EXPERIMENTAL INFARCTION OF BONE AND MARROW*

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The long bones of mammals receive their blood supply from three sources. These are: (1) multiple small periosteal vessels penetrating and supplying the outer cortex; (2) epiphyseal vessels entering the ends of bones through ligamentous and capsular attachments; and (3) one (or more) nutrient arteries entering the shaft through nutrient foramina and supplying the diaphyseal marrow and inner cortex. There is considerable evidence that these vessels anastomose freely, particularly in the adult following disappearance of the epiphyseal cartilage plate, and it is for this reason that the possibility of bone or marrow infarction is often denied. Haslhofer,[1] writing on the circulation in bone and marrow in the Henke-Lubarsch Handbuch, stated that because of the richness of these anastomoses, disruption of even the largest source, *i.e.*, the nutrient artery, produces no sequelae, even in youth.

Clinicians, on the other hand, at times invoke infarction to explain otherwise cryptic bone lesions. Axhausen and Bergmann,[2] writing in the same volume with Haslhofer, presented clinical instances of aseptic bone necrosis which they ascribed to interruption of local blood supply. Phemister and his associates[3,4] have published radiographic and pathologic descriptions of lesions which they considered the result of marrow infarction. Some of the lesions occurred in patients who had previously had caisson disease.

Because the production of bone and marrow infarcts in animals has generally been considered impossible by conventional means, earlier investigators resorted to extensive stripping procedures or to the production of multiple small emboli designed to occlude large numbers of capillaries. Brunschwig,[5] attempting to produce infarction of the marrow of the femur in dogs, stripped the entire periosteum and simultaneously cut the nutrient artery. Despite this extensive trauma, he found no evidence of infarction in 15 adult dogs. In one young animal followed for 60 days, he described fibrosis and cyst formation within the marrow cavity. Among injection experiments may be mentioned those of Wollenberg,[6] who injected talc into the femoral artery of dogs and observed areas of necrosis in metaphyses and epiphyses. Bergmann,[7] on the other hand, following the injection of particles of silver suspended in gum arabic, found no changes in the epiphyses but widespread necrosis of

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cortical bone. Kistler,[8-10] having failed to observe infarcts following simple ligation of the nutrient artery of the femur in rabbits, finally resorted to injecting suspensions of charcoal in acacia and masses of agglutinated bacteria. He injected these directly into the nutrient artery under pressures which were not measured but which were admittedly high. By these means he was able to produce areas of necrosis in the center of the metaphyses.

Huggins and Wiege[11] were the first to report changes following disruption of the nutrient vessels alone. In both mature and immature rabbits, ligation of the nutrient vessels to the femur was followed in all instances by infarction of marrow. Although in a few cases there was some periosteal and endosteal reaction about the operative site, they found no evidence of infarction of bone.

In view of the non-physiologic means usually employed previously and because of the absence of uniformity in the results obtained, it was decided to attempt once more, under better controlled conditions, the experimental production of bone and marrow infarcts.

Methods

Large, but skeletally immature, rabbits weighing between 2.3 and 3.3 kg. were subjected to transection of the nutrient artery of the femur. Immature animals were used throughout because, as shown by Drinker, Drinker, and Lund,[12] so long as the epiphyseal cartilage plate remains, anastomoses between diaphyseal and epiphyseal vessels are at a minimum.

The animals were anesthetized with intraperitoneal injections of sodium pentobarbital, supplemented when necessary with ether inhalation. Using sterile technic, the nutrient artery was exposed and cut as close to the bone as possible. As one or two small veins usually accompany the artery, they were inevitably destroyed as well. A description of the gross anatomy of the blood supply to this bone may be found in Kistler's first paper.[8] The distribution of arteries within the bone and their relative size are illustrated in Figure 8 of this paper. Bleeding was minimal and as a rule no ligatures were necessary. The wound was closed with cotton sutures. In one case, because of faulty technic, the wound became infected; this animal was discarded. As our primary purpose was to follow the cytologic sequence of events after infarction of the bone and marrow, most animals were operated upon bilaterally.

The animals were sacrificed with chloroform or air embolism at intervals of from 24 hours to 6 months. The femurs were cleaned and roent-

genograms were taken. They were then sawed transversely through the shaft and longitudinally through the epiphyses into fragments not over 2 cm. in length. These were fixed in Zenker's fluid or formalin, decalcified in nitric acid, embedded in celloidin, sectioned and stained with hematoxylin and eosin and Mallory's aniline blue. A total of 25 rabbits comprised the basis for this report.

Results

In practically every femur of which the nutrient artery had been severed, microscopic examination of diaphyseal marrow revealed evidence of infarction. Lesions were most common at the junction of the middle and lower thirds of the shaft, although in the majority of cases there was patchy infarction throughout most of the diaphysis. In several instances the lesions extended into the metaphyses, but epiphyseal marrow was never involved. The infarcted areas frequently abutted upon and involved portions of cortical bone.

The sequence of events within the infarcted marrow was as follows: 24 hours after injury, swelling and diminished staining capacity of hematopoietic cells as well as hemorrhage of slight to moderate degrees were seen. At 48 hours the infarcted areas were outlined by the appearance at the periphery of neutrophils and mononuclear cells containing cellular débris and hemosiderin. By the fourth day lack of uniformity of fat droplets gave evidence of necrosis and rupture of fat cells. At 1 week (Fig. 1), although the larger lesions still showed complete necrosis at their centers, there was considerable cellularity at the periphery. Large numbers of eosinophils made their appearance. The larger fat droplets were being actively phagocytosed by multinucleated giant cells. There was some increase in vascularity in this zone, but it was not prominent.

At 2 weeks the center of the infarct consisted of a very loose fibrillar and granular matrix from which cellular débris had largely disappeared and in which most of the fat droplets had been phagocytosed (Fig. 2). About the periphery there was a well defined zone 1 to 2 mm. wide resembling a granuloma (Fig. 3). Large mononuclear and giant cells predominated, but there were also numerous lymphocytes, eosinophils, and occasional focal accumulations of neutrophils. Connective tissue stains showed no fibrosis. An interesting feature at this stage was the presence of shrunken and pyknotic megakaryocytes completely engulfed by giant cells, a phenomenon which indicates that these cells are unusually resistant to lytic enzymes.

By 3 weeks the lesions again became less well defined by reason of the disappearance of many of the inflammatory cells. During the third and fourth weeks the impression was gained that fat-laden macrophages gradually became indistinguishable from normal fat cells. They appeared to lose their stainable cytoplasm and to assume more uniformity in size. By the eighth week healing had advanced to the point that only careful scrutiny would permit recognition of the lesion. The fat-containing cells were still somewhat more variable in size, tending to be larger than those in the surrounding uninfarcted areas. Interspersed among them were macrophages containing a faintly yellow, globular to crystalline material (Figs. 6 and 7). This substance, often present in considerable quantities, was insoluble in most of the usual fat solvents. It was not doubly refractile, even in frozen sections of formalin-fixed material, and was not acid-fast. It stained blue with Nile blue sulfate, and, although unidentified, is probably a lipid.

By 6 months, the previously infarcted areas could be identified with certainty only by the presence in them of macrophages containing this material. These cells were interspersed among what appeared to be normal fat cells. Such areas were without hematopoietic activity. Their most noteworthy characteristic, however, was the complete absence of fibrous scarring.

One animal developed diarrhea, lost weight rapidly, and died 12 days following surgery. Because of starvation the normal marrow was depleted of fat, its place being occupied by engorged sinusoids. The areas of infarction were readily recognizable against this background by the continued presence in them of fat. Figure 4 illustrates a section of femur from this animal.

In most cases there was infarction of bone as well as marrow. It was very common to see small areas of necrotic bone along the inner margin of the cortex of the shaft. Such areas occurred only where a marrow infarct abutted on cortical bone. (Infarcted bone could be recognized as early as 48 hours following loss of blood supply because the bone cell nuclei stained less deeply. Within 1 week the area appeared completely acellular.) When the marrow infarcts extended to involve distal or proximal metaphyses, there was necrosis of bony trabeculae within the zone of provisional ossification. In one case, 2 weeks following injury, there was evidence of a disturbance in longitudinal growth sequences.

The infarcted bone elicited no specific reaction. When the area involved was small, as was usually the case, it was gradually resorbed and replaced. As all bones were still growing at the time of infarction,

remodeling sequences frequently gave the appearance of necrotic bone incorporated within the mid-zone of cortex. If, on the other hand, a small necrotic area, because of its location, was destined to disappear completely during remodeling, it appeared more resistant to resorption than adjacent viable bone. When the area of infarcted bone was large, as happened less frequently, new bone was rapidly laid down, by periosteum and/or endosteum, in such a manner as to buttress the necrotic, and presumably weaker, area (Figs. 5 and 6).

Because of the small size of most of the bone infarcts, roentgenographic interpretation usually was equivocal. In Figure 9, however, may be seen a definite zone of reaction about an area of necrotic cortical bone.

Summary and Conclusions

Cutting the nutrient artery to the femur of the growing rabbit almost invariably resulted in infarction. This confirms the work of Huggins and Wiege[11] but is contrary to the experience of others. Infarction was most extensive in the diaphyseal marrow, extending in some cases well into the metaphyses. Epiphyses were never involved. In most instances there were small areas of bone infarction as well. The latter usually were limited to the inner zone of the diaphyseal cortex. Based on a small number of cases in which infarction extended into the metaphyses, it appears that any interference with longitudinal growth sequences was temporary.

The tissue sequences in both marrow and bone up to 6 months following injury are reported. In the case of marrow infarcts, the most striking feature was the complete absence of a fibrous cicatrix. Fat droplets released from necrotic fat cells were taken up by large phagocytic cells, which in time appeared indistinguishable from normal fat cells. The area of marrow infarction eventually was recognizable only by the absence in it of hematopoiesis and by the prolonged presence of numerous macrophages containing an unidentified yellow material, presumably a lipid.

Small areas of infarcted bone excited no reaction other than their very slow replacement by viable bone, a process comparable to "creeping substitution" in bone grafts. Depending on the direction of remodeling processes, they either became incorporated within viable bone or became resorbed. Resorption appeared somewhat slower than in the case of viable bone. When the area of infarction was larger, there was a rapid overgrowth of new bone, designed apparently to buttress the structurally weaker area of necrosis.

Except for the work of Huggins and Wiege,[11] whose interpretation of marrow sequences is somewhat comparable to ours, it is difficult to reconcile our results with those of other authors. Perhaps Kistler,[8-10] who has performed the most extensive work in this field and who failed to detect any changes following ligation of the nutrient artery alone, relied too much on roentgenographic evidence of damage. Brunschwig,[5] who did produce infarction of both marrow and inner cortex (by completely stripping the periosteum as well as cutting the nutrient artery of the femur in young dogs), reported fibrosis and cyst formation in the marrow. This is completely inconsistent with our results. Such results occurred in only one case, and unfortunately he published no photographs.

The human lesions described by Phemister and his associates[3,4] and ascribed by him to marrow infarction, also differ significantly from our experimental lesions. Roentgenographic and pathologic examination in his cases revealed in the femoral marrow widespread areas of liquefaction necrosis surrounded by a zone of calcification. This lack of similarity to our experimental lesions might be explained by differences in bone age at the time of injury, by differences in the duration of the lesions, or, more likely, by the much larger area of infarction permissible in human marrow. Size of infarcts has much to do with the manner in which they heal, or fail to heal.

Comparisons with osteochondritis dissecans and Perthe's disease, syndromes which have frequently been ascribed to infarction, are not pertinent, since in our rabbits there was presumably no interference with the blood supply to the epiphyses. Other examples of aseptic bone necrosis in the human may or may not be comparable.

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[*Illustrations follow*]

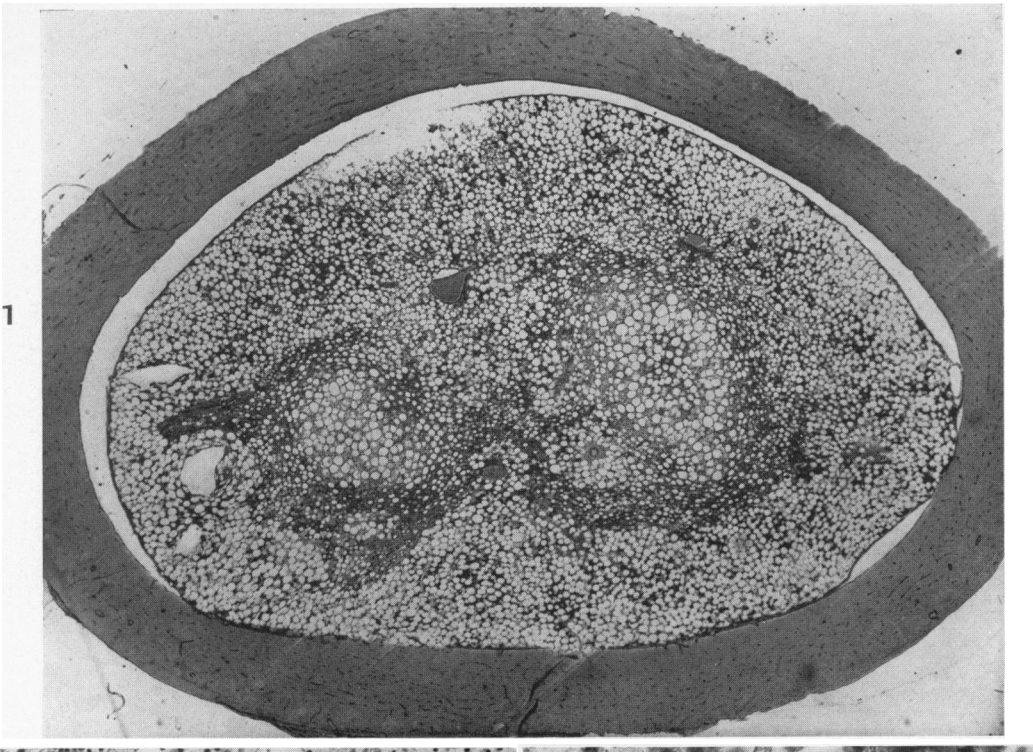
DESCRIPTION OF PLATES

PLATE 108

FIG. 1. Rabbit 191. Section through the junction of the middle and lower thirds of the shaft of a femur. The nutrient artery was cut 1 week prior to sacrifice. A large marrow infarct is well outlined by a zone of cellular reaction. × 29.

FIG. 2. Rabbit 190. Section of a marrow infarct of 2 weeks. Giant cells may be seen about fat droplets and engulfing pyknotic megakaryocytes. × 166.

FIG. 3. Rabbit 189. Section through a zone of reaction about a marrow infarct of 2 weeks. There is a collection of large mononuclear cells. The larger fat droplets are surrounded by a layer of the same cells. × 332.



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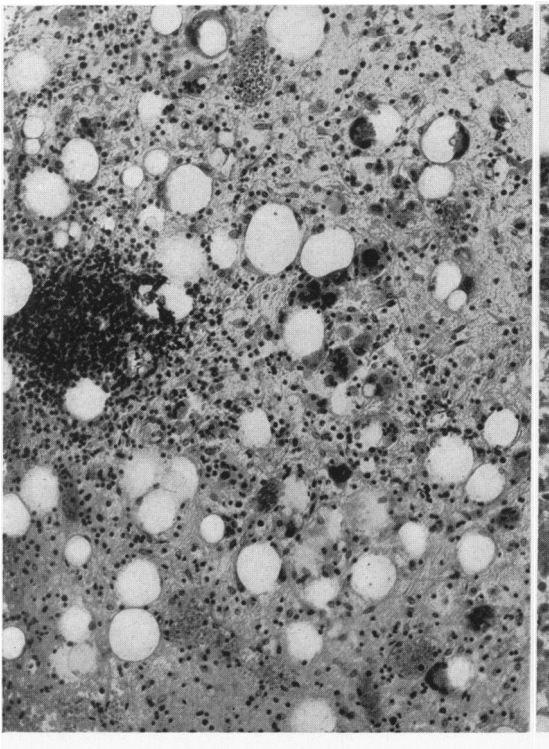

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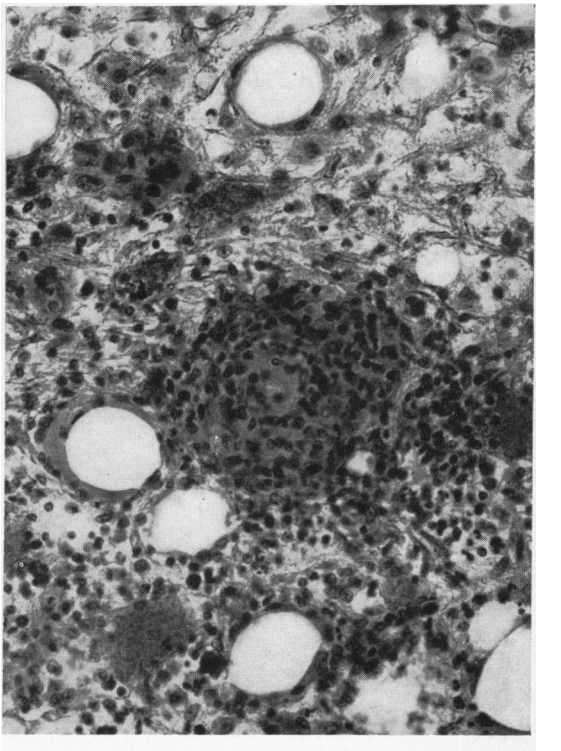

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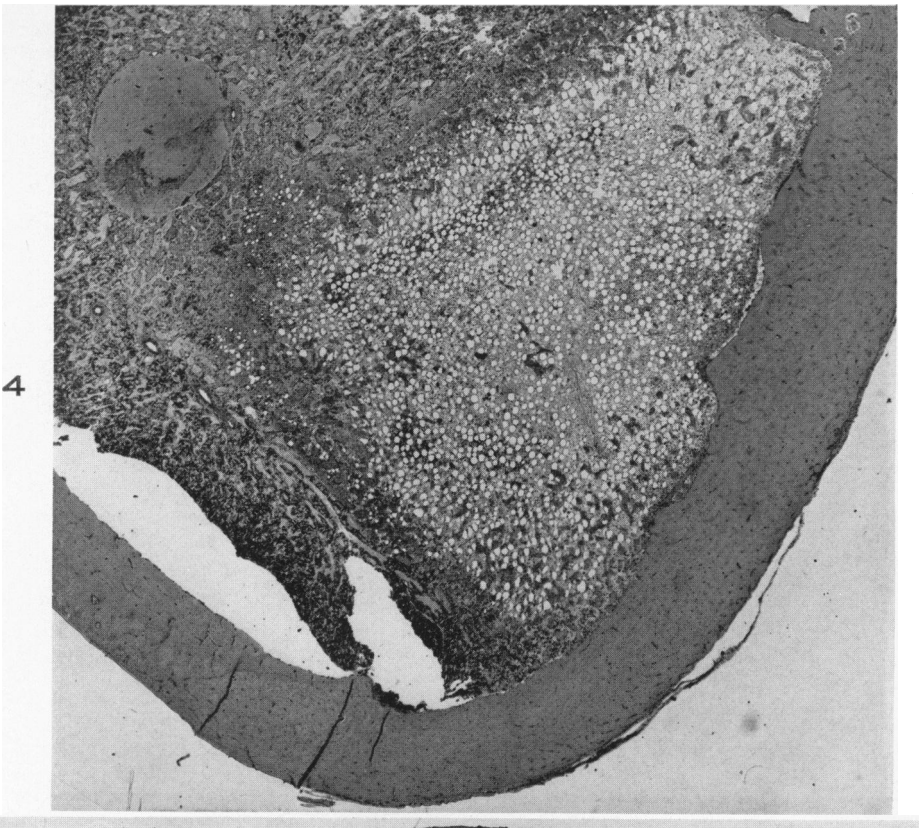
Experimental Infarction of Bone

PLATE 109

FIG. 4. Rabbit 180. Section of a marrow infarct of 12 days. This animal developed diarrhea, lost much weight, and died. In the uninfarcted marrow the fat has been mobilized and replaced by engorged sinusoids. Within the infarcted area fat droplets have been retained. Small areas of necrotic cortex may be seen along the margin of the marrow infarct. × 32.

FIG. 5. Rabbit 183. Section of marrow and cortical infarcts of 4 weeks. The zone of reaction about the marrow infarct has largely disappeared. An area of necrotic cortex has been buttressed by local overgrowth of new endosteal bone, forming an "internal splint." × 32.



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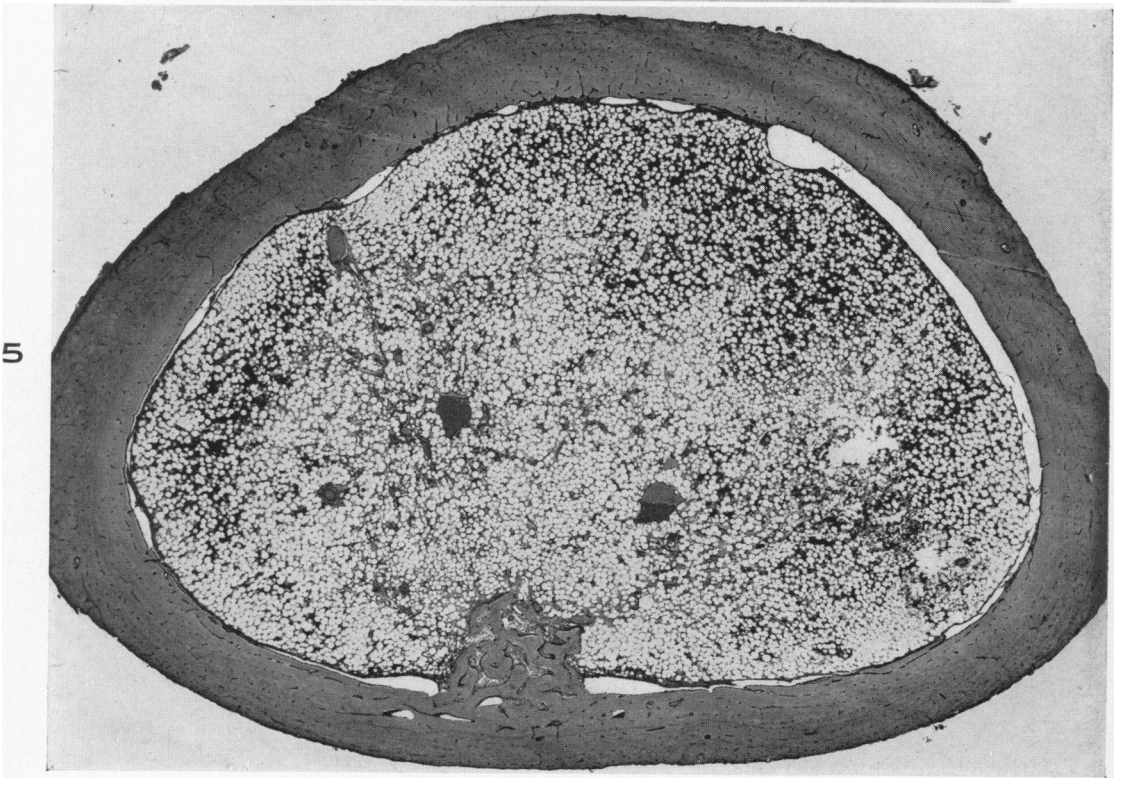

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PLATE 110

FIG. 6. Rabbit 157. Section of marrow and bone infarcts of 4 months. The marrow infarct is now recognizable only by the presence of macrophages filled with an unidentified yellow material and by the absence of hematopoiesis. There is no fibrous cicatrix. The infarcted area of cortex has been buttressed by an overgrowth of new bone from the periosteal surface. × 31.

FIG. 7. Rabbit 157. Same section as shown in Figure 6. Portions of necrotic cortex still remain after 4 months. There is no evidence of active bone formation or destruction. A small field of infarcted marrow in the lower right corner shows macrophages containing an unidentified opaque yellow material. × 135.

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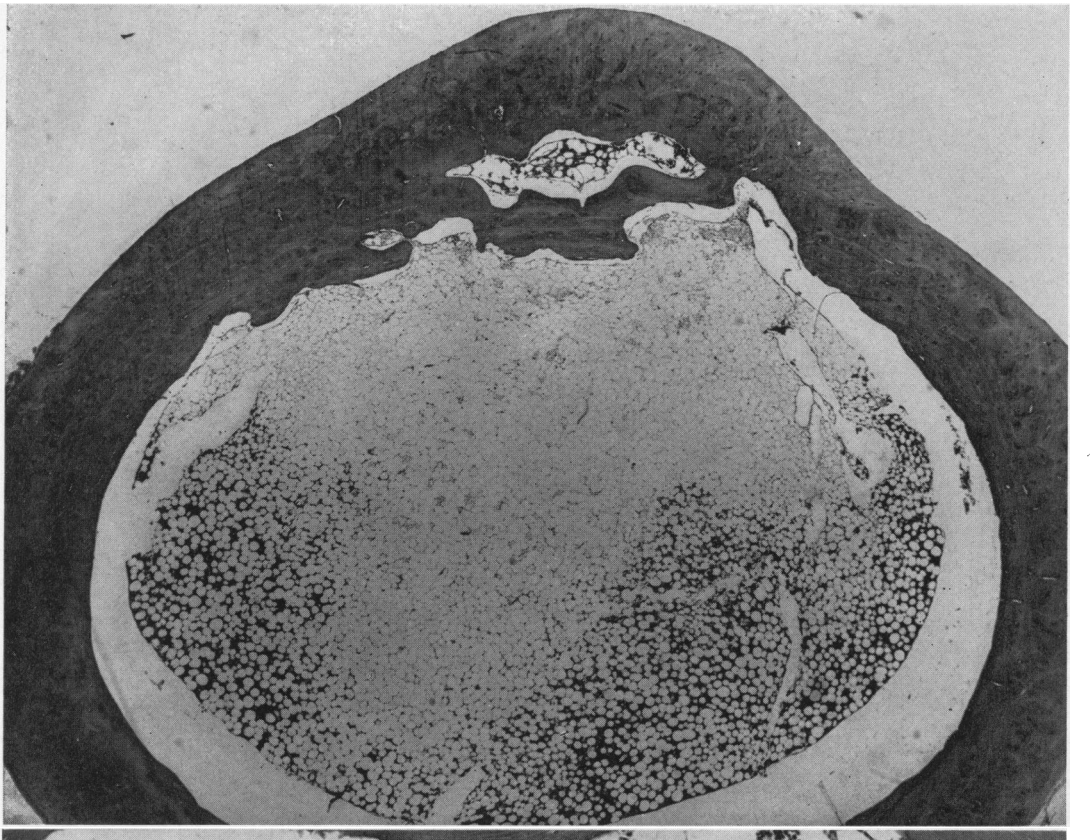

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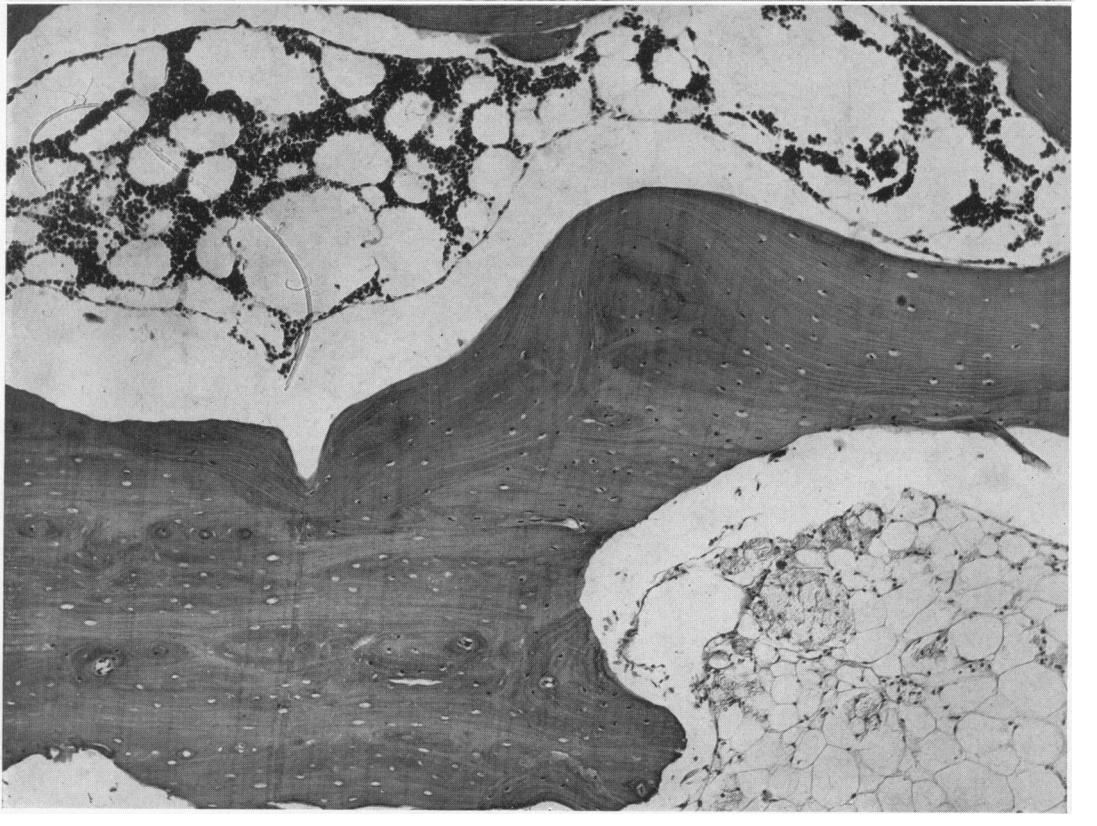
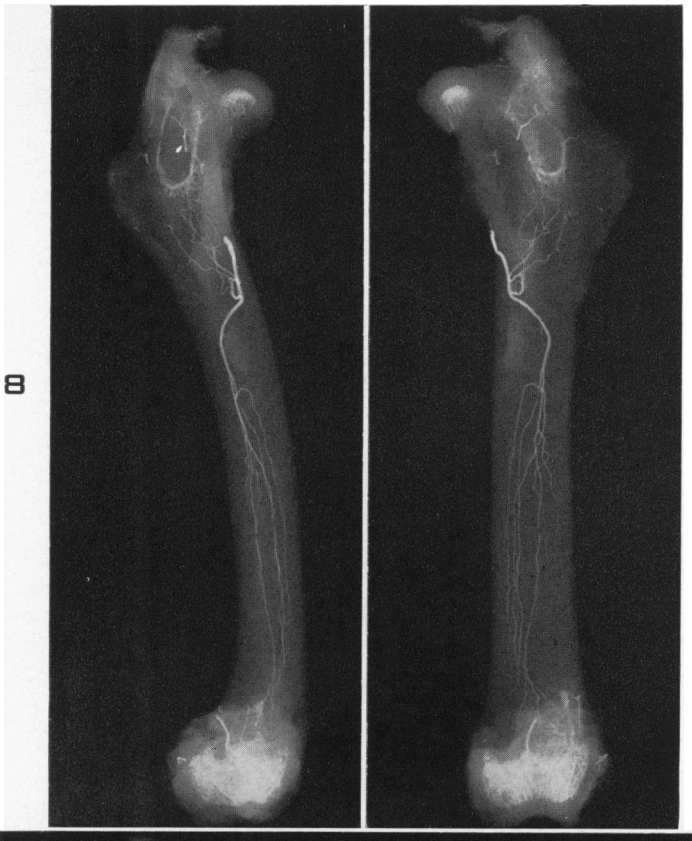


PLATE III

FIG. 8. Anteroposterior and lateral roentgenograms of the femur of a normal, immature rabbit following injection, via the abdominal aorta, of a radio-opaque fluid. The bone has been cleaned and partially decalcified. The small periosteal vessels did not fill, but several epiphyseal arteries, as well as the large nutrient artery and its branches, are delineated.

FIG. 9. Rabbit 157. Anteroposterior and lateral roentgenograms of both femurs 4 months after cutting the nutrient arteries and showing on the right an area of necrotic cortex buttressed by overgrowth of subperiosteal bone. (For comparison with Figures 6 and 7.)



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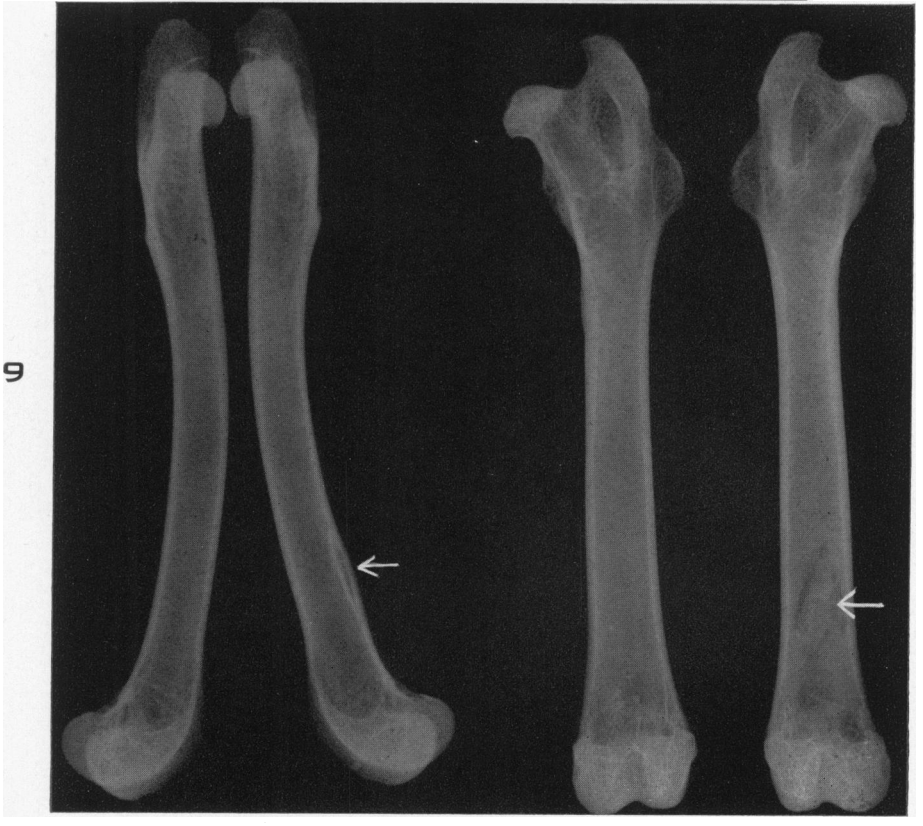

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