincidental visible filling of the gut wall vessels as an indicator. After injection with Micropaque the rabbit was killed by ether overdosage and the hindlimbs and pelvis skinned and fixed in one piece in a 5 % formalin (2 % formaldehyde) solution. To avoid loss of injection medium before fixation, care was taken to ligate vessels oozing Micropaque from cut surfaces. Two weeks were generally allowed for fixation before examining the parts radiologically and histologically. Some specimens were radiographed whole immediately after fixation in order to

compare the radiological appearances of the bones and arterial tree in the experimental and control limbs. Soft tissues were then removed until only the periosteum and immediately adjacent and attached muscle fasciculi remained about the femora and tibiofibulae; these specimens were decalcified in a 5 % nitric acid in 5 % formalin solution. Transverse sections about 1 mm. thick were cut by hand from the upper, middle and lower thirds of each bone, and these, together with other longitudinal sections, were microradiographed using Kodak maximum resolution plates and a microfocus unit incorporating the Ehrenberg and Spear tube. Other specimens were blocked in paraffin wax using Peterfi's (1921) double embedding technique, and sectioned transversely and longitudinally at 8 μ. Sections were stained with Ehrlich's haematoxylin and eosin and Mallory's trichrome stain. A few representative sections from operated and control limbs were also stained by Wilder's (1935) silver method.

RESULTS

Radiological observations

Whole plate views of the rabbit hind quarters taken prior to decalcification showed differences between control and operated sides both in the radiotranslucency of the bones and in the soft-tissue arterial pattern. After 4 weeks of ischaemia the bones of the operated limbs were less opaque to X-rays than their controls, the increase in radiotranslucency becoming more marked in succeeding months. On the other hand, the density of small arterial channels in the soft tissues of the ischaemic leg increased during the same post-operative period and at 5 months was in marked contrast to the arterial pattern of the contralateral limb, presenting as a cloudy haze of fine vessels below the level of section of the popliteal artery against the more discrete pattern in the same region of the control (Pl. 1, fig. 1).

Microradiography of thin sections cut from the control femora and tibiofibulae showed the general vascular features of the cortex and medulla of long bones. The sharply defined arterial pathways of the medulla were more consistently represented in control microradiographs than were the sinusoids of the marrow, which in some sections were hardly visualized peripherally beyond the central venous sinus. Medullary arteries tended to course radially towards the cortex (Pl. 1, figs. 3, 5) when seen in cross-sections of bone, while in longitudinal sections main arteries were found running in the periphery of the medulla giving off fine terminal branches which pierced the endosteal cortex and passed centrifugally towards the periosteum, being traceable usually as far as the outer half of the compactum (Pl. 2, fig. 7). Although periosteal and surrounding soft tissue vessels were readily visualized by radiography following Micropaque injection, control microradiography did not demonstrate anywhere a centripetal penetration of barium sulphate suspension into the underlying bone cortex.

Microradiography of transverse and longitudinal sections cut from the ischaemic limbs and leaving soft tissue adjacent to bone *in situ*, showed that in both femur and tibiofibula functioning vascular structures were still present in cortex and marrow. Marrow sinusoids opened in dense groupings either directly into the central venous sinus of the medulla or into its large radially disposed tributaries. Here and there the appearances were suggestive of a lobular organization of the marrow (Pl. 2, fig. 8), patches of sinusoids rendered radiopaque by intravital injection of Micropaque being

separated by zones not reached by the injection mass. When sinusoidal filling was virtually complete, the patchy lobular pattern of groups of sinusoids each draining into their respective tributary of the central venous sinus was not seen, but instead the sinusoids formed a uniform reticular background against which discrete arterial channels could be discerned (Pl. 2, fig. 9). Nevertheless, in the diaphyseal marrow of the ischaemic femur and tibia, sharply defined areas of densely packed and filled medullary sinusoids adjoined patches of radiologically empty medulla (Pl. 2, fig. 10). Such areas of medullary infarction were demonstrable by radiography up to 1 month post-operatively.

In the cortex of the ischaemic femur, the same centrifugal pattern of arterialization as in the controls was found at first, notwithstanding the dense vascular filling of attached musculature; but from 4 weeks post-operatively there was micro-radiographic evidence of the centripetal passage of barium sulphate suspension from the periosteal circulation into the underlying bone (Pl. 1, figs. 4, 5). In the cortex of the ischaemic tibia haemodynamic changes were more pronounced and were observed earlier. Two weeks after the vascular ligations Micropaque passed unmistakably from the surrounding soft tissue vessels centripetally into the cortex (Pl. 2, fig. 10). At first the periosteal arterial pathways in the compact cortical bone were fine in calibre and penetrated only the outer half of its thickness, being especially numerous opposite areas of medullary infarction; but with an increasing post-operative interval the periosteal arterial supply of the cortex became more evident (Pl. 1, figs. 2, 4), and there were numerous large anastomotic vessels passing between the periosteal and medullary arteries (Pl. 2, fig. 6), while other centripetal arteries passing through the cortex opened directly into the medullary sinusoids (Pl. 2, fig. 11). These dramatic changes in the pattern of bone vascularization affected predominantly the upper two-thirds of the tibial shaft: by the end of the second post-operative month they were fully developed and persisted thereafter, without diminution, for a further 3 months at least.

Histological observations

One week after the arterial ligations the marrow of the affected long bones showed widespread changes. These did not, however, involve the whole thickness of the medulla, some normal haemopoietic tissue always being present (Pl. 3, fig. 13). Adjacent to infarcted areas, where the cells had lost their differential staining properties and the sinusoids were collapsed, there were zones where some of the sinusoids were still functional as shown by the presence in them of injected barium sulphate granules as well as the presence around them of normally staining marrow cells. Some spicules of new bone had formed as projections from the endosteal surface of the cortex. The cortex itself was little affected at this early stage: occasionally some enlargement of the vascular canals was observed (Pl. 4, fig. 20), but usually the canals and cells of the compactum of both femur and tibia differ little from the normal controls. In particular the osteocytes of recently ischaemic compact bone did not undergo extensive necrosis; only in the intermediate zone, and there generally confined to the interstitial lamellae, were there found isolated osteocytes showing poor nuclear staining and occasional empty bone lacunae. Such appearances occurred only in scattered fields and then uncommonly.

From 2 to 4 weeks post-operatively the reaction of the long bones to ischaemia was most striking in the compactum, especially in the case of the tibia. Many of the vascular canals enlarged until their diameter was several times greater than that of the canals in the controls (Pl. 4, figs. 22, 24). They contained blood vessels with a thin wall composed of a single layer of endothelium and surrounded by a loose reticulum. Active osteoblasts lined the canals in palisade fashion: osteoclasts were rarely seen (Pl. 3, figs. 18, 19). As in the specimens after 1 week of ischaemia, osteocyte death was not present on a large scale: it was found chiefly in the interstitial lamellae of the intermediate zone of the compactum (Pl. 3, figs. 18, 19; Pl. 4, figs. 22–24). Endosteal new bone formation was now well developed, trabeculae being laid down in scattered plaques against the cortex, especially adjacent to ischaemic bone marrow (Pl. 4, fig. 24). Periosteal new bone was also present as irregular deposits. In other areas, the superficial cortical lamellae were deeply pitted by surface erosions containing reticular tissue similar to that found in more deeply situated enlarging canals. Sections cut from ischaemic compact bone where canal enlargement and bone removal were advanced, and stained by Wilder's method for reticulin, failed to show any change in the fibrous organization of the fundamental bone substance when compared with silvered preparations of control material.

Up to 1 month post-operatively the effects of experimental ischaemia on the bone marrow included a drastic diminution in the number of red cells and their precursors in affected areas although eosinophilic granulocytes and plasma cells were very noticeable. Collagen fibres, readily identifiable by their blue coloration in Mallory preparations, and fibroblasts were also conspicuous (Pl. 3, fig. 15, 16), as were groups of multinucleate giant cells (Pl. 3, fig. 17). The latter arose apparently from the coalescence of cells surrounding fat lacunae, possibly the endothelial cells of collapsed sinusoids. Small arteries patent to injected barium sulphate suspension were found in the loose fibrotic infiltrations of the ischaemic medulla (Pl. 3, fig. 15) and were peculiar in that their walls were made up of a single layer of endothelial cells. Control microscopy showed that this is a normal appearance in the rabbit marrow, and that the change over from a thick- to a thin-walled artery is very abrupt.

After the first post-operative month the reactive changes in the marrow rapidly abated, resolution occurring without the formation of scar tissue. The fibroblastic elements vanished together with the multinucleate giant cells, and by 2 months from the start of the experiment the bone marrow appeared normal. On the other hand, from 2 to 5 months enlarged canals in the compact bone remained conspicuous, and came to contain apparently normal haemopoietic tissue in place of the vascular reticular tissue (primary marrow) found earlier. In the tibia especially the relatively huge cortical blood spaces filled with normal-looking haemopoietic marrow (Pl. 4, fig. 25), were striking. Similar changes but lesser in degree were seen in the femur, and warrant the description 'medullization of the cortex'.

DISCUSSION

From this investigation it may be concluded that in the rabbit femur and tibiofibula, experimental ischaemia results in certain major sequelae in both the marrow and cortex. Initially, extensive medullary necrosis occurs with abolition of erythropoiesis in infarcted zones. This is followed by a reactive phase, characterized by the

appearance of patent endothelial blood vessels and abundant active cellular elements scattered diffusely through the affected areas. Myelocytes, fibroblasts and multinucleate giant cells are conspicuous in the cellular reaction to marrow ischaemia, but fibrosis did not go beyond the laying down of a loose feltwork of connective tissue fibres. This phase is followed by one of repair wherein abnormal cells and interstitial fibrous tissue disappear, the sinusoidal bed is restored and normal haemopoiesis resumed. On the other hand, the characteristic reaction of compact bone to a diminution in its blood supply is a widening of the cortical vascular canals which come to contain a loose vascular reticulum and osteoblasts. Massive osteocyte death does not occur and the fibrous basis of the fundamental bone substance does not suffer any obvious alteration. Nevertheless, the cortical lesion is progressive, resulting in the removal of large quantities of bone and its replacement by medullary tissue. With medullization of the cortex appears radiographic evidence of osteoporosis and participation of periosteal arteries in the nutrition of the bone as a whole.

The question of the circulatory pathway in bone cortex, ischaemic or otherwise, has been neglected almost universally in the past probably because the terminology employed in describing small blood vessels which pass *into* underlying bone from the periosteal membrane (Pinard, 1952; Morgan, 1959), prejudices the investigator as to the actual direction of blood flow within them. It is interesting, however, to record that Clopton Havers (1691) described the mutrient arteries of compact bone as entering only in localized areas which then give rise to 'vast numbers of veins' leaving the surface of the cortex. Nevertheless, since his day it has generally been taken that normal compact bone possesses in some degree a periosteal arterial blood supply.

Recent investigations into the problems of vascular organization and haemodynamics of long bones (Brookes, 1958*a*, *b*; McAuley, 1958) lend support to the concept formulated by Brookes & Harrison (1957) of a medullary arterial system responsible for the supply of blood to both the sinusoids of the marrow and the capillaries of compact bone. In normal conditions it is held that the periosteal blood vessels play no part in the arterialization of the underlying bone cortex, but for blood which has flowed through the vascular canals of the compactum a venous drainage pathway is available in the capillaries of the periosteum and the interfascicular capillaries and veins of muscles with a fleshy diaphyseal attachment.

Because the blood vessels of the cortex, and the medullary arterial terminals, are all simple endothelial tubes, it is difficult from histological studies alone to say with any certainty in which direction the blood flows within the cortical vascular canals. A radiological technique was used by Brookes & Harrison not only to visualize aspects of the intra-osseous vascular system, but also to elucidate the problem of blood-flow direction in mammalian compact bone. It was based on the fact that the barium sulphate suspension used did not pass freely into the capillary bed. If the whole length of a capillary from medulla to periosteum is filled, as, for example, in indian ink preparations, the direction of the blood flow cannot be inferred. But by the deliberate employment of an injection mass, such as Micropaque, which only imperfectly enters into the capillary bed, a strong pointer is gained as to the circulatory direction.

In view of the peculiar characteristics of the circulation in bone it is surprising that a curtailment of the normal medullary arterial supply should be in part compensated for by a reversal of blood flow within the compactum, whereby periosteal blood passes centripetally through the cortex into the medulla. The microradiographic results described here, however, show unequivocally that experimental ischaemia, varying in severity, is followed by an appropriate participation of periosteal arterial blood in the nutrition of the affected bone, thus indicating that the periosteal circulation is an important reserve collateral blood route. That blood flow reversal in the cortex is greater in the tibia than the femur in this experiment may be explained as follows: popliteal ligation seriously interferes with the tibial blood supply through the numerous arteries of its upper extremity (Brookes & Harrison, 1957); also when the principal nutrient artery of the femur is eliminated the artery of the trochanteric fossa can take on the role of a second principal nutrient artery (Brookes, 1957) so that ligation of the tibial principal nutrient artery is on anatomical grounds a severer lesion than the corresponding operation in the rabbit femur. The experimental microradiographic findings are also to be correlated with the demonstration in Man (Brookes, 1959) of a significant periosteal arterial supply to ischaemic tubular bone obtained from amputation specimens in cases of peripheral occlusive vascular disease, in which the visual evidence for a pathological centripetal blood flow is in marked contrast to the centrifugal blood flow in normal and quasi-normal human material.

With regard to the histological findings it is noteworthy that few authors have published accounts of principal nutrient artery ligation experiments; Huggins & Wiege (1939), working on the rabbit femur, were the first to record any infarction by this method. Infarction is denied by Kistler (1934) but confirmed by the other authors mentioned. Endosteal bone production is not recorded by Marneffe (1951), but he does describe a mild medullary fibrosis 2 weeks post-operatively, which Bragdon *et al.* (1949) did not observe. The latter deny that haemopoietic function was restored, but Huggins & Wiege were able to report a normal marrow after 74 days of ischaemia without, however, observing the reactive stage of myelofibrosis recorded here as occurring at 2–4 weeks. As for compact bone, all the authors mentioned in this section concur in describing only minimal changes, if any, in the osteocytes of the cortex. Even Kistler in his charcoal embolism experiments would not allow that infarction progressed further than the inner quarter to one-third of the cortex. The present findings show only a slight punctate cellular death in the intermediate zone (where true osteones are found), so it may be concluded that osteocytes in general are particularly resistant to ischaemia. Nevertheless, in order to account for the canal enlargement it seems necessary to postulate some chemical effect on and change within the osteocytes due to curtailment of their blood supply which stimulates the normal canal contents (capillaries and undifferentiated connective tissue elements), into an active phase of bone excavation. This late sequela accounts for the radiographic osteoporosis of ischaemic compact bone found experimentally, and it is suggested that both phenomena are comparable to similar findings reported in ischaemic bones in Man (Jaffe & Pomeranz, 1934; Brookes, 1959). In addition, it would appear that this drastic disturbance to the normal structure of compact bone is an adaptive reaction to adverse environmental change, because the

enlarged vascular spaces, now capable of holding *many* blood vessels (and not the usual one for each), permit the periosteal circulation to act as an important collateral blood route. The centripetal blood flow into bone may well be the primary factor tending towards bone survival in ischaemia caused by interruption of the nutrient vessel.

Although not mentioned in this paper, the role of the metaphyseal vessels in affording a collateral circulation for the interrupted nutrient artery must not be forgotten. They may well tide the bone over the fortnight or so before the periosteal centripetal circulation is established.

SUMMARY

1. The principal nutrient artery of the femur and tibiofibula, together with the popliteal artery were ligated and sectioned in adult rabbits, and the sequelae of the ensuing partial ischaemia of bone observed radiologically and histologically over a 5-month period.

2. The results indicate that a reversal of the normal centrifugal arterialization of the cortex takes place to some extent, periosteal arteries participating as a collateral blood route to underlying ischaemic bone.

3. Myelofibrosis and endosteal bone formation are observed as a transient phenomenon up to 1 month post-operatively, the marrow thereafter reverting to normal. The multinucleate giant cells conspicuous in the cellular reaction of the marrow to ischaemia, are possibly derived from sinus endothelium.

4. Osteocytes in partially ischaemic bone cortex do not undergo massive necrosis, but in general retain normal staining properties. The characteristic response of compact bone to ischaemia is a true osteoporosis diagnosable radiologically and histologically, with gross enlargement of Haversian canals which come ultimately to contain haemopoietic marrow, so that a medullization of the cortex results.

I am happy to record my gratitude to Prof. R. G. Harrison for his continued interest in this research and for his many helpful suggestions. My thanks are also due to Messrs A. Taunton and D. L. Reeve for their valuable technical assistance throughout this investigation and in the preparation of the plates.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Radiograph of the undecalcified posterior extremities of a rabbit following 5 months of experimental ischaemia of the right femur and tibiofibula. Barium sulphate suspension has been injected intra-arterially. The bones of the operated right side show greater radiotranslucency than those of the control limb. The vascularization of the right leg is very much denser than normal below the level of the sectioned popliteal artery. ×0·75.

Fig. 2. Microradiograph of a transverse section of a rabbit's tibia 5 months after the production of ischaemia. A pronounced centripetal arterialization of the bone cortex is demonstrated. Many large calibred vessels spring from the periosteal arteries and pass unmistakably into the underlying bone. ×12.

Fig. 3. Microradiograph of a transverse section of the control tibia taken from the same animal shown in Figs. 1 and 2. The stem of the principal nutrient artery is seen in the cortex and its ramifications in the medulla. Fine terminal branches pass centrifugally into the compactum and can be followed at least half-way across its thickness. No periosteal arterial supply is present. ×12.

Fig. 4. Microradiograph of a transverse section through the anterior surface of a rabbit femur rendered ischaemic 4 weeks previously by ligation of the principal nutrient artery. An abnormal centripetal blood flow into the compact bone from the vessels of the attached vastus intermedius is demonstrated. ×12.

Fig. 5. Microradiograph of a transverse section of the control femur to that shown in Fig. 4. The normal radial disposition of medullary arterial terminals and their centrifugal penetration of the endosteal face of the compactum is shown. In spite of dense vascular filling of the attached vastus intermedius muscle no centripetal penetration of the cortex occurs from its periosteal aspect. ×12.

PLATE 2

Fig. 6. Microradiograph of a longitudinal section through the diaphysis of a rabbit tibia 3 months after the production of bone ischaemia. Numerous large calibred anastomoses between periosteal and medullary circulations are shown in which the blood flows centripetally through the cortex into the medulla. ×13.

Fig. 7. Microradiograph of a longitudinal section through the control tibial diaphysis to the one shown in Fig. 6. Medullary arteries are shown coursing in the outer zone of the bone marrow giving off terminal branches which pierce the cortex centrifugally and are traceable about half way across its thickness. Periosteal structures are still *in situ*. Along the lower edge of the print the central venous sinus can be seen. ×13.

Fig. 8. Microradiograph of diaphyseal marrow taken from a femur three weeks after ligation of the principal nutrient artery, showing medullary sinusoids arranged in a lobular fashion. × 15.

Fig. 9. Microradiograph of the vascular contents of diaphyseal marrow seen in an ischaemic rabbit femur 3 weeks after operation. The medullary sinusoids form a dense reticular background against which discrete arterial channels are seen. × 15.

Fig. 10. Microradiograph of a transverse section of an ischaemic rabbit tibia 2 weeks after operation. An abnormal centripetal periosteal arterial supply to the cortex has been revealed by intra-arterial injection of radiopaque suspension. Much of the marrow still retains a blood supply, although in this section a large infarcted area is shown, demarcated by injected medullary sinusoids. × 10.

Fig. 11. Microradiograph of a transverse section through the ischaemic tibia of a rabbit 2 months after operation. A large calibred arterial channel passes centripetally through the cortex opening directly into medullary sinusoids. × 15.

Plate 3

Fig. 12. Photomicrograph of normal rabbit femoral marrow showing a portion of the central venous sinus, an adjacent large medullary artery and haemopoietic blood spaces. Mallory, × 110.

Fig. 13. Photomicrograph of the femoral medulla of a rabbit 1 week after section of the principal nutrient artery. Many of the marrow sinusoids are collapsed; the artery shown in cross-section is empty of blood and radiopaque medium, but some haemopoiesis is still occurring. Mallory, × 110.

Fig. 14. Photomicrograph of ischaemic marrow in a rabbit tibia 2 weeks after operation. Many fat lacunae have vanished and haemopoietic areas replaced by fibroblastic infiltrations. Several multinucleate giant cells are also visible in this preparation. Haematoxylin and eosin, × 110.

Fig. 15. Photomicrograph of the bone marrow taken from the midshaft of a tibia 2 weeks following production of ischaemia. Two small medullary arteries filled with Micropaque are shown whose wall is a single layer of endothelium. Fibroblasts, plasma cells and eosinophilic myelocytes are found in abundance in the myelofibrotic infiltrations. Haematoxylin and eosin, × 430.

Fig. 16. Photomicrograph of tibial bone marrow after 4 weeks of ischaemia. Islands of medullary bone are shown developing in fibrotic marrow. Mallory, × 130.

Fig. 17. Photomicrograph of a cluster of multinucleate giant cells developing in the tibial bone marrow of a rabbit 2 weeks after production of ischaemia. Several stages are shown in the formation of these giant cells, which appear to be derived from sinus endothelium surrounding the fat lacunae which are undergoing replacement. Haematoxylin and eosin, × 250.

Fig. 18. Photomicrograph of an enlarging Haversian canal in an ischaemic rabbit tibia 4 weeks after operation. The canal contains small endothelial vascular tubes in a loose reticulum bounded by a pallisade of osteoblasts. Osteocytes in the vicinity of the canal appear normal. Haematoxylin and eosin, × 480.

Fig. 19. Photomicrograph of two osteoclasts in the ischaemic tibial cortex of a rabbit, 3 weeks after operation. Although the bone is undergoing active dissolution, the osteocytes appear normal. Haematoxylin and eosin, × 480.

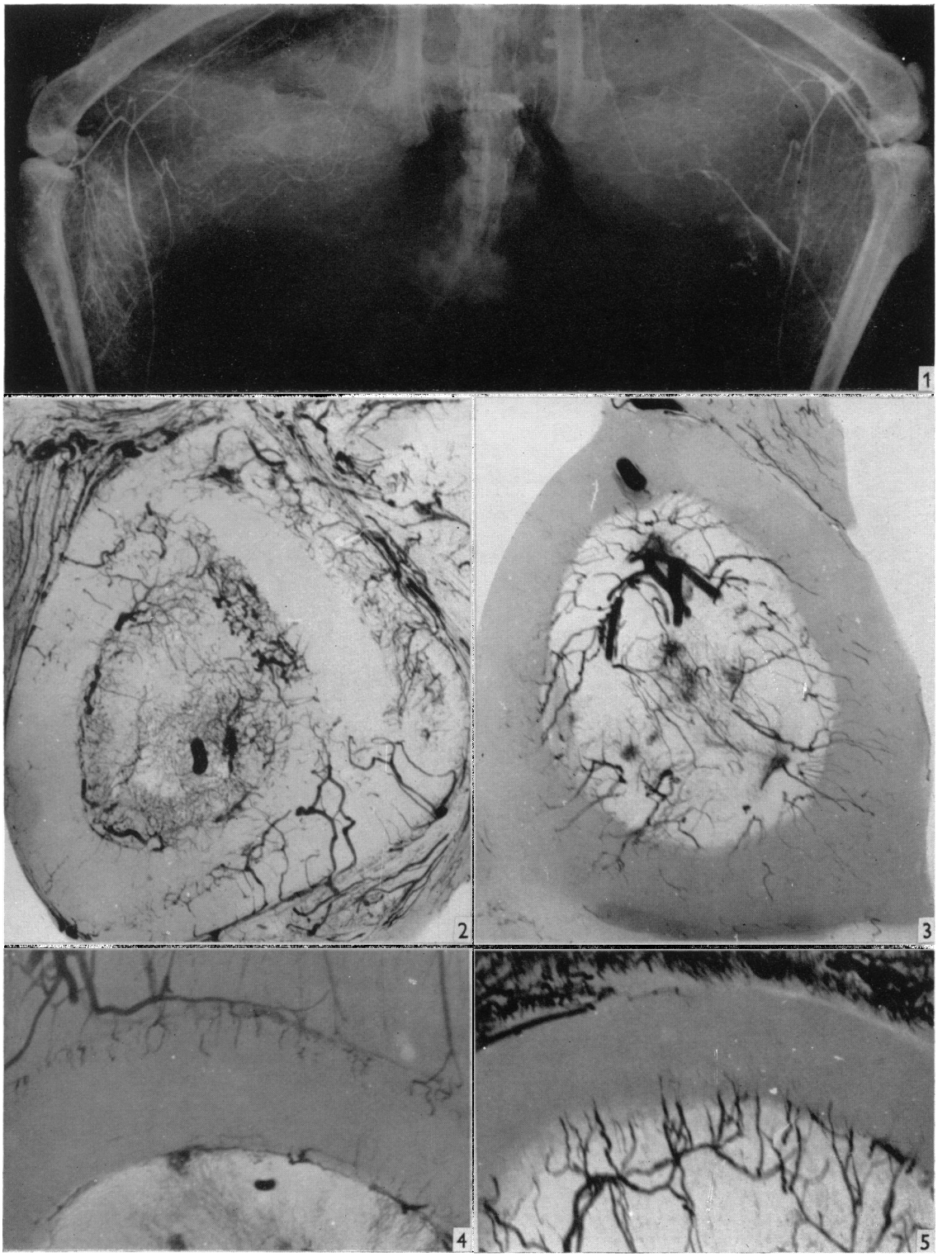
Plate 4

Fig. 20. Photomicrograph of a transverse section of the control tibial cortex of a rabbit in which the contralateral tibia had been rendered ischaemic 1 week previously, showing normal Haversian canals and osteocytes. Mallory, × 110.

Fig. 21. Photomicrograph of a transverse section of ischaemic tibial cortex in a rabbit 1 week after operation showing enlargement of some Haversian canals in a field of normally staining osteocytes. Mallory, × 90.

Fig. 22. Photomicrograph of rabbit tibial cortex after 2 weeks of ischaemia, showing considerable enlargement of the Haversian canals. The osteocytes appear normal in general, poor staining tending to occur in interstitial lamellae. Mallory, × 90.

Fig. 23. Photomicrograph of ischaemic tibial cortex 2 weeks after operation showing enlarging Haversian canals and normally staining osteocytes. Haematoxylin and eosin, × 470.



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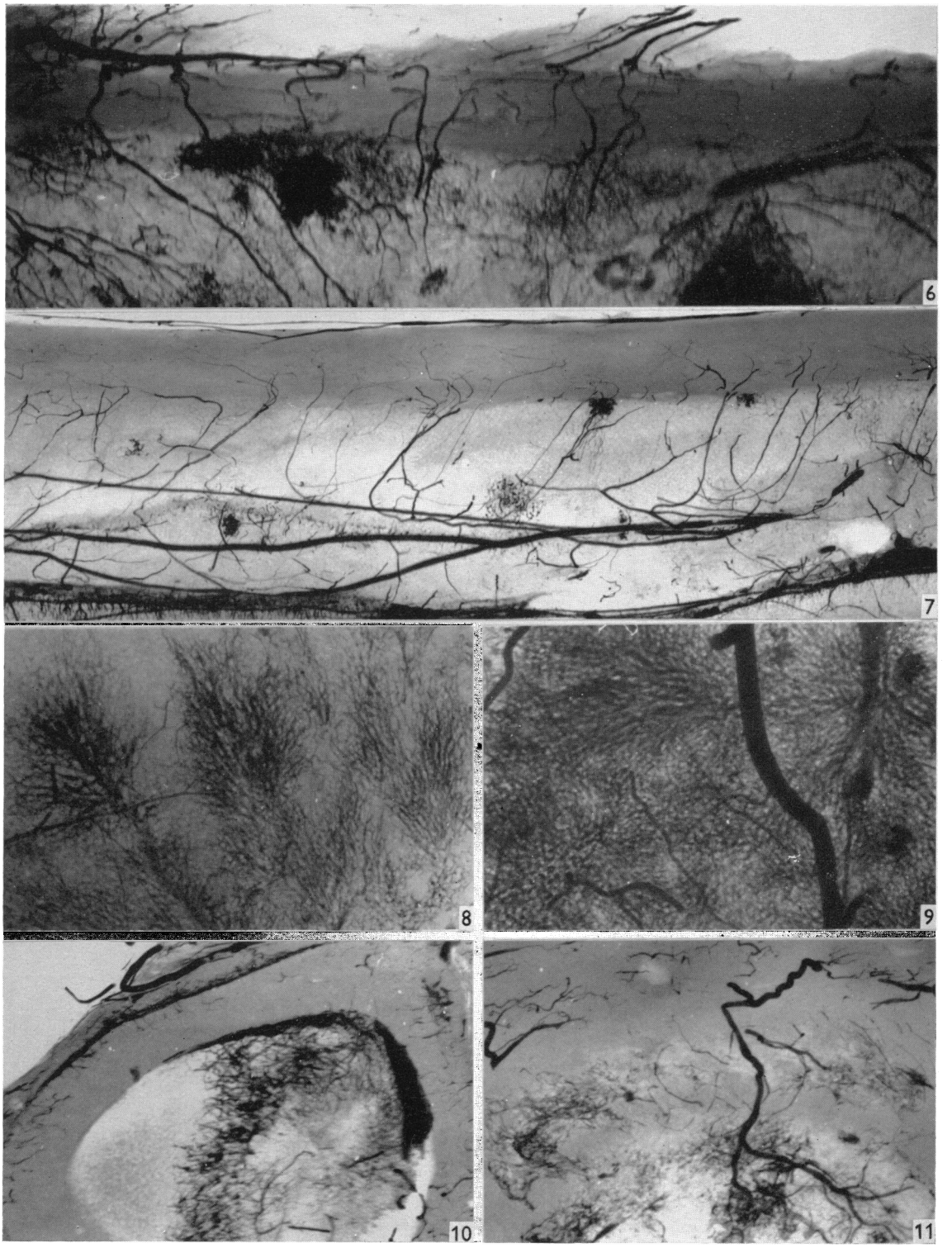

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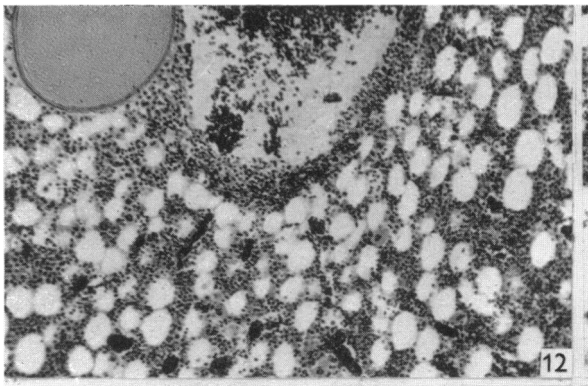
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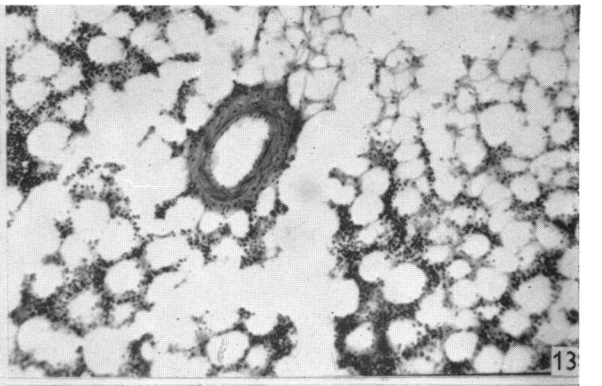
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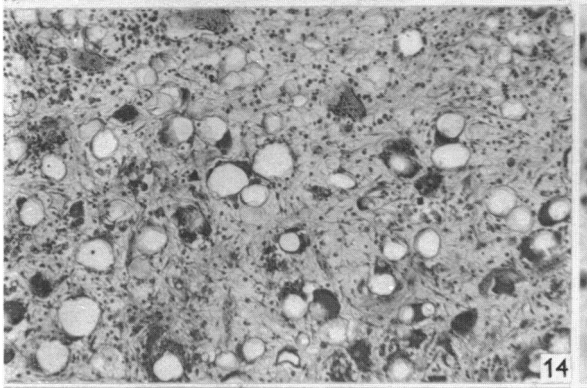
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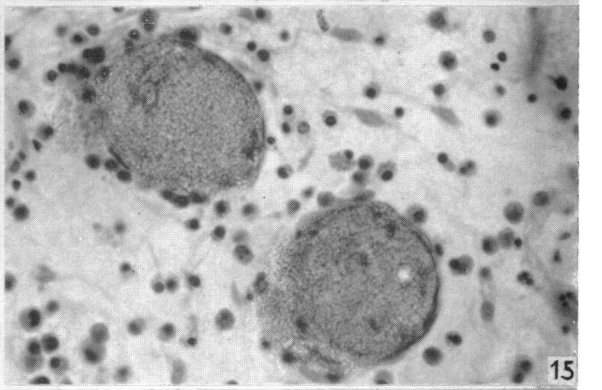
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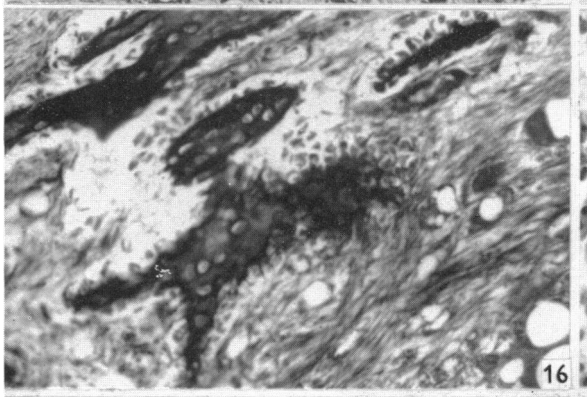
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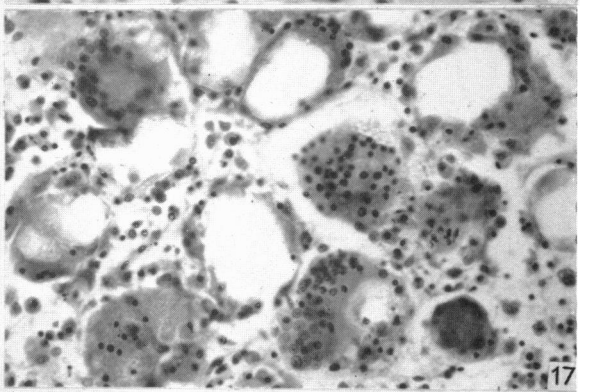
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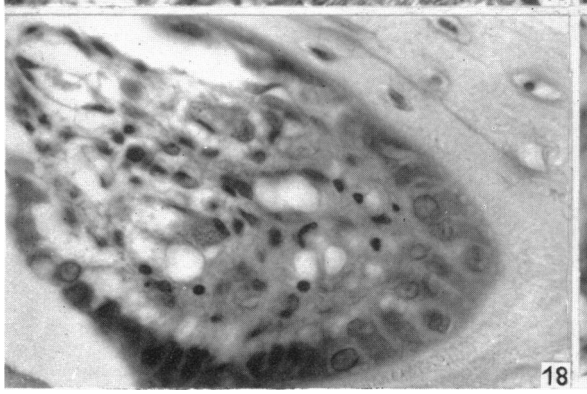
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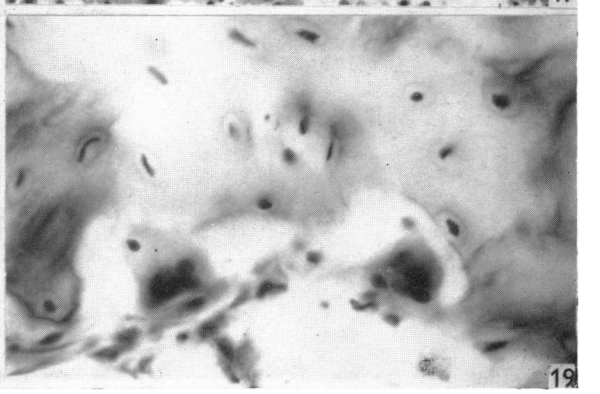
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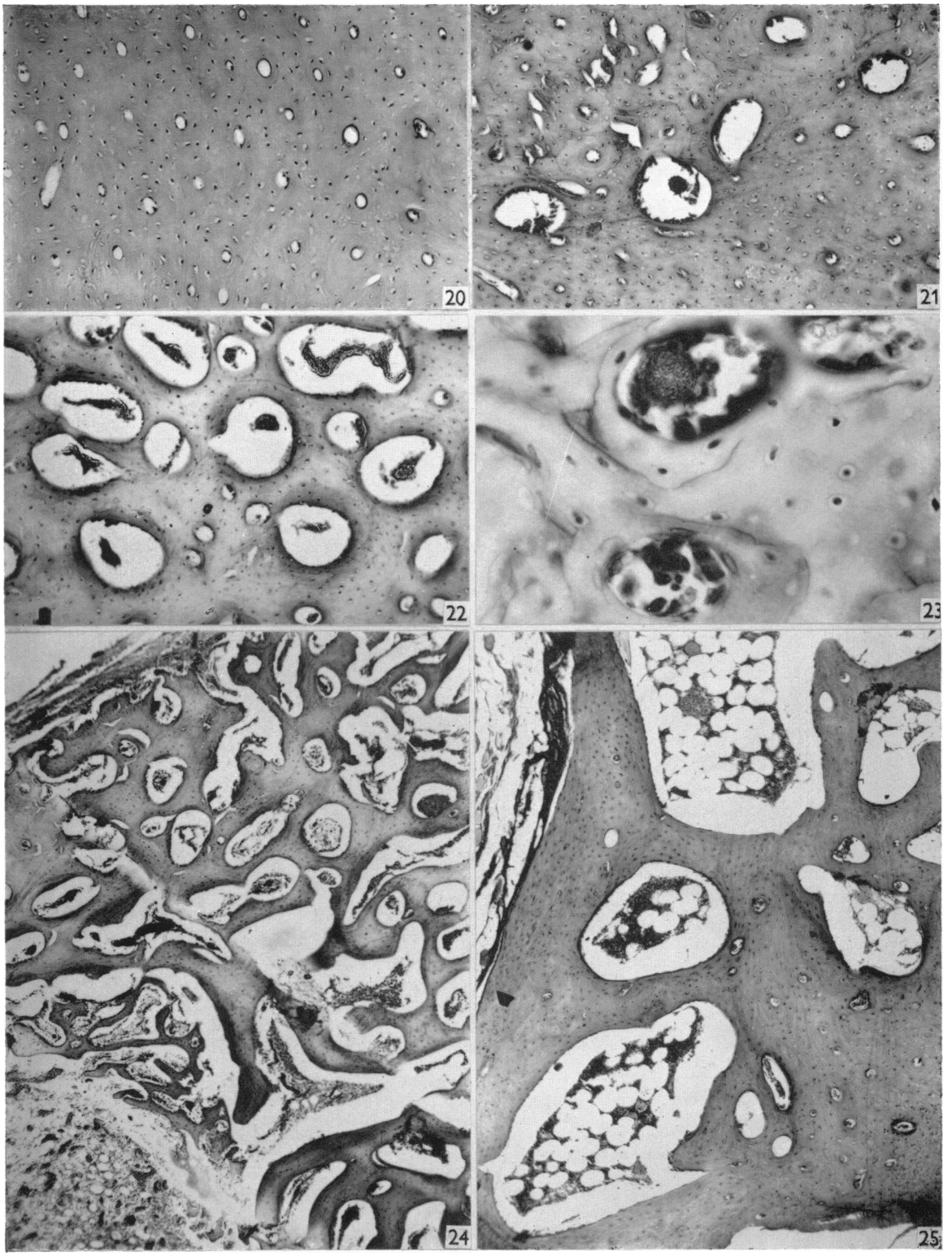
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Fig. 24. Photomicrograph of a transverse section through the ischaemic tibia of a rabbit 2 weeks after operation, showing a generalized enlargement of the Haversian canals which contain a loose vascular tissue and osteoblasts, frequently in pallisade formation. A plaque of newly formed endosteal bone is seen towards the lower left-hand corner of the illustration where ischaemic marrow is also visible. Massive osteocyte death has not occurred. Haematoxylin and eosin, $\times 40$.

Fig. 25. Photomicrograph of a transverse section through the tibial cortex of a rabbit rendered ischaemic 5 months previously. Giant spaces are present in the otherwise compact bone, and contain apparently normal haemopoietic marrow. The osteocytes in the field are in general normal in appearance. Haematoxylin and eosin, $\times 60$.