

## The intrasosseous vasculature of the ulna of *Gallus domesticus*

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The intrasosseous vasculature of bone has received much attention, the basis of present knowledge being laid by such workers as Langer (1876), Siraud (1895), Lexer (1903, 1904), Lexer, Kuliga & Turk (1904) and Delkeskamp (1906). Most of the work with experimental animals has been done on mammalian bone, the common animals studied being the guinea-pig (de Marneffe, 1951), dog (Drinker, Drinker & Lund, 1922; Johnson, 1927; Rubaschewa & Priwes, 1932), rat (Reichel, 1947; de Marneffe, 1951; McAuley, 1958) and rabbit (Kistler, 1934; Bragdon, Foster & Sosman, 1949; de Marneffe, 1951; Foster, Kelly & Watts, 1951; Brookes, 1957; Brookes & Harrison, 1957; Lemoine, 1957; Morgan, 1959; Trueta & Cavadias, 1964).

In birds, the pattern of growth in length of the long bones differs fundamentally from the mammalian pattern. Growth at the extremities of a chicken long bone is wholly cartilaginous and there are no separate centres of epiphyseal ossification (Haines & Mohuiddin, 1962). The growing cartilaginous ends present three zones, a narrow outer zone of articular cartilage, a wide middle zone of hyaline cartilage, and an inner zone of growth cartilage. Details of the structure of these zones may be found in the works of Wolbach & Hegsted (1952) and Pratt (1961). When proliferation of the cells in the growth cartilage ceases, the marrow tissues of the shaft enter the hyaline zone of cartilage and endochondral osteogenesis spreads through this zone from the shaft.

The vascular arrangements at the growing ends of avian bones may therefore be different from those of mammalian bones. Apart from the limited work of Foote (1921) and Doan (1922), little work has been carried out on the general intrasosseous vascular pattern of growing avian bone.

The vascularization of cartilage, the function of cartilage vessels, and the mechanism by which cartilage canals are formed has also been investigated by several workers (Eckert-Mobius, 1924; Stump, 1925, Haines, 1933; Hurrell, 1934; Trueta, 1957). Brief mention of these vessels was made by Lubosch (1924), Fell (1925), and Whiston (1940), but the first diagrammatic representation of the cartilage canals of the long bones of birds was not published until 1942. Haines (1942) claimed that the canals in birds were essentially similar to those in mammals. He described them as initially opening into the marrow cavity of the shaft, but felt that in later stages the continuity between the canals and the marrow is lost. In addition, he stated that when the cartilage is reduced to a relatively narrow articular layer the canals dwindle away until none is left.

Wolbach & Hegsted (1952) also felt that in the domestic fowl there is a communica-

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tion between the canals of the hyaline zone of the cartilaginous epiphysis and the marrow of the shaft. However, their findings were different from those of Haines (1942). They described the blood vessels of the nutrient system as invading the growth cartilage and extending through it to penetrate the hyaline zone, where they branch and anastomose with each other.

Wolbach & Hegsted (1952) also described the terminal loops of the nutrient system. These were considered to present two to seven or more vessels of capillary dimensions showing evidence of tortuosity and anastomoses.

The present investigation was undertaken in order to determine the adult pattern of both arterial and venous systems of the ulna of the domestic fowl, and to establish the modifications which occur with growth and development of the bone.

#### MATERIALS AND METHODS

Sixty wings of domestic fowls were studied in the investigation, all of them being from uncaponized male birds so that the phenomenon of physiological medullary new bone formation, as seen in laying female birds, might be avoided as a possible complicating factor when the basic vascular patterns were being studied. Some were of the white Leghorn variety, others were a cross between Rhode Island Red and Light Sussex varieties. The ages of the birds ranged from 9 d to approximately 12 months, and wings of both right and left sides were used.

The perfusion technique employed was similar to that described by Trueta *et al.* (1947), Trueta & Harrison (1953), Morgan (1959), Trueta & Cavadias (1964), and others. The material used consisted of a well-stirred mixture of equal parts of Michrone Berlin Blue (30 g in 1000 ml water) and 'Micropaque', a fine 10% barium sulphate suspension able to penetrate as far as the capillary bed (Ardran, 1953). The perfusion was carried out manually with a 20 ml syringe connected to the cannula by a length of polythene tubing. The perfusion was performed very slowly and gently, avoiding excessive flow. The perfusion pressure was not recorded.

In some birds the perfusion was carried out through the subclavian artery after ligation of all major branches not leading to the wing. In others, it was carried out through the corresponding vein. In this way the perfusion was largely confined to the wing itself. To provide additional information concerning the nature and extent of anastomoses occurring within the ulna, several wings were also perfused after preliminary ligation of the principal nutrient artery and vein of the ulna.

The details of the blood supply were then studied by means of fine-grain radiography and microradiography, by the Spalteholz technique on both intact and dissected bones, and by normal histological methods. The latter involved fixation in 10% formalin for 2-3 d and then decalcification in 5% nitric acid. The adequacy of the decalcification process was assessed by fine grain radiography, the average time required being 3 d. After completion of the dehydration and clearance processes, the specimens were embedded in paraffin wax, sectioned, and stained with haematoxylin and eosin.

## OBSERVATIONS

*The arterial supply*(1) *The nutrient artery*

The shaft is chiefly supplied by the nutrient artery which, on reaching the junction of the middle and upper thirds of the bone, passes through the bony cortex at an oblique angle directed distally and enters the marrow (Fig. 1). This site and direction of entry was found at all ages and in all except five of the ulnas examined. In three, aged 35, 196 and 280 d, the nutrient artery penetrated the cortex at right angles at the midshaft level, in the other two, aged 35 and 210 d, it entered at the junction of the middle and lower thirds of the bone and was directed proximally.

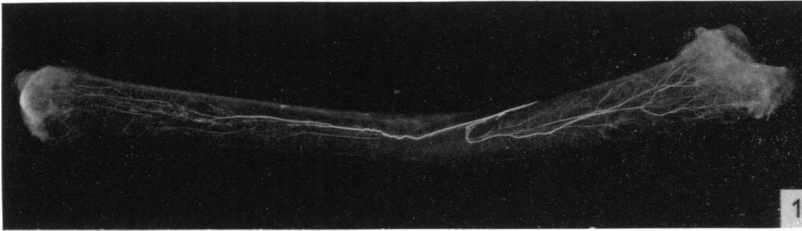


Fig. 1. Arterial perfusion showing the pattern of branching of the nutrient artery system. Bone growth has almost ceased. Radiograph.  $\times 1$ .

Lying just beneath the cortex, the artery continues its oblique course distally for a short distance before dividing into ascending and descending divisions. No minor branches are given off within the shaft of the bone before this major division occurs.

The ascending division often continues distally with the descending division for a short distance before deviating centrally and curving back through almost  $180^\circ$  to pass along the centre of the shaft in a proximal direction. At a variable point, often about the level of the nutrient foramen, it divides into ascending branches. In general, two main ascending branches may be seen which divide and subdivide as they continue towards the proximal end of the bone. However, both the level of division and the apparent number of main branches vary. During its course, the perfused ascending division may appear somewhat tortuous.

The descending division proceeds obliquely onwards as a direct continuation of the main nutrient trunk to reach the centre of the shaft a short distance below the nutrient foramen. It then continues distally along the centre of the shaft and at a variable level, often near the junction of the middle and distal thirds of the bone, divides into descending branches. In general, two main descending branches may be seen which continue towards the distal end of the bone, dividing into further sub-branches as they go. The pattern is more constant, and the vessels tend to be more straight than those of the ascending division, but variations are frequent.

This general arrangement was found at all ages in the bones examined.

The pattern of branching and the general course of the side vessels which arise from the two divisions and their main branches is such that three groups of minor vessels can be described:

(1) Vessels which arise at an acute angle and pass obliquely along the shaft for a

variable distance in the direction of the parent vessel before turning towards the cortex, branching as they go. These do not reach the bone ends and are found at all ages.

(2) A small number of short vessels which arise more or less at right angles from the parent vessel. These pass radially outwards and fan out as they reach the endo-

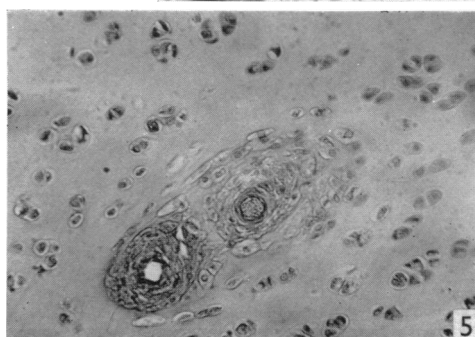
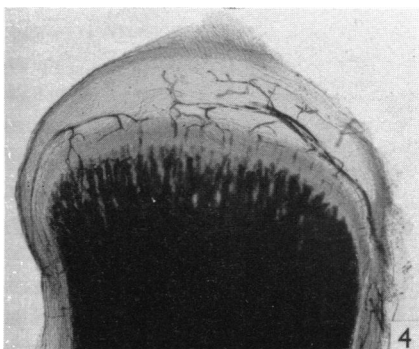
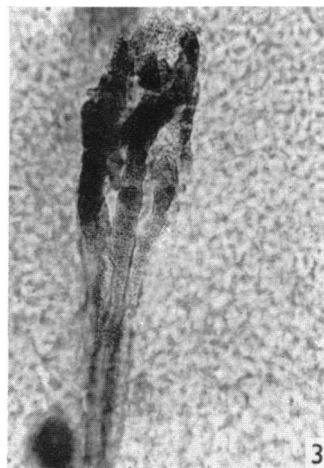
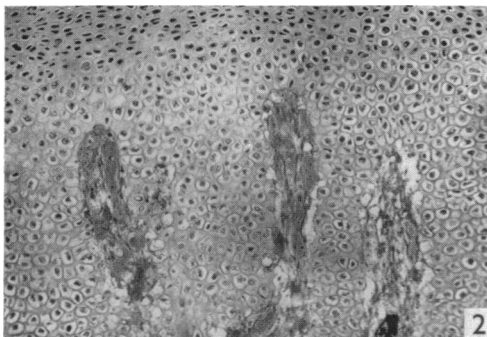


Fig. 2. Invasion of the metaphyseal aspect of the growth plate by terminal vessels of the nutrient artery system. Perfused specimen. H. & E.  $\times 100$ .

Fig. 3. Basket-like pattern of a terminal vascular loop of the nutrient artery system. Spalteholz preparation.  $\times 100$ .

Fig. 4. Young ulna, showing the arching vessels of the cartilaginous epiphysis. Spalteholz preparation.  $\times 16$ .

Fig. 5. Section through the cartilaginous epiphysis of a young ulna showing a cartilage canal. The artery contains perfusion material, Single cartilage cells are found in the immediate vicinity of the vascular bundle, but farther away they appear first in pairs, and then in groups of four. H. & E.  $\times 200$ .

steal surface of the cortex. They are generally restricted to the central section of the shaft, and are seen in the older rather than in the very young bones.

(3) Vessels which arise by repeated division and subdivision of the main branches of the nutrient artery in growing bones, forming a spray of vessels which supply the

growing bone ends. From this spray, which becomes very dense in the sponge-like endochondral zones, individual vascular loops are seen which invade the metaphyseal aspect of the growth cartilage (Fig. 2). These loops are widely, but fairly uniformly spaced. In perfused specimens, some of them appear as simple loops by virtue of the outgoing vessel turning sharply back on itself. However, others are more complex. At its extremity the outgoing vessel is seen to break into several sinusoidal channels which immediately pass backwards and ensheath the principal vessel, giving rise to a bulbous, basket-like pattern (Fig. 3). When proliferation of the cells in the growth plate has ceased and the invasion of the hyaline zone of cartilage has been completed, these vessels are no longer seen.

## (2) *Arteries of the bone ends*

(a) *Growing bones.* In the cartilaginous ends of growing bones, the middle hyaline zone is sparsely penetrated by vessels, some passing directly inwards from the perichondrial vessels, the majority entering the peripheral region of the cartilage near its junction with the bone at the point where the perichondrial and periosteal vessels communicate (Fig. 4). These vessels pass along the so-called cartilage canals and tend to run an arching course in conformity with the convexity of the growth plate. They give rise to few branches and do not anastomose with each other. The cartilage canals are surrounded by cartilage cells which are actively proliferating and producing matrix (Fig. 5), and although the actual distances between the various canals were not accurately measured at all ages, they appear to be relatively closely spaced in young bones compared with the wider spacing seen in adult bones.

Those which arch close to the epiphyseal surface of the growth cartilage are of special interest, because not only do they maintain this close relationship until proliferation of cells in the growth zone has ceased, but they also send occasional blood vessel loops vertically into this zone (Fig. 6). Although some of these loops may appear to pass through the entire thickness of the growth plate, the majority do not and, in the material examined, no communication with the diaphyseal vessels was observed at any stage. The vessels of the cartilaginous epiphysis are nevertheless linked extraosseously with those of the metaphyseal region by virtue of their external connexions with the communicating system of perichondrial and periosteal vessels.

The loops of the cartilage vessels, both those ending in the hyaline zone and those partially penetrating the growth cartilage, present features similar to the pattern described by Trueta (1957) in the cartilaginous epiphysis of the human femoral head. They exhibit a bulbous form by virtue of the artery breaking into a number of capillaries which surround the termination of the artery and then join into a single vein which runs back along the cartilage canal.

(b) *Adult bones.* When proliferation of the cells in the growth cartilage ceases, the brush-like terminal loops of the nutrient artery system, and the marrow itself, start to invade the hyaline zone. The sparse vessels of this zone are slowly enveloped by the advancing front and become incorporated in the blood supply of the terminal region of the endochondral zone of bone (Fig. 7). They initially maintain their arcuate course, but this may become considerably modified in the final stages of growth. In addition, they markedly increase their area of ramification. Small branches pass towards the terminal plate of bone, subdividing to reinforce the

vascular network at the bone end, while others proceed centrally (Fig. 8). These latter vessels develop direct anastomoses with the branches of the nutrient artery (Fig. 9), a feature confirmed by retrograde filling of the main nutrient system of the adult ulna via these vessels after ligation and division of the nutrient artery and vein, combined with periosteal stripping. Together with the metaphyseal vessels, the arcuate vessels appear to largely take over the blood supply of the bone ends in the adult.

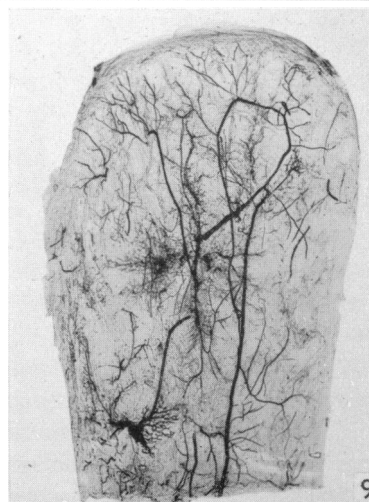
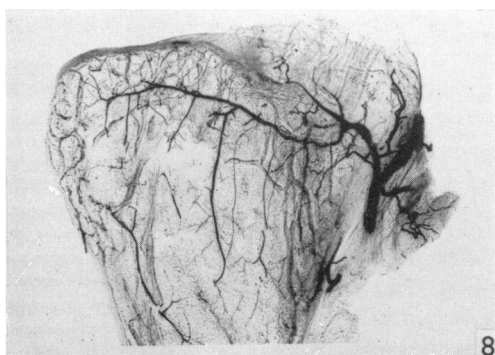
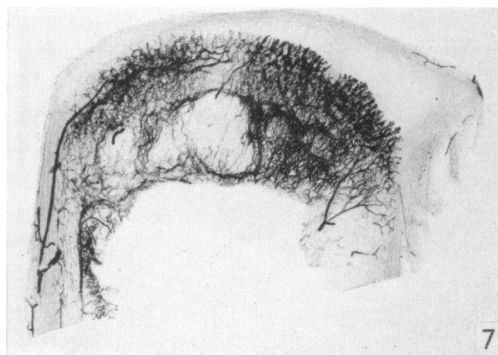
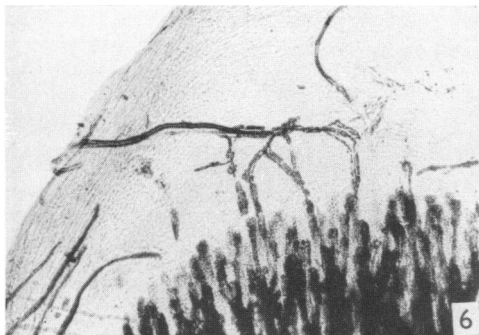


Fig. 6. Young ulna. Vascular loops enter the growth plate from its epiphyseal aspect but do not pass right through. Spalteholz preparation.  $\times 25$ .

Fig. 7. Stage of closure of the growth plate. The vessels of the hyaline zone are becoming enveloped by the advancing diaphyseal vessels. Spalteholz preparation.  $\times 20$ .

Fig. 8. Fully grown ulna, showing the arcuate artery. This vessel originally supplied the cartilaginous epiphysis. Spalteholz preparation.  $\times 16$ .

Fig. 9. Fully grown ulna. The ramifications of the arcuate artery are shown, and an anastomosis with a branch of the nutrient artery is seen. Spalteholz preparation.  $\times 16$ .

### (3) Periosteal and cortical vessels

In addition to small vessels which arise from the nutrient artery before it enters the bone, the periosteal network is supplied by numerous small vessels along the length of the shaft. The largest of these are at the bone ends, especially the proximal end. As they approach the periosteum, they give off numerous branches which spread out at random, subdividing and anastomosing to form an open-laced pattern.

The shaft cortex is honeycombed by a complex system of vascular channels which run more or less parallel to the bone cortex in the midshaft region, but incline obliquely outwards and away from the midshaft in other regions (Fig. 10). This is in conformity with the diverging direction of the branches of the nutrient artery as the bone ends are approached. These channels are linked together by others running at right angles to them.

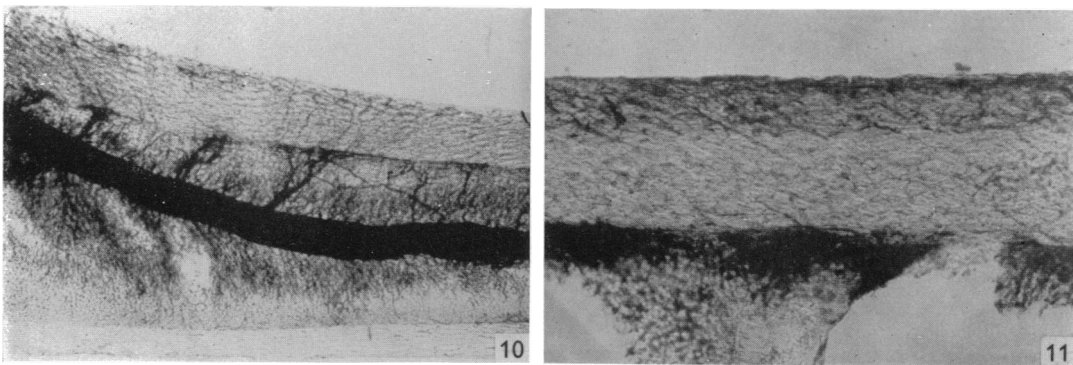


Fig. 10. Venous perfusion. Spalteholz preparation of the midshaft region, showing the varying direction of the vascular channels in the cortex.  $\times 16$ .

Fig. 11. Spalteholz preparation showing the one-third/two-third distribution of cortical vessels.  $\times 36$ .

The cortical system communicates superficially with the periosteal vessels, and at its medullary surface is connected with the branches of the nutrient artery. Occasional vessels, derived in some cases from the periosteal arteries and in others from the nutrient branches, tend to pursue a distinct course, sometimes direct, sometimes devious, through portions of the cortex rather than immediately dispersing into the lattice-like system of vascular channels. The direction of arterial flow through this system was a problem beyond the scope of the present investigation, but it was noticeable that the outer third of the system filled more densely on perfusion (Fig. 11), and that the channels here appeared to be more closely packed than in the inner two-thirds.

At the shaft extremities of growing bones approaching maturity, occasional periosteal vessels were seen to assume the role of true metaphyseal arteries, passing directly through the cortex and breaking into branches which proceeded towards the peripheral fringe of the metaphyseal aspect of the growth plate. No difference was found between the end vessels of these metaphyseal arteries and those of the nutrient artery. These vessels could not be demonstrated in the younger chick ulnas.

*The venous system**(1) The central medullary vein*

The medullary vein consists of centrally placed ascending (distal) and descending (proximal) portions which unite a little proximal to the midshaft level to form one common efferent vein. This passes obliquely out of the bone through the nutrient foramen. The ascending portion passes directly into the common efferent vein, whereas the descending portion characteristically deviates a little posteriorly before looping around to join the ascending portion at a little less than a right angle (Fig. 12).

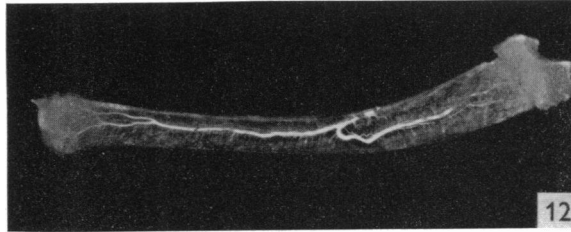


Fig. 12. Venous perfusion showing the general pattern of the medullary vein. Radiograph.  $\times 1$ .

Each of the two divisions is formed by the union of two main tributaries which are in turn formed by the joining of lesser tributaries. The level of the main union is interesting. In young bones up to 14 days, it may correspond with the level of the primary splitting of the divisions of the nutrient artery into branches. In more mature bones, the union is invariably closer to the bone end than the level of primary arterial branching.

The pattern of the tributaries draining into this basic system is quite different from the pattern of arterial division within the bone. The primary units are the extensively distributed, thin-walled venous sinusoids, and three patterns of drainage of these vessels into all sections of the central vein may be seen along the shaft of the bone (Fig. 13):

(a) Sinusoids arising close to the central medullary vein drain directly into the vein itself, or its tributaries, giving it a rather feathery appearance.

(b) Groups of sinusoids which drain areas a little farther away from the central vein tend to channel into small collecting vessels which in turn directly empty into the central vein or its tributaries. This gives rise to a somewhat tufted appearance.

(c) Groups of sinusoids from the peripheral regions of the medullary cavity and from the growing bone ends first drain into small collecting vessels, and these join with other similar vessels to form a branch-like pattern, the stem of which joins the great vein or its tributaries.

Except at the bone ends, the sinusoids or their collecting vessels enter the principal vein at right angles, in marked contrast to the pattern of branching of the arterial system.



(2) *Veins of the bone ends*

These follow their corresponding arteries closely so that in the growing cartilaginous epiphysis, the cartilage canals contain both arteries and veins. No sinusoid networks are seen and there is no drainage across the growth plate to the medullary venous system. Drainage occurs chiefly towards the junctional region between perichondrial and periosteal veins.

With the cessation of proliferation of the cells in the growth cartilage and the invasion of the hyaline zone by the diaphyseal vessels, the veins of the cartilaginous ends become incorporated into the venous drainage of the bone ends in a manner similar to the incorporation of the corresponding arteries (Fig. 14). They may initially be seen as arcuate or transversely running vessels, but their course may be

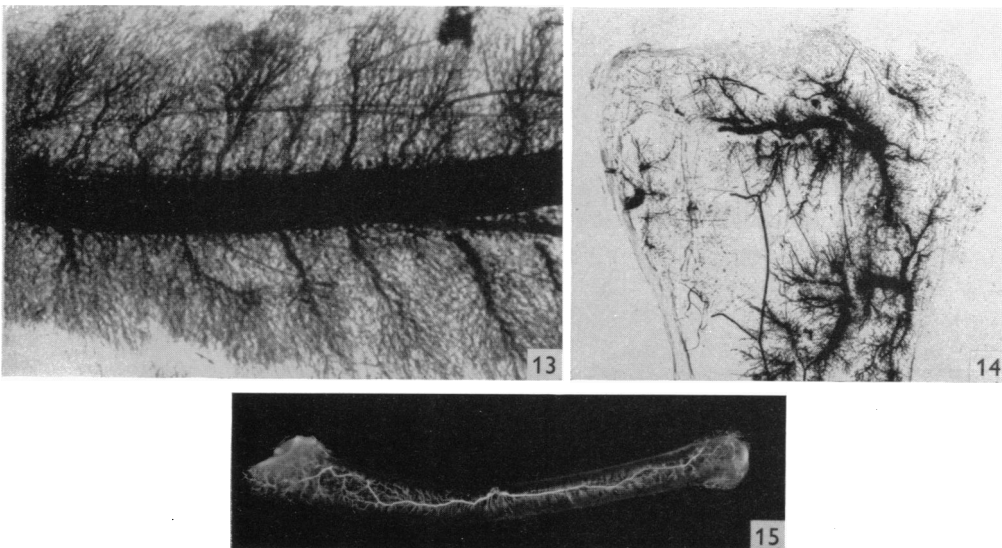


Fig. 13. The medullary vein and its tributaries. Spalteholz preparation.  $\times 25$ .

Fig. 14. The arcuate vein and its sinusoidal tributaries. Spalteholz preparation.  $\times 16$ .

Fig. 15. Adult ulna. Venous perfusion. The course of the arcuate veins is often considerably modified by the final stages of bone growth. Radiograph.  $\times 1$ .

considerably modified with further growth (Fig. 15). At this stage their tributaries assume a sinusoidal appearance, and a linkage occurs with the medullary venous system via the sinusoidal network rather than by the direct anastomosis of larger channels.

(3) *Periosteal and cortical veins*

The system of periosteal veins, including the presence of metaphyseal veins, which are generally more numerous than their corresponding arteries, closely follows the system of arteries. The venous pathways in the lattice-like system of channels of the

shaft cortex likewise communicate with the periosteal veins superficially and with the sinusoids of the peripheral areas of the marrow on the endosteal side of the cortex.

#### DISCUSSION

The *shaft* of the ulna of the domestic fowl is supplied by a nutrient artery and drained by a medullary vein. In the material examined, it was found that irrespective of the breed of fowl and despite occasional anomalies and variations, the general vascular pattern in the shaft is similar to that seen in mammalian bones. However, modifications of the pattern take place during development. The vessels increase in length and diameter as the bone enlarges, and there is a difference in the rate of growth in length of various parts of the arterial and venous systems within the developing bone. One example of this is the changing relation between the level of commencement of the divisions of the medullary vein and the level of branching of the divisions of the nutrient artery as the bone grows.

There was no evidence of any separate, true metaphyseal vessels in the very young bones, although they were observed in growing bones prior to cessation of proliferation of the cells in the growth cartilage. The impression was that they are formed from periosteal vessels at some intermediate stage before the cells of the growth cartilage cease their proliferation. The exact time relationship was not established.

The shaft cortex was observed to be honeycombed by vascular channels which tend to incline obliquely outwards and away from the midshaft in conformity with the diverging direction of the branches of the nutrient artery. The system was found to fill more densely and to be more closely packed in the outer third than in the inner two thirds of the cortex.

The vascular pattern at *the bone ends* and the modifications which occur with development are different from those seen in mammalian bones which have separate centres of epiphyseal ossification.

The growth plate is invaded from its metaphyseal side by numerous, but widely spaced basket-like terminal loops of the nutrient artery system, and from its epiphyseal side by sparse loops from the vessels in the related cartilage canals. Although occasional loops from the epiphyseal side appear to pass through the entire thickness of the growth cartilage, no actual anastomoses across the active growth plate were seen.

The hyaline zones of the growing cartilaginous epiphyses are penetrated by non-anastomosing blood vessels which run through a system of cartilage canals. These canals were found to be surrounded by cartilage cells which were actively proliferating and producing matrix, and there was observed to be an apparent increase in the distance between the canals in the hyaline zone as the cartilaginous epiphyses enlarged. Accordingly, it must be concluded that the canals act as centres around which cartilage proliferation occurs. However, whether the cartilage cells in the immediate vicinity of the canals proliferate preferentially by virtue of their proximity to the blood supply conveyed by the canals, or whether the cartilage cells are actually derived from the proliferation and differentiation of the mesenchymal cells surrounding the vessels was not determined.

In no bone examined did hyaline cartilage degeneration or secondary epiphyseal

ossification occur in the cartilaginous epiphyses. It seems reasonable to assume that activity of the growth plate ceases before progressive separation of the cartilage canals reaches a stage sufficient to cause degeneration. With cessation of proliferation of the cells of the growth cartilage, the terminal loops of the nutrient artery system were seen to invade the hyaline zone, enveloping the vessels of this zone. In association with this, the processes of ossification were observed to spread into the cartilaginous epiphysis from the diaphysis. In this way, both the arteries and veins of the hyaline zone were found to become incorporated in the blood supply of the adult bone end and to develop anastomoses with the blood vessels of the shaft.

At the ends of some mammalian bones, such as the distal end of the phalanges and the distal end of the first metatarsal (man), there are no secondary epiphyseal centres of ossification. In these situations the bone end is ossified from the shaft as in the long bones of birds. Dodds (1930) described the pattern of the cartilage cells in the distal end of the phalanx but made no comment on the vascular pattern. However, Haines (1933) observed the cartilage canals at the distal end of the first metatarsal bone. He considered that 'as the bone of the shaft encroaches on the cartilage, reducing it to a thin articular plate, the canals disappear'. In view of the present findings in the domestic fowl, it may be that further investigations of the blood supply of these special areas in mammalian bone might reveal similarities to the avian pattern.

#### SUMMARY

The intraosseous vasculature of the ulna of the domestic fowl (*Gallus domesticus*) was studied by means of radiological, Spalteholz and histological techniques following perfusion of the vessels. The basic pattern of both arterial and venous systems is described together with an account of the modifications which occur with growth and development of the bone during the period 9 d to 12 months. Similarities to, and differences from, the mammalian pattern have been mentioned.

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