XV.—*The Development of the Carapace of the Chelonia.* By JOHN BERRY HAYCRAFT,
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All observers are agreed that the bones of the plastron and some bones of the carapace are simple membranous bones, arising from centres formed in a pre-existing fibrous membrane. Considerable difference of opinion exists as to the development of the costal and neural plates of the carapace.

OWEN* speaks of the ossification "as extending from the ribs and neural spines into the substance of the neural and costal plates. The ribs and spines enter into the composition of the carapace."

GEGENBAUR† questions whether the ribs of the Chelonia "are not in reality enormously developed transverse processes, and considers that the neural and costal plates have developed in the integument."

CLAUS‡ remarks that the spinous processes of eight of "the thoracic vertebræ (2nd to 9th) appear in the middle line as horizontal plates (neural plates), the ribs of the same vertebræ are transformed into broad transverse plates (costal plates)."

HUXLEY§ says that the neural plates and the costal plates exist as expansions of the cartilages of the neural spines and ribs of the primitive vertebræ, before ossification takes place. This being the case, the "neural and costal are vertebral and not dermal elements, however similar they may be to the nucleal, pygal, and marginal plates."

It would be difficult to find an example of greater confiction of opinion than is contained in the above statements, and it is obvious that even the *simple facts* of the developmental process have not been made out. The most recent contributions to the subject are those of Dr C. K. HOFFMANN.|| This observer has carefully described the main features of the development both of the costal and neural plates. He has shown that the primitive vertebræ and ribs, composed of cartilaginous tissue, become encrusted with bone, but he erroneously describes the costal and neural plates as arising outside the vertebral and costal periosteum. In reality, as I shall show, there is no true periosteum at all. His view, undoubtedly incorrect, is that these plates arise outside the ribs and vertebræ, and are not expansions of them; they are membranous ossifications immediately surrounding the ribs and vertebræ. He describes very fully the subsequent calcification and absorption of the costal and vertebral cartilages, and the obliteration of the primitive bony rib.

* *Comp. Anat. and Phys. of Vertebrates*, vol. i. p. 63.

† *Elements of Comparative Anatomy*, pp. 433, 440.

‡ *Text-Book of Zoology*, p. 226.

§ *The Anatomy of Vertebrate Animals*, p. 201.

|| Dr H. G. Bronn's *Klassen u. Ordnungen des Thier-Reichs*, Sechster Band, iii. Abtheilung.

My own work on this subject is followed out very much on the same lines; but I have endeavoured to fill in certain gaps left in his description. I am obliged to differ from him in what is to my mind his most important conclusion he has arrived at. I shall endeavour to show that there is no true periosteum (perichondrium) around either the primitive cartilaginous rib, or the primitive cartilaginous vertebræ, and that the costal and neural plates do not therefore arise outside the periosteum as he affirms. Whereas in the growing rib or vertebra, say of a crocodile, we find an investing periosteum, the inner surface of which is alone osteogenetic, the periosteum of a turtle's rib consists only of a few fibres of the osteogenetic membrane, of which the carapace is composed, arranged loosely around the bone. In the case of the crocodile's rib, the deposition of bone, being on the inner surface of a cylindrical osteogenetic tube, leads to the formation of a cylindrical rib, whose girth is limited by the growth of the periosteal tube. In the case of the turtle there is no such limiting membrane, and the bone forming first on the surface of the cartilage spreads outwards in all directions, gradually involving the whole carapace. This is a short, and as I believe an accurate description of the formation of the neural and costal plates. I shall endeavour to amplify this statement in the following pages. When the *facts* of the developmental process are thoroughly grasped, it will be possible to discuss with advantage the *relation* which the costal and neural plates bear to the ribs and vertebræ.

Thanks to the embryo turtles kindly given me by Professor MOSELEY, I have been able to investigate the development in its earliest stages. The subsequent steps have been worked at from a very nearly complete series of fresh-water tortoises. The carapace was hardened either in alcohol or picric acid. A very convenient method when dealing with animals that have already begun to develop osseous tissue is to place the carapace in picric acid, to which a few drops of hydrochloric acid have been added, when the carapace will soften in a few days, and sections may at once be prepared.

Study of a Series of Sections Transverse to the Length of the Carapace (Plate, figs. 7-10).

The youngest embryo that I have been able to obtain was a green turtle, measuring about 1 centimetre in length. The vertebral cartilage in this embryo is entirely cartilaginous, and so are the ribs. The cartilage is in all situations surrounded by embryonic tissue, such as is found in the rest of the carapace. There is no special periosteum, for one can hardly speak of the tissue next the cartilage, which has somewhat of a set around it, as a periosteum. Dorsal muscle plates are evident in this embryo.

Each vertebra is composed of three pieces. The first encloses the notochord, forming a complete ring around it. The other two encircle the neural canal laterally and posteriorly. The cartilaginous ribs are attached to the vertebræ by fibrous tissue.

In a somewhat more advanced embryo (fig. 7), the lateral pieces of cartilage have

joined posteriorly over the neural canal. The rib is joined by fibro-cartilage both to the notochordal cartilage and to the lateral plate of its side. No trace of bone is as yet apparent.

The further stages of development I have studied both in the green turtle and in the common fresh-water tortoise. As my specimens of the latter form a more complete series they will now be referred to, but if in any detail they differ from the green turtles, that difference will be indicated.

A tortoise of 2.2 centimetres long (fig. 9) has the vertebræ composed of one piece of cartilage, complete fusion of the original pieces having taken place. The rib is now completely joined to the vertebral column, but the line of juncture is indicated in this and in older specimens by a curved band of closely-packed cells. I think there can be no doubt that what has been termed the costal cartilage is indeed a true rib cartilage and not a developed transverse process as GEGENBAUR affirms. The rib cartilage is from first to last quite distinct from the vertebral cartilages, whereas a transverse process arises as a *prolongation* of one of these. In the 2.2 centimetres tortoise ossification has already commenced both on the vertebral and on the rib cartilage. The shaft of the rib cartilage is covered by a thin layer of bone, which extends over part of the head of the rib cartilage. This layer of bone, neither in this nor in any of the tortoises of larger size, never extends quite up to, or blends with, the bone which is now found to encrust the vertebral cartilage. In addition to the crust of bone on the rib cartilages, bony spicules project dorsally over the muscle plate (C, fig. 9), and this growth is one of the first indications of the formation of the costal plates. In the same specimen bone has already formed within the neural canal, and the neural arch is covered posteriorly by a thin crust of bone which extends laterally towards, but never blends with, the crust of bone on the rib cartilage. From the posterior part of the neural arch bony spicules are seen to have extended into the connective tissue found lying between the vertebræ and the superficial scutes, to form the neural plates. This connective tissue consists partly of those straight interlacing fibres (in connection with which are numerous connective tissue corpuscles) which are so characteristic of a membrane destined to be converted into bone.

In a tortoise 2.6 and in another 3 centimetres long, the spongy bone developed behind the ribs and vertebræ had increased in amount, extending round and partly enclosing the muscle plate.

In a specimen 4 centimetres long (fig. 10), the vertebræ are seen to have been covered and lined by bone, which has extended backwards to form the neural plate. Much of the cartilage of the vertebræ has been removed and replaced by bone, and the same obtains in the rib cartilage. Of the latter the head alone remains intact, together with some scattered and often isolated cartilaginous remains of the main body of the primitive rib.

The formation of the neural plate is now far advanced. It extends nearly up to the tortoise-shell, and laterally it has extended to meet the advancing costal plate. At this point the bony spicules of the two plates dove-tail very loosely into each other, forming what may be termed a *primitive suture*. The spicules of the neighbouring plates

never, however, come actually into contact one with another. They are invariably ensheathed and separated from one another by osteogenetic fibrous tissue loaded with osteoblasts. As the plates grow in size, the suture still remains; there is no bridging over of spicules from one plate to another.

Larger tortoises simply show further stages of growth. The costal and neural plates grow in size, and the vertebral cartilage entirely disappears and is replaced by bone. The fibro-cartilaginous carapace is now replaced by bone, which, forming on the surface of the cartilage, gradually extends in all directions involving the cartilage itself, and the whole of the fibrous carapace except a thin layer under the chitinous carapace, which is converted into ordinary fibrous tissue.

The development of the neural plate has now been fully described, except, indeed, the process by which the cartilage and the fibrous tissue change into bone. It will be convenient to study this when the development of the costal plate has been more fully described. The formation of the latter at its proximal extremity has been already studied; it will be advisable to examine a series of sections transverse to the length of the tortoise, but passing through the rib cartilage and developing costal plate at its distal margin, and showing its relation with the marginal plates.

Sections at the Costo-Marginal Junction (Plate, figs. 5 and 6).

It will be sufficient to study two sections, one of the tortoise 2·6 centimetres and the other of the tortoise 4·6 centimetres long. In the figure (fig. 5, A) the commencing marginal plate is shown as well as the external end of the costal plate. The latter contains the still cartilaginous rib, absorption of this tissue not having been completed. From the outer end of the rib cartilage (which is not represented in the figure) bony spicules (C, fig. 5) shoot out towards the marginal plate. The spicules have not nearly reached the marginal plate, and are still far away from the notch between the superficial scutes of the carapace.

In the 4·6 centimetres tortoise, the marginal plate has increased considerably in size. The rib cartilage is seen here and there within the costal plate. Most of the cartilage is indeed absorbed, but portions are still to be seen, and occasionally almost all the rib cartilage is left. The rib cartilage is much larger, and it is thicker than the rib cartilage of the 2·6 centimetres tortoise. It seems, then, that during the formation of the costal plate, even up to a comparatively late period, the cartilage continues to grow, and the absorption of large masses of the tissue does not seem to interfere with the growth of those portions which remain within the bony ring in which they are enclosed. Not only has the rib cartilage grown outwards towards the marginal plate (fig. 6), but the bony spicules have pushed outwards, and have passed beyond the notch on the superficial scutes. The marginal plates, which arise as little nodules in the connective tissue, are seen in the 4·6 centimetres tortoise to have increased considerably in size. Spicules have shot out into

the fibrous carapace, some of them towards the advancing costal plate. The spicules of the two plates have loosely dove-tailed into each other to form a *primitive suture*.

A Study of Sections Transverse to the Length of the Ribs (Plate, figs. 1-4).

A section through the distal end of the rib of the green turtle of about 5 centimetres long (fig. 1) shows that the structure is entirely cartilaginous. It is embedded in the general osteogenetic tissue of the carapace, which has a "set" around it. It cannot be said to possess a perichondrium.

Fig. 2 represents a section through the proximal end of the same rib. Here is found the first commencement of ossification, the mesoblastic cells next the cartilage having become converted into osteoblasts, and having formed a complete ring of bone around the cartilage. At the sides of the rib (see fig. 9), in the region of the intercostal spaces, the process is more active, and tiny spicules are seen already to project into the surrounding tissue. These broaden the rib, and are the first indications of the formation of the costal plates seen from this aspect.

In the fresh-water tortoise, 2.2, 2.6, and 3 centimetres long (fig. 3), the further development of the costal plates may conveniently be traced. Spicules of bone penetrate in all directions, but especially into the intercostal connective tissue. In 4 and 4.6 centimetres tortoises (fig. 4) the process has so far advanced that neighbouring costal plates have met together, forming the primitive sutures already described, and all the tissue of the carapace except the sensitive layer under the scutes is converted into spongy bone. The rib cartilage is all absorbed, and so is the little tube of bone which first invested it (A, fig. 4).

The Osteogenetic Tissue of the Carapace.

The whole carapace of the embryo tortoise, as has previously been mentioned, consists of embryonic connective tissue, in which are embedded cartilaginous vertebræ and ribs, the whole being covered with epidermic scutes. The tissue is rich in blood-vessels, and consists chiefly of interlacing collagenous fibres, many of which are straight and clasped by clasping cells. Many young corpuscles are seen evidently about to form fresh fibres, traces of which are often apparent. There is no sign of any segmentation of this osteogenetic tissue. It forms an unbroken sheet of tissue, in which, of course, the cartilaginous ribs and vertebræ are embedded. In fig. 11 the tissue is represented surrounding a rib cartilage. The connective tissue cells near the cartilage have become osteoblasts, and a thin layer of bone has been formed on the rib cartilage. At the same time, however, active changes take place in the surrounding tissue, especially in that part which is intercostal. The tissue becomes very vascular near the rib cartilage, and there is a rapid formation of the straight fibres or spicules destined to be included in the forming bone as SHARPEY'S fibres. In the inter-

costal region this change is most evident, and by the time some half dozen laminae of bone have been deposited on the rib cartilage, well-marked processes of spongy bone are seen to have already projected into the intercostal spaces.

The processes are furnished at these points by little brushes of straight connective tissue fibres, between which osteoblasts are found in great numbers. Parts of these straight fibres are already lodged within the processes from which they project, and the further growth of these processes is, of course, due to the further inclusion of these and of other fibres. In this way, then, the whole of the membranous carapace becomes converted into spongy bone, which grows, and is subsequently divided into two, the outer and the inner, compact plates. These changes are brought about by that modelling process, seen in all osseous structures, and which seems to consist in a constant deposition of fresh bone and removal by absorption of superfluous tissue.

What is a Costal Plate?

Let us first consider the development of the costal plate, for the neural plate, developed in precisely the same way, may be included in any deductions we may draw concerning the homologies of the rib plate.

In the first place, are these intra-cartilaginous or intra-membranous bones? HOFFMANN and others maintain that they are intra-membranous like the other bony scutes, because they are developed in membrane, and because the rib cartilage around which this development takes place is rapidly absorbed. The fact that these plates have been developed in membranes around the cartilages of the rib, does not, however, make them membranous bones; unless, indeed, one looks upon the femur or a human rib as membranous bones. I suppose that it would be safe to say that not one single ounce of bone in an adult human skeleton was ever in histogenetic connection with cartilage at all. The latter tissue is, as far as long and short bones are concerned, a primitive tissue indicating their future positions, but in no way developing *into* them. The shaft of the femur and of a human rib are developed in the *membranes* which surround the primitive cartilage, which membrane is called the periosteum. The only way we can possibly look at the question in the light of modern histology is not whether or not bone is formed *out of* membrane, but whether or not its position was taken at an early period of development by hyaline cartilage. From this point of view the answer is simple. The costal plate differs entirely from a plastral plate, in that the latter was never in any way connected with cartilage, while the rib plate was, for it developed like the human or any other rib around a rib cartilage. It is true that the rib plate develops around the rib cartilage in a different manner from that in which the human rib develops, but this does not alter the morphological homologies of these structures. The supposed hard and fast lines in animal morphology do not exist in fact, and cell structures and cell processes pass one into another through innumerable transition forms. At one time one or two typical osseous developments had been described, but it is now known that these types

are bridged between by *many* intermediate processes. Thus the terminal phalanx of the finger is an intra-cartilaginous bone, and develops in all respects like the middle phalanx, except that its distal end shoots out towards the tip of the finger just like the end of the costal plate of the tortoise shoots out towards the marginal plate. We cannot, however, consider the costal plates simply as ribs, for most would agree that the term rib indicates a long cylindrical bone enclosed in a periosteum. We may, however, with propriety consider the costal plate as a *rib expansion*.

If, finally, we contrast the carapace of the Chelonia with the body-wall of a crocodile, the following differences are apparent. In the crocodile we have segmentation into alternate muscle plates and rib cartilages enclosed in periosteum, the outer layers of which have differentiated already into fibrous tissue. These structures grow, the muscle plates forming intercostal muscles, the periosteum being only osteogenetic on its inner surface secreting a cylindrical rib. In the tortoise the carapace is segmented, but only to the extent of having cartilaginous ribs within an otherwise undifferentiated body-wall. The ribs are not ensheathed by any specially differentiated periosteal membrane, and so the bone where it develops around the cartilages gradually comes to involve the whole of the intercostal spaces. In the turtles, parts of the ribs, viz., those which join the marginal plates, preserve their rib-like character. They have not expanded to form true costal plates, because they are invested by a restraining periosteum, in which true adult fibrous tissue has already formed.

The study of Chelonia, in which the ossification of the carapace is incomplete, is very instructive in this relationship. Owing to the lack of material I have not been able to devote as much attention to this as I should have desired, but such observations as I have been able to make are worth recording.

If a young *Horopas areolatus* be dissected, one can remove the scutes and the membranous carapace, leaving behind true bony vertebræ and ribs. I have made careful sections of a *Horopas*, and find that the bony vertebræ and fully-formed bony ribs show the merest indications of osseous extension into the surrounding carapace. I am told that my specimen corresponded in point of general development with one of the larger fresh-water tortoises I have figured, yet the condition of the carapace was very different. The ribs were cylindrical bony tubes, with here and there indications of a lateral expansion. They were embedded in a tissue which was no longer embryonic, it was fairly differentiated fibrous tissue. The ribs were not enclosed in a *tube* of periosteum, hence the attempts at lateral expansion. They were surrounded, however, by tissue which had already differentiated, and had therefore not the same tendency to be involved in any osteogenetic changes.

One may conclude with the following general statement. The chest-wall of a typical vertebrate segments into rib cartilages, surrounded by perichondrial tubes, osteogenetic on their inner surfaces alone, and muscle plates embedded in connective tissue. The cylindrical ribs form on the inner surface of the growing perichondrium (periosteum), and the muscle plates form the intercostal muscles.

In the Chelonia there are no muscle plates, and the perichondrium is absent from the rib cartilage, except in such situations where ribs are actually seen, as at the outer part of the carapace of turtles.

In those Chelonia where the carapace forms a complete bony box, the body wall segments into rib cartilages embedded in embryonic connective tissue. Ossification commences on the surface of the rib as in the typical vertebrate, but as there is no periosteum, it spreads outwards and involves the whole body-wall, the tissue of which becomes osteogenetic at first near the rib cartilage, and finally throughout its entirety.

In those Chelonia in which the carapace is incomplete, the rib expansions involving only a part of the body-wall, the following changes occur. The body-wall segments as before into rib cartilages without true periosteal sheaths, embedded in embryonic connective tissue. Bone forms on the outer surface of the ribs and expands more or less into the surrounding connective tissue. This does not occur with great rapidity however, and the intercostal embryonic connective tissue differentiates into white fibrous tissue.

The neural plates are developed in a manner which is in every way essentially similar to the development of the costal plates.

DESCRIPTION OF PLATE.

Figs. 1, 2, 3, and 4.—Transverse sections through the ribs and costal plates in developing tortoise. A, rib-cartilage; B, scutes; C, young bone; D, suture between approaching costal plates.

Figs. 5 and 6.—Transverse sections through the carapace of developing tortoise at the costo-marginal junction. A, marginal plate; B, scutes; C, costal plate.

Figs. 7, 8, 9, and 10.—Transverse sections through the vertebræ of developing tortoise. A, costal cartilage; B, vertebral cartilage; C, costal bone; D, vertebral bone; M is placed on the dorsal muscle.

Fig. 11.—Part of a transverse section through a rib of an embryo turtle. A, rib cartilage; B, a few laminae of bone; C, a lateral projection of bone; D, osteogenetic tissue around rib, there being no fully-formed periosteum; H is placed in a blood capillary; K, osteoblast.