

EPIPHYSIAL STRUCTURE IN LIZARDS AND MARSUPIALS

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INTRODUCTION

THE general arrangement of the secondary centres of lizards, first described by Dollo (1884), has been discussed by Moodie (1908) and Fuchs (1908) who give a good account of the earlier literature. The histological structure has been studied by Heidsieck (1928) in the Geconidae and Agamidae, and by several authors, especially Wallis (1928) in *Lacerta*. Here a more detailed account of a typical centre will be given and the mechanism of union described, and the description extended to include other groups not previously studied. Some atypical forms will be figured for the first time, particularly that found in *Varanus*, in which cartilage canals have been developed.

In mammals the presumably primitive type of epiphysial centre will be described in marsupials, again for the first time, and the remarkable parallels between mammalian and reptilian evolution in epiphysial structure discussed.

EPIPHYSES OF TYPICAL LIZARDS

In *Agama atra*, which possesses epiphyses of the ordinary type found in lizards, the cartilage at the end of each long bone shows a large secondary centre which divides the articular cartilage (*a.r.*) from the zone of growth (*z.g.*) over the greater part of their extent. In the young bone a zone of undifferentiated cartilage (*z.u.c.*), with the cells collected into small clumps, surrounds the secondary centre. The periphery of the centre itself is composed of calcified cartilage (*c.c.s.c.*), which is seen spreading by narrow processes (*pro.*) among the groups of cells in the undifferentiated zone, so as finally to isolate these groups in the calcified matrix (*i.g.*).

The calcification of the secondary centre always begins in the more central parts of the cartilaginous epiphysis, and is at first widely separated from the articular cartilage, the growth cartilage and the perichondrium (Nauck, 1936, *Lacerta*; Haines, 1940, *Mabowia*). As the calcified area expands at the expense of the undifferentiated cartilage it eventually comes to lie near the perichondrium at one or more points, whereupon the perichondral tissues erode the matrix by wide blunt processes, without at first forming endochondral bone, a stage seen in *Ichnotropis longipes*. In the *Agama* figured the calcification has spread beneath the perichondrium towards both the articular surface and the shaft (*s.p.s.c.*). The perichondral tissues have made their way into the centre by a relatively narrow entrance (*e.t.s.c.*), and have replaced much of the

calcified cartilage by marrow spaces (*m.s.s.c.*), while on the remains of the cartilage endochondral bone (*e.b.*) has been laid down. In the zone of growth the cells lie in well-marked longitudinally arranged clumps, indicating active division, and in the zone of hypertrophy (*z.h.c.*) the cells form cartilage columns (*c.clm.*). The bays of erosion (*b.e.*) and the endochondral bone have the usual reptilian arrangement.

This kind of epiphysial structure is probably found in all groups of lizards in which the secondary centres become ossified, with the sole exception of the *Varanidae*. This has been confirmed in *Geconidae*, *Agamidae*, *Iguanidae*,

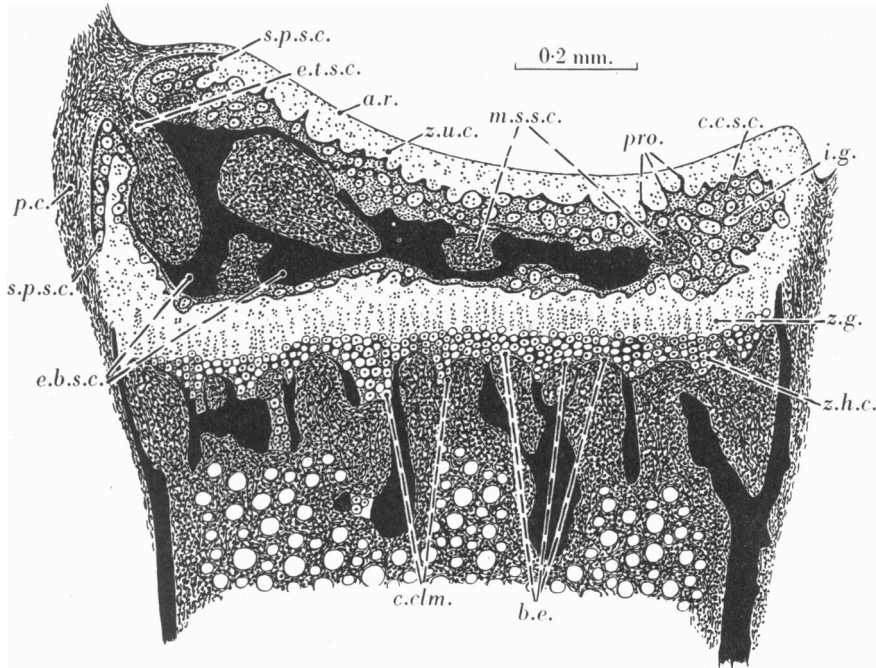


Fig. 1. Head of the radius of *Agama atra*.

Zonuridae, *Heloderma*, *Tejidae*, *Lacertidae*, *Gerrhosauridae*, *Scincidae* and *Chamaeleontidae*. The number of points of entry of the perichondral tissues is variable—usually one, but sometimes several. Again, in many epiphysial cartilages there are extra centres starting from intratendinous ossifications, e.g. the epicondyles of the humerus, the surface of the olecranon, the tibial tuberosity, the attachment of the cruciate ligaments to the femur, etc. Finally, as in mammals, some of the cartilages of the metacarpals and phalanges are ossified by extensions from the shaft, instead of by separate secondary centres (Heidsieck, 1928). But the type of ossification in the large limb bones is remarkably constant.

UNION OF SECONDARY CENTRES IN LIZARDS

In later stages the secondary centres grow in volume at the expense of the cartilage, but do not usually cut off the articular cartilage from the growth cartilage completely. Contact of the secondary centre with the perichondrium is always limited and very little perichondral bone is formed, in most cases none at all (Fig. 4). Where independent intratendinous ossification centres

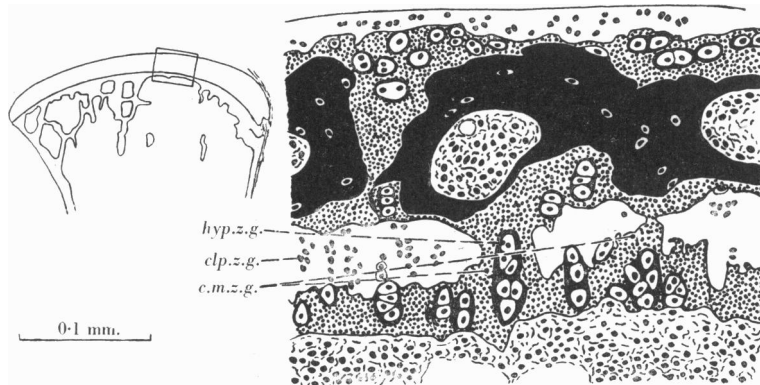


Fig. 2. Upper end of the tibia of *Pseudocordylus microlepidotus*.

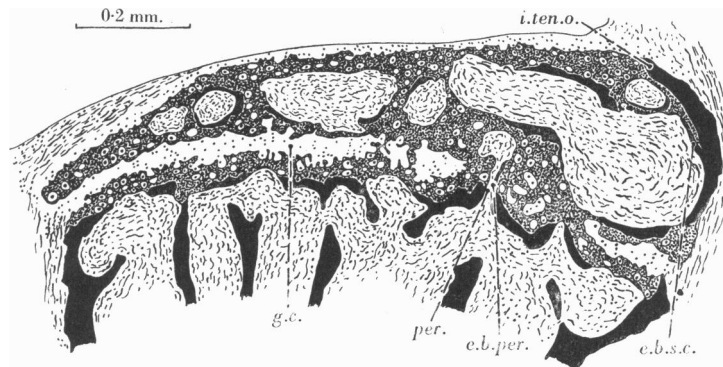


Fig. 3. Upper end of the tibia of *Nucras delalandii*.

have been formed at the insertions of tendons into the epiphysial cartilages, these unite with the main secondary centres before the whole mass unites with the shaft (Fig. 3, *i.ten.o.* and *e.b.s.c.*).

Cessation of growth is first indicated by changes in the growth cartilage (Fig. 2). The cells, while in some regions remaining clumped in a hyaline matrix (*clp.z.g.*), in others all become hypertrophied (*hyp.z.g.*) so that there can be no further formation of cartilage, since the source of young cells is exhausted. The matrix around these hypertrophied cells becomes calcified

(*c.m.z.g.*), so that the calcified cartilage on either side of the growth cartilage is now joined at intervals, and the continuity of the hyaline cartilage broken. Heidsieck (1928) described perforation of the growth cartilage as the earliest stage of union, but it is clear that this co-calcification of the primary and secondary centres precedes the stage of perforation.

In an example illustrating very early perforation of the growth cartilage, of which serial sections were cut (Fig. 3), only one marrow process has actually reached the secondary centre (*per.*). While most of the growth cartilage is still hyaline (*g.c.*) the process has made its way by a narrow canal through the co-calcified region, and has then expanded in the calcified cartilage of the secondary centre, but has as yet made no communication with the marrow spaces of the secondary centre. Along the course of the process some endochondral bone has been laid down (*e.b.per.*), further narrowing the canal as it passes through the growth cartilage. The epiphysial centre itself appears immature, there being a large proportion of uneroded calcified cartilage and little endochondral bone, though the intratendinous bone laid down at the insertion of the patellar tendon (*i.ten.o.*) and the endochondral bone of the secondary centre (*e.b.s.c.*) have already joined. It is clear that the early perforation is carried out entirely by the activity of the marrow of the shaft, that of the secondary centre taking no part in the work.

In mammals there is unfortunately no work published on the histological changes attending the normal union of epiphysial centres. Since the surface of the epiphysial centre towards the shaft usually shows no growth activity, or alternatively active erosion (Payton, 1933; Krompecher, 1937), while the tissues of the shaft are continually eroding the calcified cartilage, it is reasonable to suppose that it is the tissues of the shaft and not those of the secondary centre that are ultimately responsible for union. But the actual occurrence of co-calcification of the shaft and epiphysial centre followed by the growth of marrow processes through the co-calcified areas is, it is believed, described and figured here for the first time in any animal.

As a result of the limited area of erosion of the growth cartilage, isolated fragments of this cartilage may remain as inert masses enmeshed in the trabeculae of endochondral bone for an indefinite period. Heidsieck (1928) considers such a phase normal in lizards, having found it in three species, and it is illustrated here (Fig. 4) in a Cape chameleon. The animal was a fully adult female, with well-developed embryos (the species is viviparous). The remains of the growth cartilage (*is.g.c.*) are completely surrounded by bone (*e.b.*) and therefore incapable of further growth. In other species (Wallis, 1928, *Lacerta*; Heidsieck, 1928, *Ptychozoon*) complete disappearance of the cartilage is described.

In some lizards such as *Phyllodactylus porphyreus*, a small gecko, the secondary centres are never ossified, but remain even in the adult as masses of calcified cartilage (Fig. 5, *c.c.s.c.*). The specimen figured was taken from a fully grown female containing ripe eggs. The growth cartilage (*z.g.*) has

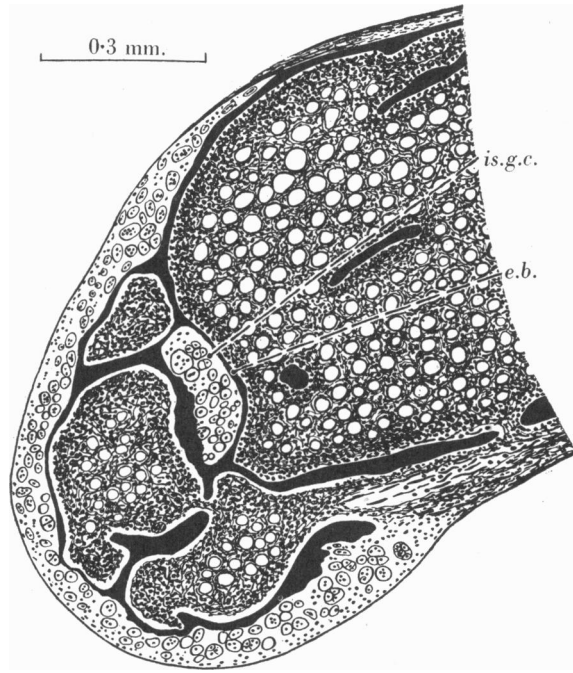


Fig. 4. Lower end of the femur of *Lophosaura pumila*.

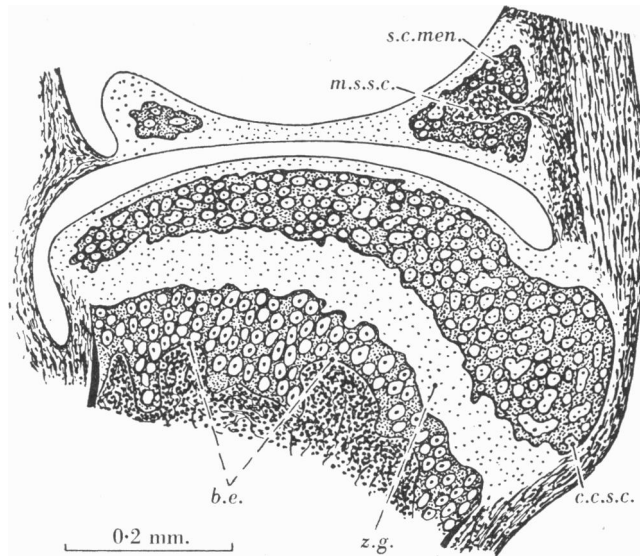


Fig. 5. Upper end of the tibia and semilunar cartilages of *Phyllodactylus porphyreus*.

remained uncalcified, but all trace of special arrangement of the cells has been lost. All the other large long bones show a similar structure of the epiphyses, with massive centres of calcified cartilage, but no marrow or bone. Now at first sight such an epiphysis might appear primitive, for large secondary centres persisting to a late stage of development are found in *Sphenodon* (Haines, 1939), though there they are ossified in the fully adult animal. But it seems more probable that in *Phyllodactylus*, and some other small lizards with a similar structure, the endochondral bone of the epiphyses has been secondarily lost, for in many members of its family the epiphyses are normally developed. The endochondral bone of the shafts has also been reduced, though the bays of erosion (*b.e.*) are found normally on the surface of the cartilage. The semilunar cartilages show a structure similar to that of the epiphyses, but one of the secondary centres (*s.c.men.*) shows a small marrow space (*m.s.s.c.*) eroded by the perichondral tissues. Here again, however, there is no endochondral bone, though a bony structure is usual in this situation in lizards. As regards both the short and the long bones, therefore, *Phyllodactylus* is to be regarded as neotenic in structure rather than as truly primitive. Among mammals a similar neotenic change, the failure in union of the secondary centres at maturity, has been described by Dawson (1929, 1935) in mice and rats.

EPIPHYSIAL STRUCTURE IN *VARANUS*

The Varanidae, now comprising the single genus *Varanus*, contain the largest of living lizards, and are widely separated from all other groups. Parsons (1905), Moodie (1908) and Fuchs (1908) have figured dried and cleared specimens showing secondary centres, but the finer structure has never been described.

In a newly hatched *Varanus niloticus* the epiphyses contained secondary centres of calcified cartilage (Fig. 6, *s.c.*). The calcification was diffuse, spreading in the matrix between the somewhat hypertrophied cells. This kind of calcification is quite normal in the secondary centres of lizards and is illustrated elsewhere (Haines, 1940), but is unknown in non-reptilian centres.

On the ventral surface the epiphysis overhangs the shaft, as is usual in lizards when the plane of the articular surface is set obliquely to the line of the shaft (Heidsieck, 1928). Between the shaft and this overhanging part of the epiphysis the perichondral tissues give rise to a system of cartilage canals (*can.*). These branch so as to supply the interior of the epiphysis, their terminations (*t.can.*) lying near the articular surface. The canals are somewhat irregular in shape, and occasionally in individual sections islands of cartilage (*is.c.*) are found lying apparently free in the tissue of the canals, but in neighbouring sections they are always found to be joined with the main mass. There are no anastomoses between individual canals, and the numerous blood vessels (*b.v.*) must therefore be 'end artery' systems. Near the canals there is no calcification of the cartilage matrix, and the cells pass by gradual transition from

the loose connective tissue of the canals (*c.t.can.*) through a region of flattened cells (*f.c.*) to the undifferentiated cartilage with small round scattered cells (*r.c.*), and again the transition from these to the hypertrophied cells of the region around the secondary centre is very gradual. In all these characteristics the canals of the young *Varanus* parallel those of mammals (Haines, 1933), and here again the canals appear to be acting as an internal perichondrium forming new cartilage cells at their periphery as well as carrying blood vessels into the interior of the cartilage.

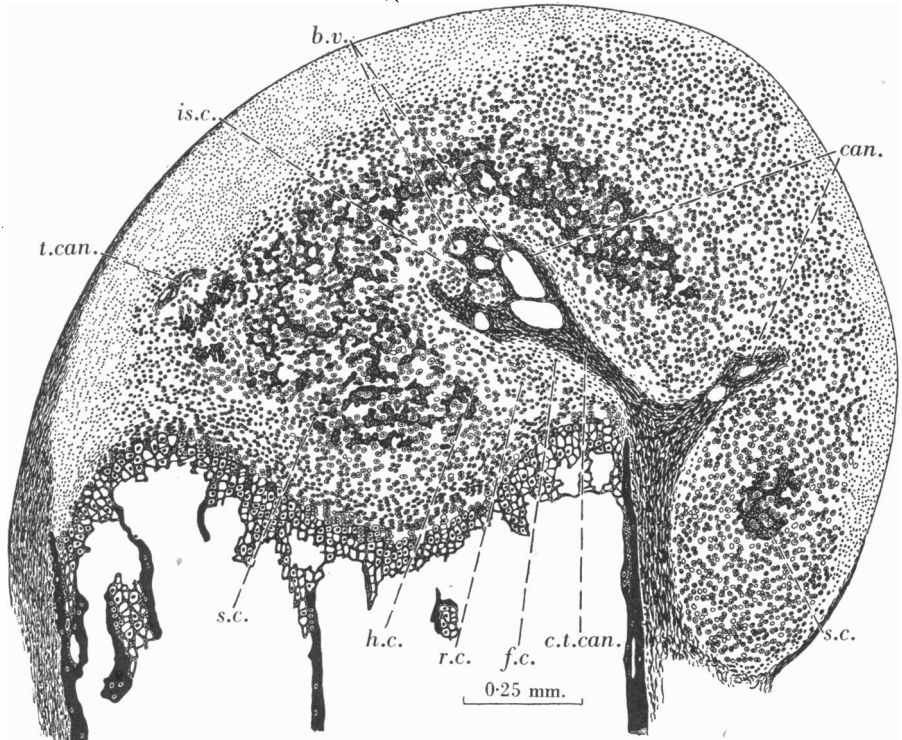


Fig. 6. Lower end of the femur of a newly hatched *Varanus niloticus*.

In an older *Varanus albogularis* the cartilage cells are markedly clumped (*clp.*) and the calcification is now spreading among these clumps so as to surround and isolate them. In many places series of growth lines (*g.l.*) mark the former extent of calcification. The older parts of the calcified matrix are dense (*d.c.m.*) and show splitting, an artifact of decalcification noticed in *Sphenodon* (Haines, 1939). The canals (*can.*) are now sharply bounded on all sides, and there can be no further contribution of cartilage cells to the epiphysial centre, which is heavily calcified and no longer capable of further expansion. The blood vessels (*b.v.*) are active and contain blood corpuscles.

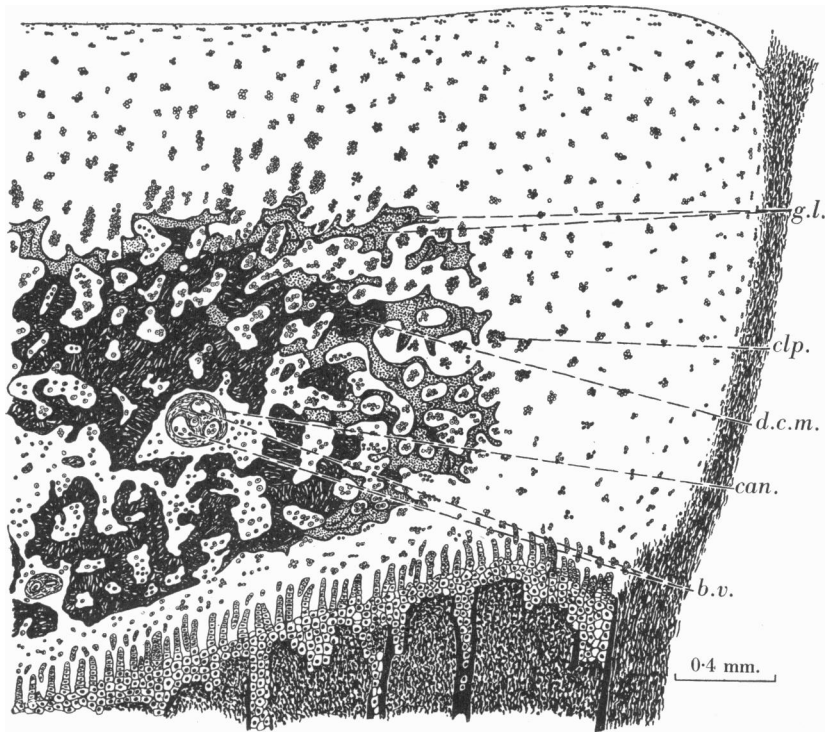


Fig. 7. Upper end of the radius of *Varanus albigularis*.

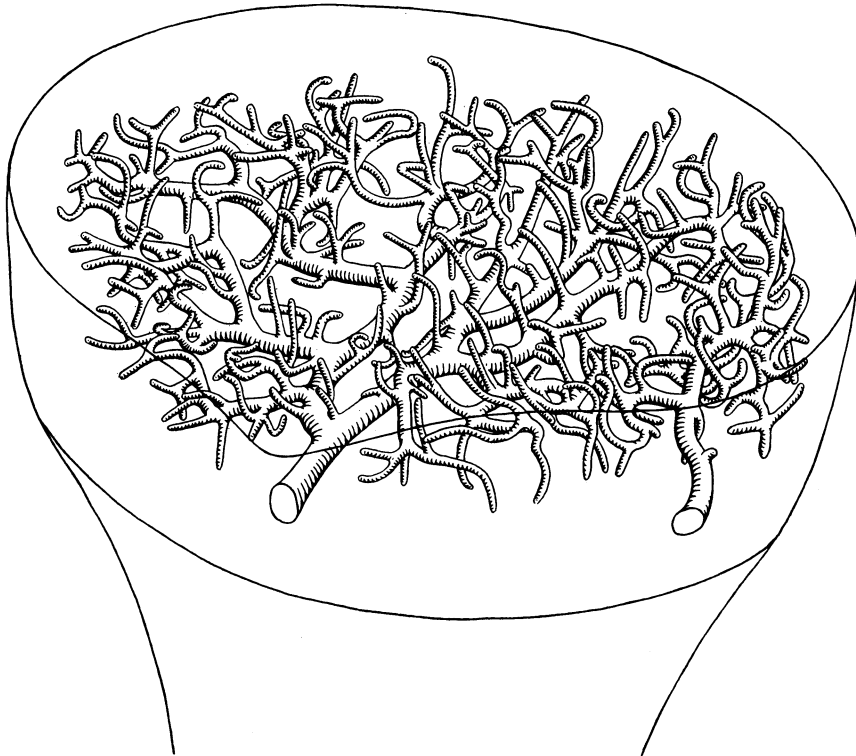


Fig. 8. Reconstruction in celluloid of the cartilage canals in the upper end of the radius of *Varanus albigularis*.

A reconstruction (Fig. 8) shows the intricacy of the canals at this stage, shortly before ossification begins in the secondary centre. They are more highly developed than any described in mammals, and have fewer points of entry from the perichondrium (only two from the lateral surface in the head of the radius), but otherwise their arrangement is similar to that in mammals.

When ossification of the epiphysial cartilage sets in, it is of course modified by the presence of the cartilage canals. At the lower end of the humerus of a *Varanus exanthematicus* the canals enter from both anterior and posterior surfaces of the bone, those marked *A* (Fig. 9) coming from the one surface and those marked *B* from the other. They ramify through the whole bulk of the cartilage, and there is a specially rich supply to the cartilage overlying the growth zone (*can.z.g.*), but no canal was seen to pierce the growth zone to reach the shaft. The cartilage is calcified as before and shows similar artifacts.

Three secondary centres (*s.c.*) are developed, one for each epicondyle (*ep.med.* and *ep.lat.*) and a central ossification underlying the articular surface, an arrangement paralleled among mammals in the rat (Strong, 1925) and guinea-pig (Harman & Saffry, 1934). The central ossification is nowhere in contact with the perichondrium, being entirely surrounded by cartilage, so that the tissues forming its bone and marrow must have been derived from the tissues of the cartilage canals. Further, since it is supplied by canals from both surfaces, its original position presumably lay between the two sets, which therefore determine the position of the centre. Again, by their spread the centres have cut off the ends of some of the canals that previously traversed the cartilage now replaced by bone, so that these ends now appear to arise from the centres instead of from the perichondrium (Haines, 1933, centrifugal canals; Fig. 9, *cen.can.*). In all these points the Varanidae resemble the mammals.

The epicondylar centres probably began as intratendinous ossifications with secondary spread into the underlying cartilage, similar to those described at the insertion of the quadriceps and triceps, for they have been seen to arise in this way in other lizards. As they spread into the cartilage, however, these centres also gain a blood supply from the cartilage canals.

In later stages the centres join to form a single mass, which has been figured by Fuchs (1908). The canals are now entirely destroyed and the structure is similar to that of epiphyses which have never contained cartilage canals and have been ossified by direct perichondral invasion. Moodie (1908) and Fuchs (1908) are agreed that in *Varanus* the secondary centres do not join the shafts of the long bones, even in old animals.

It has been suggested that *Varanus* is closely related to *Heloderma*, a lizard belonging to another rather isolated group, and Moodie (1908) has claimed a special similarity of arrangement of the secondary centres which would confirm this view. The study here put forward, however, supports the more orthodox view that the Varanidae have no special relationships to other lizards, for *Heloderma* has typical epiphyses without canals.

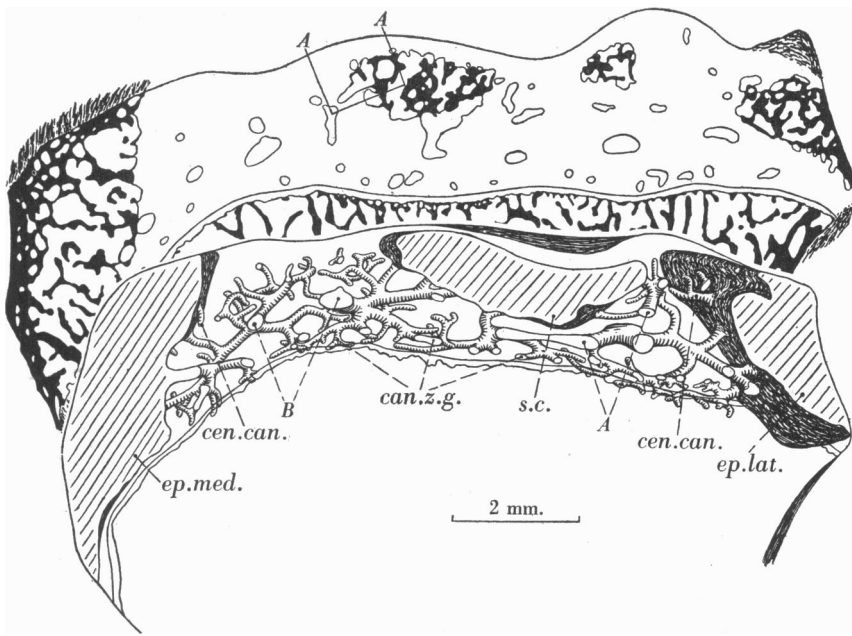


Fig. 9. Section and reconstruction of the lower end of the humerus of *Varanus exanthematicus*.

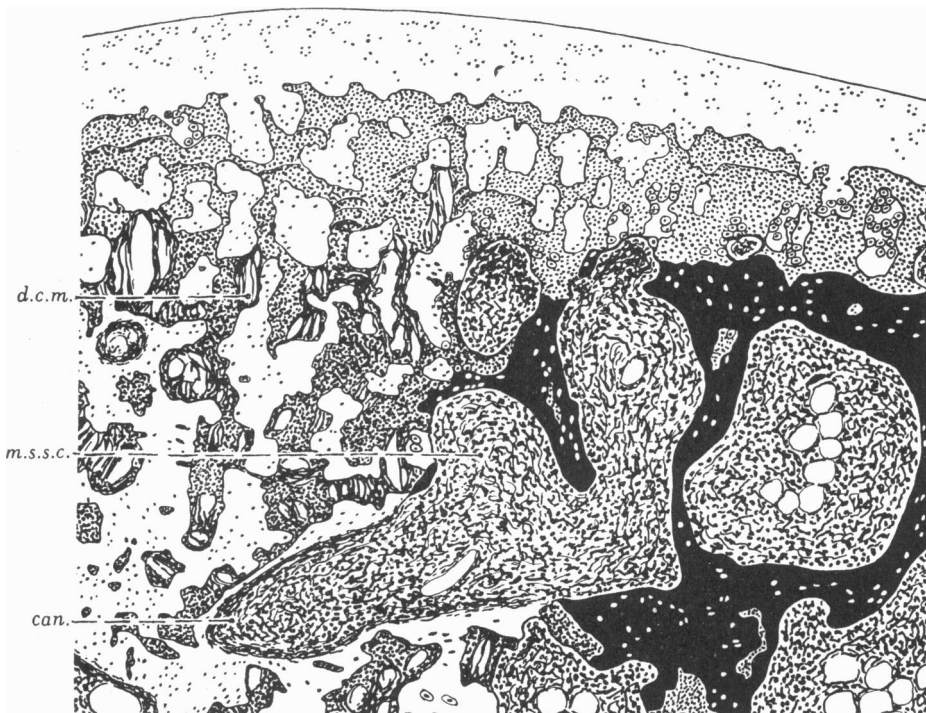


Fig. 10. Enlargement of the portion marked AA in Fig. 9.

EPIPHYSES IN MARSUPIALS

In mammals as in lizards ossification of the epiphyses may occur either with or without pre-existent cartilage canals. But the only examples so far known of ossification without canals, the rat and mouse (Haines, 1933), appear to have lost the canals secondarily, owing to the small size of the cartilages, for Bidder (1906) found them in the rabbit. In marsupials, however, the canals

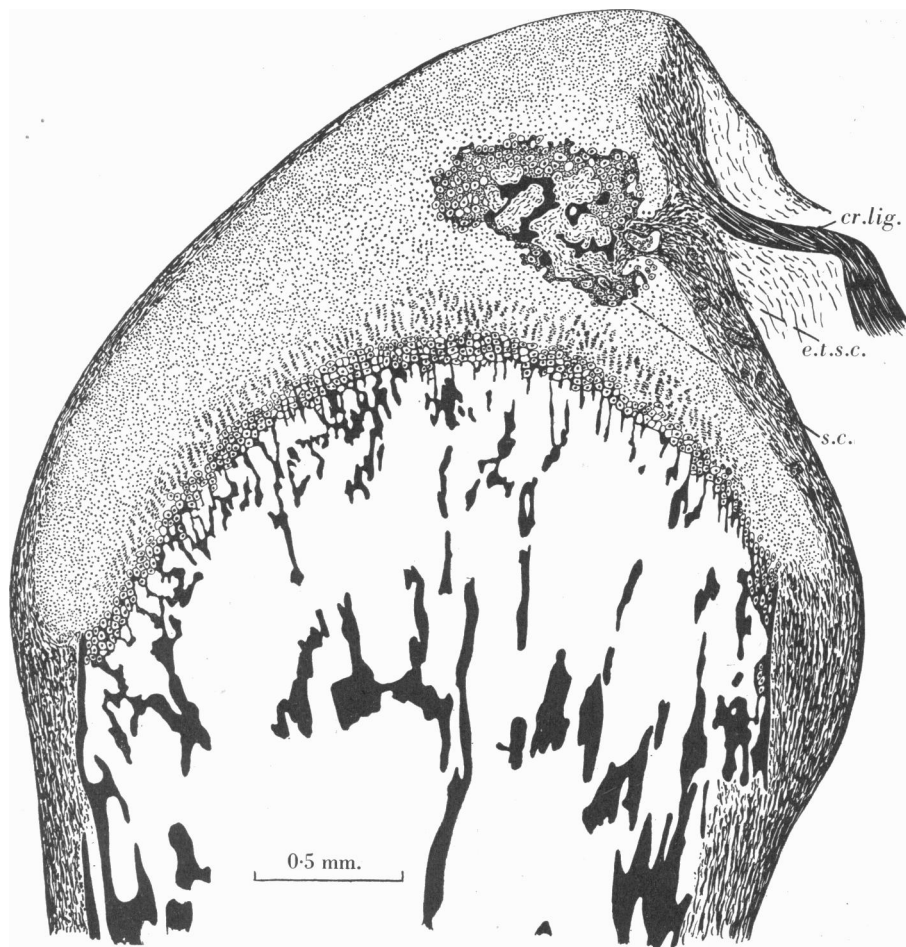


Fig. 11. Lower end of the femur of *Didelphis marsupialis azarae*.

do appear to be primitively absent. In a young *Didelphis* with a small secondary centre in each condyle of the femur, an arrangement found also in the mouse by Johnson (1933), no canals were found (Fig. 11). Indeed, apart from the relatively small size of the area of calcified cartilage and its rapid ossification, the histological picture is very similar to that seen in the typical

lizards. The absence of canals has also been confirmed in *Macropus* and *Dendrolagus*, animals sufficiently large to show canals if these occurred in marsupials.

Thus in the mammals and the lizards a phylogenetic stage of perichondral ossification has passed into a stage of ossification by cartilage canals by one of the most precise parallelisms known in evolution. But whereas only one group of lizards has acquired canals, the vast majority of mammals have them.

If absence of canals is truly primitive in mammals, the monotremes must have acquired them independently. But they are of very peculiar form and their development is unknown.

SUMMARY

1. In typical lizards (all families studied except Varanidae) the secondary centres begin as masses of calcified cartilage which, when they approach the perichondrium, are replaced by marrow and bone.

2. When growth is completed the cells of the growth zone lose their typical arrangement and the secondary centres are joined to the shaft by calcified cartilage. Perforation of the cartilage takes place first through the regions of co-calcification.

3. The failure of ossification or union of secondary centres found in some lizards is a secondarily acquired neotenic feature.

4. The Varanidae show systems of cartilage canals whose structure and fate are very precisely paralleled among the mammals.

5. In marsupials there are no canals and the secondary centres are ossified directly from the perichondrium. This is presumably the primitive arrangement in mammals.

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KEY TO LETTERING

<i>a.r.</i>	articular region	<i>e.b.per.</i>	endochondral bone related to perforation
<i>b.e.</i>	bay of erosion	<i>e.b.s.c.</i>	endochondral bone of secondary centre
<i>b.v.</i>	blood vessel	<i>ep.lat.</i>	lateral epicondyle
<i>can.</i>	canal	<i>ep.med.</i>	medial epicondyle
<i>can.z.g.</i>	canal of growth zone	<i>et.s.c.</i>	entrance of tissues of secondary centre
<i>c.clm.</i>	cartilage column	<i>f.c.</i>	flattened cells
<i>c.c.s.c.</i>	calcified cartilage of secondary centre	<i>g.c.</i>	growth cartilage
<i>cen.can.</i>	centrifugal canal	<i>g.l.</i>	growth line
<i>clp.</i>	clump of cells	<i>h.c.</i>	hypertrophied cell
<i>clp.z.g.</i>	clump of cells in growth zone	<i>hyp.z.g.</i>	hypertrophied cell of growth zone
<i>c.m.z.g.</i>	calcified matrix of growth zone	<i>i.g.</i>	isolated group of cells
<i>cr.lig.</i>	cruciate ligament	<i>is.c.</i>	isolated cartilage
<i>c.t.can.</i>	connective tissue of canal	<i>is.g.c.</i>	isolated growth cartilage
<i>d.c.m.</i>	densely calcified matrix	<i>i.ten.o.</i>	intratendinous ossification
<i>e.b.</i>	endochondral bone		

<i>m.s.s.c.</i>	marrow space of secondary centre	<i>s.c.men.</i>	secondary centre of meniscus
<i>p.c.</i>	perichondrium	<i>s.p.s.c.</i>	sub-perichondral spread of calcification
<i>per.</i>	perforation	<i>t.can.</i>	termination of canal
<i>pro.</i>	process	<i>z.g.</i>	zone of growth
<i>r.c.</i>	round cell	<i>z.h.c.</i>	zone of hypertrophied cells
<i>s.c.</i>	secondary centre	<i>z.u.c.</i>	zone of undifferentiated cells

REFERENCES

- BIDDER, A. (1906). *Arch. mikr. Anat.* **68**, 137.
 DAWSON, A. B. (1929). *Anat. Rec.* **43**, 109.
 — (1935). *Anat. Rec.* **63**, 93.
 DOLLO, M. L. (1884). *Zool. Anz.* **7**, 65.
 FUCHS, H. (1908). *Anat. Anz.* **32**, 352.
 HAINES, R. W. (1933). *J. Anat., Lond.*, **68**, 45.
 — (1939). *J. Anat., Lond.*, **74**, 80.
 — (1940). *J. Anat., Lond.*, **75**, 101.
 HARMAN, M. T. & SAFFRY, O. B. (1934). *Amer. J. Anat.* **54**, 315.
 HEIDSIECK, E. (1928). *Morph. Jb.* **59**, 343.
 JOHNSON, M. L. (1933). *Amer. J. Anat.* **52**, 241.
 KROMPECHER (1937). *Die Knochenbildung*, pp. 150. Jena: Fischer.
 MOODIE, R. L. (1908). *Amer. J. Anat.* **7**, 442.
 NAUCK, E. T. (1936). *Morph. Jb.* **77**, 372.
 PARSONS, F. G. (1905). *J. Anat., Lond.*, **39**, 402.
 PAYTON, C. J. (1933). *J. Anat., Lond.*, **67**, 371.
 STRONG, R. W. (1925). *Amer. J. Anat.* **36**, 313.
 WALLIS, K. (1928). *Z. Zellforsch.* **7**, 257.