# THE STRUCTURE OF THE EPIPHYSES IN *SPHENODON* AND THE PRIMITIVE FORM OF SECONDARY CENTRE

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## INTRODUCTION

The epiphyses of the early tetrapods are now known to have consisted of pads of hyaline cartilage overlying the convex ends of the long bones, thicker in the younger and thinner in the older animal, but with no secondary centres of ossification or calcification (Haines, 1938). But it has long been known that secondary centres are found in *Sphenodon* and in lizards, and in several of the fossil representatives of the groups to which they belong, and also in some fishes, anurans, birds and mammals. The detailed structure of these centres has however been studied little, and it will be the purpose of this paper to describe their microscopic structure in *Sphenodon*, the most primitive animal possessing well-developed secondary centres, and then to discuss their evolution and their further phylogenetic development in some other groups of animals.

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## THE EPIPHYSES IN *SPHENODON*

Secondary centres in the spines of the vertebrae of *Sphenodon* were reported by Albrecht (1883), the earliest record of such centres in reptiles. Howes & Swinnerton (1901, Pl. 6, figs. 14, 18) figured centres of the distal ends of the radius and ulna, but did not describe them in the text. Moodie (1908) failed to find any trace of the centres in his cleared preparations, and doubted the accuracy of the earlier observations. His material however was fully adult, and his conclusion that epiphysial centres were rarely present in *Sphenodon* was unjustified. Lubosch (1910) appears to be the only author who has prepared or figured microscopic sections of the epiphyses, but though his figure of the metatarsal shows a large secondary centre, it is not clear enough to show whether it is made of bone or cartilage. Heidsieck (1929), who has given an excellent account of the microscopic structure of the long bones of *Sphenodon*, and who in his work on lizards (1928) has examined reptilian epiphyses, had no young material available for study, and states specifically (in his paper) that he was unable to determine the structure of the secondary centres.

Several young individuals are now available for study. A dried forelimb and hindlimb skeleton in the British Museum (Index Collection), a cleared tarsus in the Dendy collection at King's College, and a radiograph of a com-

plete individual at University College leave no doubt as to the existence of secondary centres, the last confirming Albrecht's observations of separate centres for the vertebral spines. Further, I have been able to prepare sections of bones from four individuals, of 105 mm. total length (about 55 mm. snout-vent length) given me by the late Dr McMaster, of 149 mm. snout-vent length by courtesy of the Zoological Society of London and of two subadults, one sent by Prof. Gowland and one of 225 mm. from Prof. Appleton's collection.

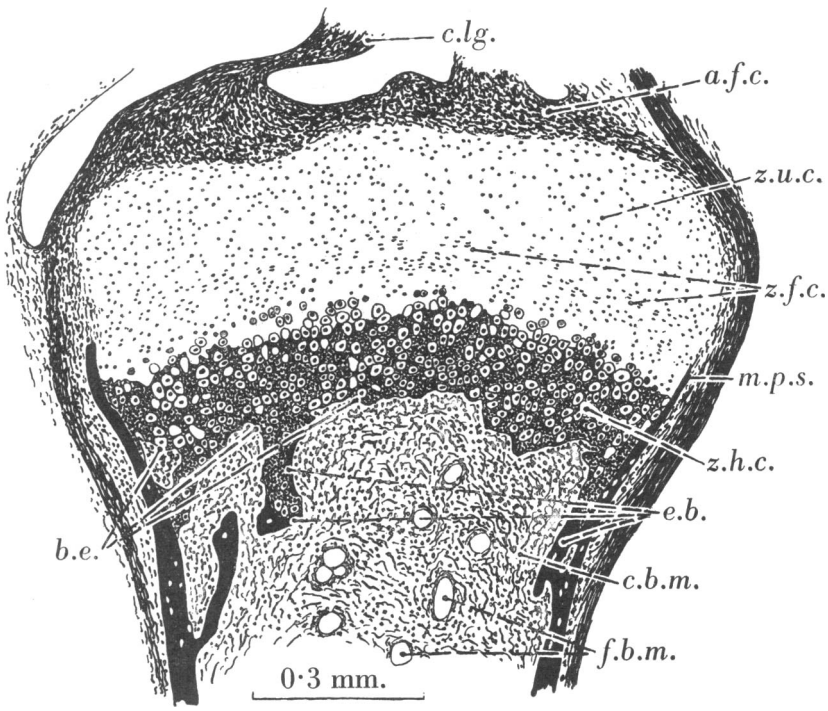


Fig. 1. Longitudinal section of the upper end of the tibia in a young *Sphenodon*. Total length 105 mm.

In the youngest specimen (serial of knee prepared), the essential epiphysial structure is well shown in the tibia but no secondary centres are yet developed. A thick articular fibro-cartilage (*a.f.c.*) covers the articular and non-articular surface, and gives attachment to the cruciate ligaments (*c.lg.*). The main mass of hyaline cartilage contains undifferentiated cells only (*z.u.c.*). At the level of the growing margin of the periosteal shaft (*m.p.s.*) this zone passes into the growth cartilage, a layer of flattened cells (*z.f.c.*) which is convexly bulged towards the articular surface. The zone of hypertrophied cells (*z.h.c.*) is well developed, and the calcification of the matrix is continuous, but the cells are as yet quite irregular in their arrangement. The surface facing the marrow is irregular, with large bays of erosion (*b.e.*) cut into the cartilage, indicating active destruction. Some endochondral bone (*e.b.*) has been laid down on the

cartilage, but it is not yet well developed. The marrow is cellular (*c.b.m.*), with a few scattered fat spaces (*f.b.m.*).

In the 149 mm. specimen (upper end of femur cut) the structure is profoundly altered by the formation of a secondary centre consisting of a mass of hypertrophied cartilage with a calcified matrix within the zone of undifferentiated cells. The endochondral bone is more fully developed. Since however the structure is similar to that of the next individual in the series, which is much better preserved, it will not be described in detail.

In Prof. Gowland's animal (all long bones radiographed, serials of lower end of humerus, upper ends of tibia and fibula, and both ends of radius and ulna prepared) well developed centres of calcified cartilage are found. In the lower end of the ulna for example (Figs. 2, 4) the whole cartilaginous epiphysis is massive, with a thin layer of fibro-cartilage on the articular surface (*a.f.c.*). Towards the centre of the cartilage the undifferentiated cells (*z.u.c.*) become hypertrophied (*h.c.s.c.*) and scattered calcifications of the matrix appear amongst them (*c.c.m.*). Finally the calcifications run together to form a continuous mass (*c.c.s.c.*). The secondary centre must be taken to include all the region of hypertrophied cells and calcified matrix whether the calcification is continuous or not.

Within the centre are numerous spaces (*s.s.c.*) of peculiar structure, hatched in the figures. Each is completely surrounded by calcified matrix, so that the cavities are never in direct contact with hyaline cartilage. The walls are very irregular, with many fragments of matrix apparently free in the cavities (Fig. 2, *i.f.c.m.*). It seems most probable that the cavities are artifacts, produced by the solution of calcium salts in places where the non-calcareous matter is insufficient to retain its continuity after decalcification. Certainly in radiographs of the epiphyses before calcification there is no sign of cavity formation in the secondary centres. Similar cavities have been seen in certain lizards, and they have been figured by Erdheim (1914) in the heavily calcified costal cartilages of rats.

The cells of the growth zone (Fig. 4, *z.g.*) are less flattened than before, and are arranged in clumps, while the hypertrophied cells form more or less definite longitudinal columns (*c.cl.*). The endochondral bone is plentiful and it now plays an important part in maintaining the strength of the formation, for the periosteal bone has remained relatively thin (Fig. 2, *p.b.s.*). It is arranged predominantly as longitudinal trabeculae (*l.t.*). The other epiphyses of the humerus, radius and ulna have a similar structure, each having one large calcified centre. Even at the lower end of the humerus, where the centre stretches from medial to lateral epicondyle, it is not subdivided. In the upper end of the radius, however (Fig. 3), there is no centre, probably on account of the relative thinness of the cartilaginous epiphysis, but the arrangement of the endochondral bone is similar to that described in the ulna.

In the fully developed bone the distinction between the shaft and epiphysial region is lost (Figs. 5, 6). The whole of the growth cartilage and the

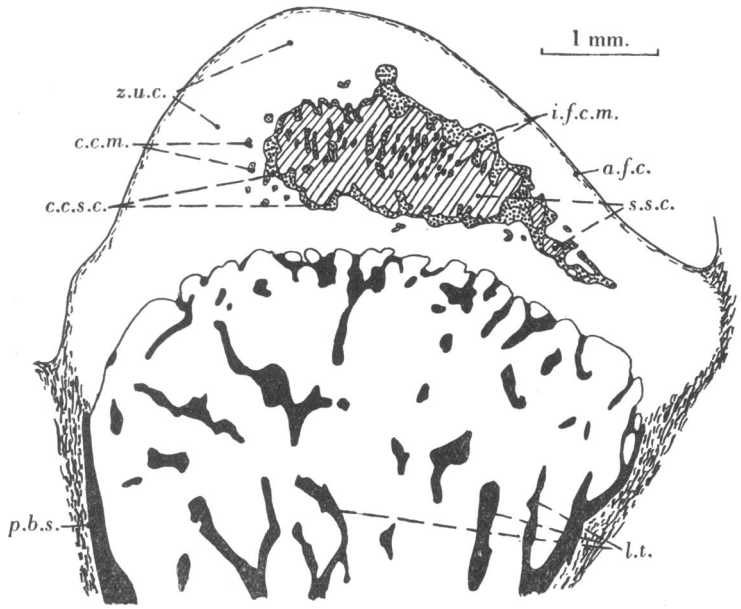


Fig. 2. Lower end of the ulna of a subadult *Sphenodon*.

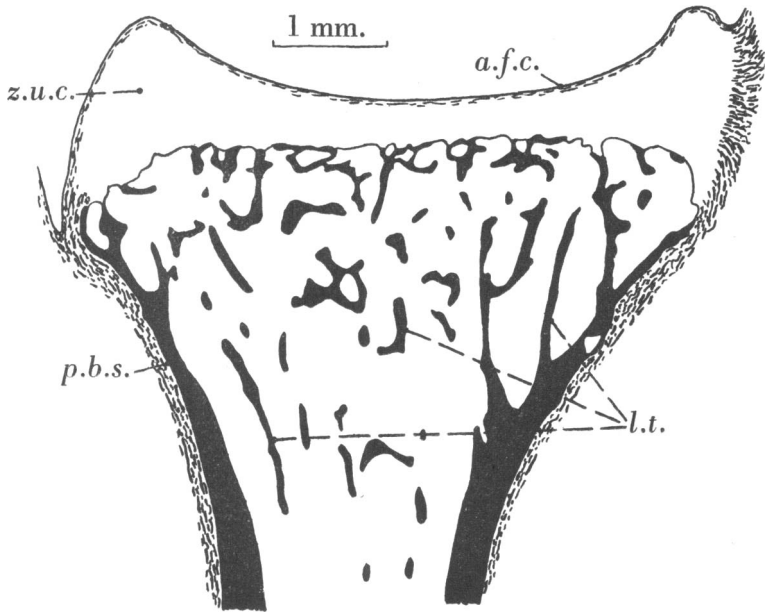


Fig. 3. Upper end of the radius of a subadult *Sphenodon*.

greater part of the calcified cartilage of the secondary centre are replaced by marrow and endochondral bone, so that the tissues of the shaft pass without interruption into the epiphysial region, and approach the articular surface. The remaining cartilage forms a thin layer of hyaline articular cartilage (Fig.

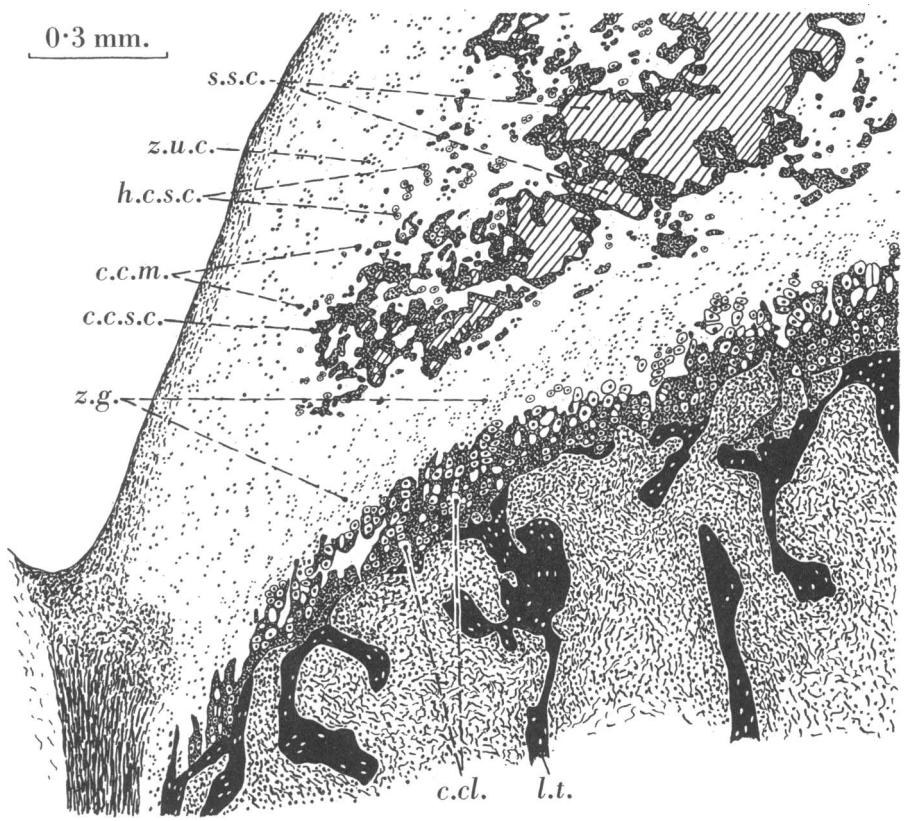


Fig. 4. Part of the lower end of the ulna. Same series as Fig. 2.

6, *a.c.*), beneath which a few unmodified cells represent the remains of the formerly wide zone of undifferentiated cells (*z.u.c.*). Passing towards the marrow the cells first become hypertrophied (*h.c.s.c.*), and then the matrix becomes heavily calcified (*c.c.s.c.*), this formation being derived from the uneroded subarticular part of the secondary centre.

In the shaft a cement line (*cem.*) separates the endosteal part of the endochondral bone (*e.e.b.*) from the periosteal bone (*p.b.s.*). The periosteal bone itself has now grown beyond the original limits of the shaft, and has come into contact with the cartilage of the epiphysial region (*con.*), the bone being sharply deflected by the cartilage. In the region of contact the cartilage has become calcified (*c.c.p.b.*).

The earlier epiphysial mechanism of growth is now destroyed, but it is possible that the bone is still capable of some slow growth. Some new cartilage may be formed by the division of the undifferentiated cells, and the bays of erosion (*b.e.*) have the appearance of activity. Again, though the marrow is for the most part fatty (*f.b.m.*), there is a thick layer of cellular marrow in contact with the cartilage (*c.b.m.*), not walled off from the cartilage by bone as is usually the case when erosion has ceased (Wallis, 1927).

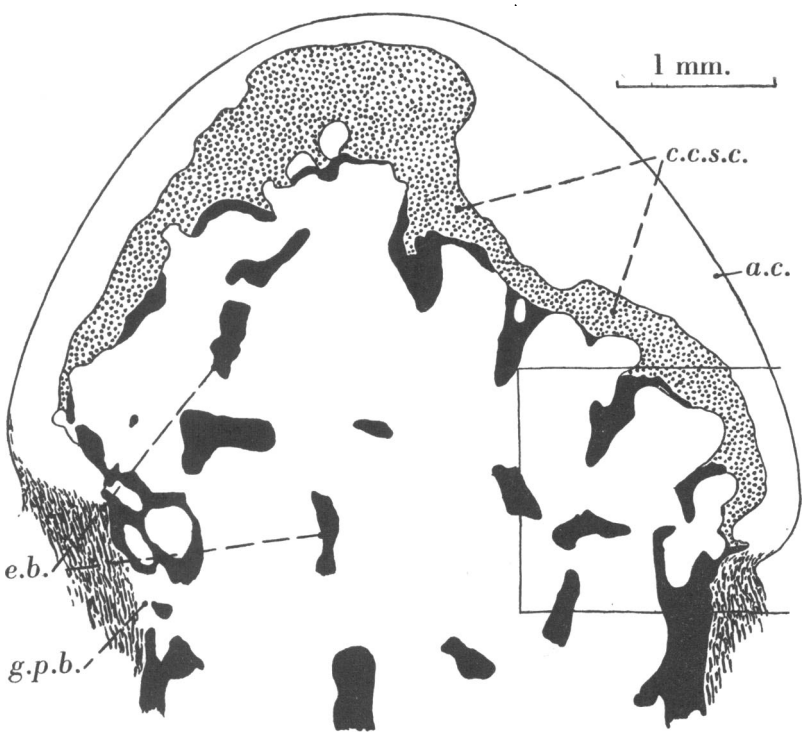


Fig. 5. Lower end of the femur of *Sphenodon*. Snout-vent ength 225 mm.

The tibia and fibula of Prof. Gowland's animal are similar in structure to the femur described. No specimen showing the actual stages of the destruction of the growth cartilage is known, so that the method of destruction must be inferred from the fully adult form. There are two possibilities. The secondary centre may be eroded independently by its own system of marrow and blood vessels derived directly from the perichondrium around the articular region, with the later secondary destruction of the growth cartilage, the method found in lizards and mammals. On the other hand the marrow of the shaft may first perforate the growth cartilage and then erode the secondary centre, so that the secondary centre never has at any time a blood supply independent of the shaft. Now even after the growth cartilage has been destroyed the original relationships of the vascular channels as they enter the bone are preserved,

and it is usually possible to determine whether any particular set of vessels originally entered the shaft or the secondary centre. In *Sphenodon* no vessel entering the epiphysial region has been seen in any bone, the most distal points of entry being definitely in the shaft (Fig. 5, *g.p.b.*), not through the cartilage of the epiphysial region as in lizards and mammals. In some bones the vessels are confined to the more central part of the shaft. So it seems that in *Sphenodon* the secondary centres are destroyed by the tissues of the shaft which perforate the growth cartilage on their way to the secondary centre.

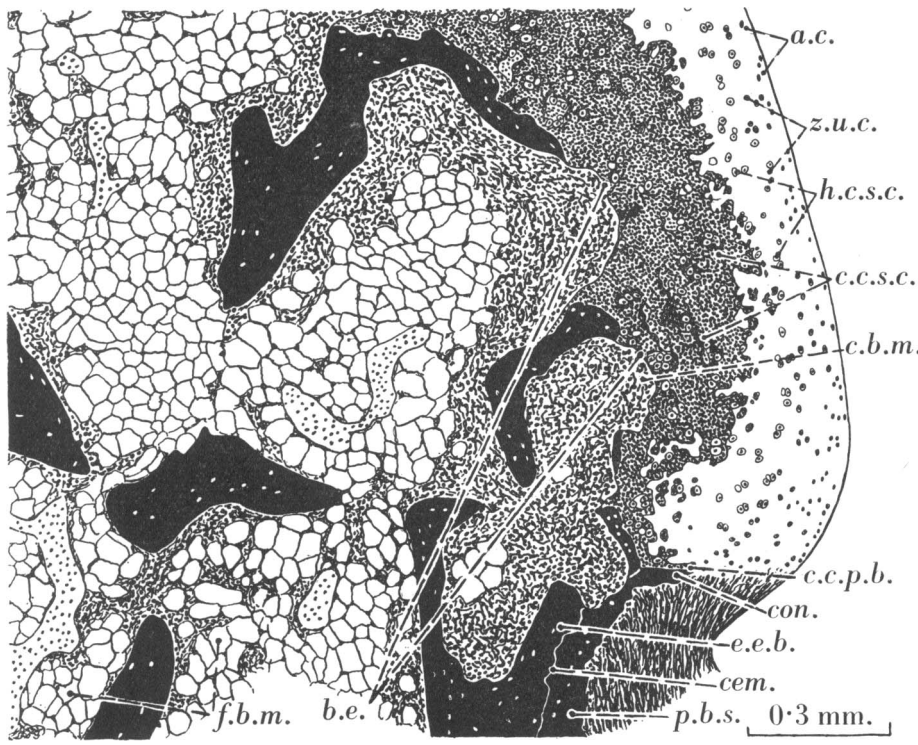


Fig. 6. Part of the lower end of the femur shown in Fig. 5.

Thus, if the appearances have been interpreted correctly, the epiphyses of *Sphenodon* pass through an early hyaline stage, this is followed by a prolonged period in which a large secondary centre of calcified cartilage occupies the region between the articular cartilage and the growth cartilage, and finally this is replaced by tissues derived in the shaft. Secondary centres have been recorded in the Jurassic *Sapheosaurus*, a fossil form related to *Sphenodon* (Meyer, 1859; Fuchs, 1908), the earliest record of such centres in any animal. So *Sphenodon* is probably typical of the Rhynchocephalia, and has probably preserved an early form of epiphysial structure.

## THE PRIMITIVE FORM OF SECONDARY CENTRE

Large centres of calcified cartilage are known in anuran amphibians but are of a very specialized type, and smaller centres are also known in the branchial bones of some fishes (*Sciaena*, Haines (1934), and noted recently in the cod, *Gadus morrhua*). Similar centres which persist throughout life are known in some lizards (*Phyllodactylus*, Haines, unpublished work), but they are probably not primitive formations in this group. In all other animals in which such secondary centres are found at any stage (some birds, most lizards and mammals) the calcified cartilage is replaced by bone and marrow, derived directly from the perichondral tissues. But while in most eutherian mammals the calcified centre is small and transient, in monotremes (Haines, unpublished work) the centres are relatively large before they are eroded. It is thus reasonable to follow Parsons (1905) in suggesting that in the several distinct groups of animals in which secondary centres have been evolved, the first stage has in each case been the appearance of a small calcified centre in the depths of the cartilaginous epiphysis, as seen in the cod. The enlargement of this centre which still persisted throughout the life of the individual is a probable second stage, and *Sphenodon* in the persistence of the calcified centres to a late stage of growth appears so far primitive. The final destruction of the growth cartilage and replacement of the secondary centre by marrow and bone is probably a later evolutionary development, and though the structure in *Sphenodon* suggests that both processes were first carried out entirely by the tissues of the shaft, it must remain uncertain whether the lines leading to the lizards, birds and mammals have ever passed through such a stage in their phylogeny.

## THE ENDOCHONDRAL BONE IN *SPHENODON*

It has been suggested (Haines, 1938) that the primary function of secondary centres is to allow of the spatial separation of the articular and growth cartilages so that each may be arranged in the form most advantageous in the region concerned. The articular cartilage can then adopt the shape best adapted to the joint structure, and the growth cartilage that best adapted for the formation of the scaffolding of calcified cartilage about which the primary trabeculae of endochondral bone are built. In animals which possess no secondary centres it has been demonstrated, by models for turtles and crocodiles, and in the fossil *Dicynodon* by a polished section, that the main longitudinal trabeculae of the endochondral bone radiate towards the articular cartilage. It remains to show that in *Sphenodon*, the most primitive animal possessing large secondary centres, the trabeculae radiate towards the growth cartilage and not towards the articular cartilage.

In the lower end of the ulna a reconstruction of the bone shows that the trend of the longitudinal trabeculae (*l.t.*) can be recognized distinctly, and that their radiation is towards the growth cartilage. The articular cartilage (see Fig. 2) in this bone has a much sharper curvature than the growth cartilage,

and the trabeculae bear no relation to it. The whole formation would appear to constitute a mechanically efficient structure.

*Sphenodon* illustrates also a stage in the refinement of the structure of the endochondral bone in the region of its formation. The bays of erosion (*b.e.*) are narrower and more numerous than, for instance, in the turtles, and the primary trabeculae (*p.t.*) correspondingly more numerous and finer in structure. The functional advantage of this refinement of endochondral ossification, which has occurred independently in mammals, birds and reptiles (Lubosch, 1924; Haines, 1938) is unknown, but possibly it is an advantage to have a large number of primary trabeculae available, so that those best placed for the mechanical requirements of the bone may be selected and the remainder destroyed.

The arrangement of the vascular channels which pierce the wall of the shaft has been fully discussed by Heidsieck (1929).

## THE MECHANISM OF DEVELOPMENT OF SECONDARY CENTRES

Primary and secondary centres are sharply contrasted in structure. In the primary centres of the long bones the whole width of the central part of the cartilage model becomes hypertrophied, so that the two ends of the cartilage which later form the epiphyses are completely severed from one another by calcified cartilage, and the latter is soon destroyed by erosion from the perichondrium. In the secondary centres, on the other hand, the calcified cartilage occupies only the central parts of the cartilage mass, and is at first, and for a prolonged period of growth, completely cut off from the perichondrium on all sides by a layer of hyaline cartilage (see Fig. 2). This distinction seems to be quite constant, and to apply to all groups of animals having secondary centres, at any rate for the typical centres at the ends of the long bones.

Now even in animals which have no secondary centres the two kinds of calcification are found, the one in the shafts of the long bones, the other in the short bones of the carpus and tarsus. In the turtles for instance, though the primary centres of ossification are of the form familiar in other bony animals, the cartilaginous epiphyses are relatively small and no secondary centres are developed in them. But in the carpal bones of a young animal (Fig. 7) large, well defined secondary centres of calcified cartilage can be found occupying the interior of the cartilage. In the centre figured the structure changes from a large celled region in the centre (*r.l.c.*) through a small celled intermediate region (*r.s.c.*) to a peripheral darkly-staining region (*r.d.s.*) where the matrix is more heavily calcified and has a radiate appearance. A thick zone of hyaline cartilage completely surrounds the centre, and cuts it off from the articular fibrocartilage (*a.f.c.*), and from the dense fibro-vesicular tissue (*f.v.t.*) which occupies the region between adjacent skeletal parts where there is no articular cavity, and from the perichondrium on the dorsal and ventral surfaces of the cartilage. The other short bones show a similar structure, and it is only when the

calcification spreads so as to reach the perichondrium that ossification of the centre sets in. Thus from the structural point of view the short and long bones are distinct, and the centres of the short bones resemble the epiphysial centres.

It seems probable, therefore, that in the line leading to *Sphenodon* the cartilages of the ends of the long bones enlarged for the mechanical reasons already

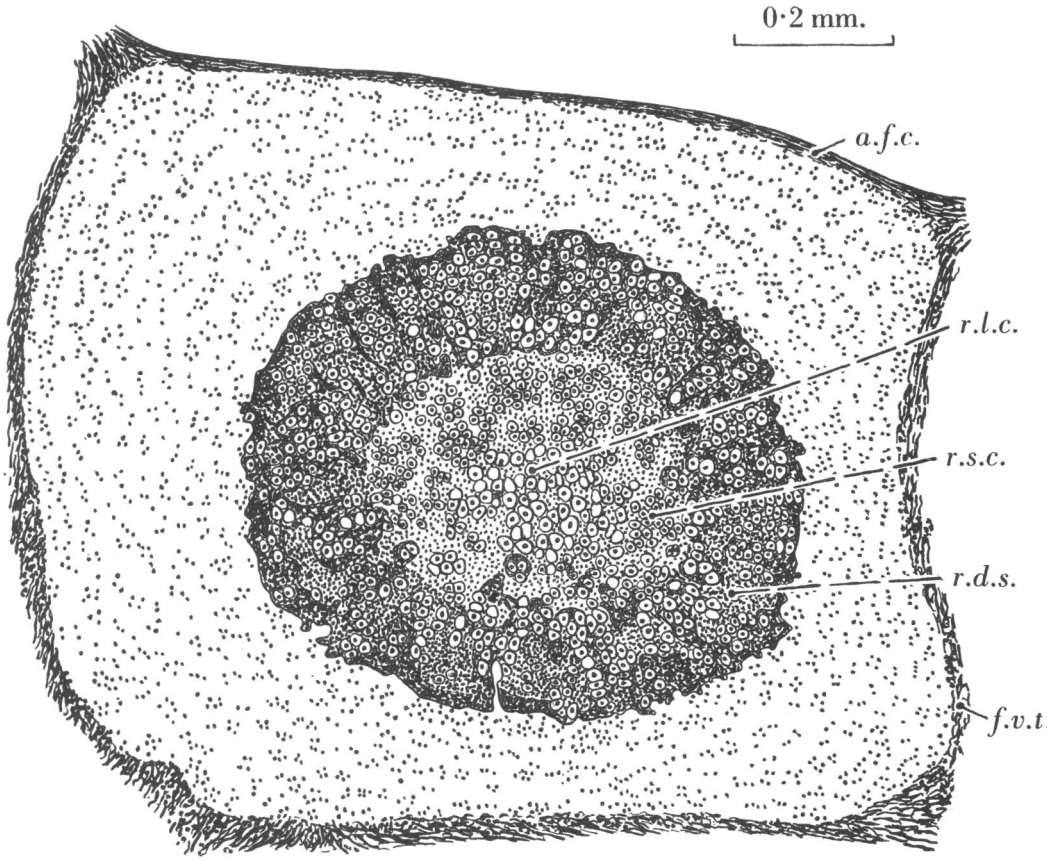


Fig. 7. Section through the second carpal of a young turtle, *Emys orbicularis*. Carapace length 99 mm.

discussed, and that the enlarged cartilages then became influenced by the processes which had already been at work in the short bones for a prolonged geological period. Further, so far as is known, the short bones and the secondary centres have remained similar to each other in each species throughout its evolution. Specializations such as the various modes of ossification and the development of cartilage canals when found in the one are also found in the other, and the time relations of the various stages in development are again similar.

## SUMMARY

1. The secondary centres in *Sphenodon* are composed of large masses of calcified cartilage which persist for a prolonged period during growth.

2. These centres are later replaced by marrow and bone, probably derived from the tissues of the shaft which pierce the growth cartilage.

3. The earliest secondary centres were formed of calcified cartilage in all groups of animals which have developed them, and probably persisted throughout the life of the individual.

4. The longitudinal trabeculae of the endochondral bone in *Sphenodon* radiate towards the growth cartilage, and their disposition is compatible with the suggestion that the primary function of secondary centres is associated with the spatial separation of the growth and articular cartilages, so that the arrangement of the trabeculae need not necessarily, as in animals which have no secondary centres, radiate towards the articular cartilage.

5. Secondary centres are formed in the central parts of the cartilages in a manner similar to the centres for the short bones, and it is probable that the earliest secondary centres were formed by mechanisms developed earlier in phylogeny for the ossification of the short bones.


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## KEY TO LETTERING

Hyaline cartilage plain, calcified cartilage stippled, bone black

| | | | |
|---|---|---|---|
| *a.c.* | articular cartilage | *g.p.b.* | gap in periosteal bone |
| *a.f.c.* | articular fibro-cartilage | *h.c.s.c.* | hypertrophied cells of secondary centre |
| *b.e.* | bay of erosion | *i.f.c.m.* | isolated fragment of calcified matrix |
| *c.b.m.* | cellular bone marrow | *l.t.* | longitudinal trabecula |
| *c.cl.* | cartilage column | *m.p.s.* | margin of periosteal shaft |
| *c.c.m.* | calcified cartilage matrix | *p.b.s.* | periosteal shaft of bone |
| *c.c.p.b.* | calcification near contact of periosteal bone | *p.t.* | primary trabecula |
| | | *r.d.s.* | darkly-staining region |
| *c.c.s.c.* | calcified cartilage of secondary centre | *r.l.c.* | region of large cells |
| *cem.* | cement line | *r.s.c.* | region of small cells |
| *c.lg.* | cruciate ligament | *s.s.c.* | spaces in secondary centre |
| *con.* | contact of periosteal bone with epiphysial cartilage | *v.c.* | vascular channels |
| | | *z.f.c.* | zone of flattened cells |
| *e.b.* | endochondral bone | *z.g.* | zone of growth |
| *e.e.b.* | endosteal part of endochondral bone | *z.h.c.* | zone of hypertrophied cells |
| *f.b.m.* | fatty bone marrow | *z.u.c.* | zone of undifferentiated cells |
| *f.v.t.* | fibro-vesicular tissue | | |