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The development of pneumatisation in the skull of the domestic fowl (*Gallus gallus domesticus*)

D. A. HOGG
Department of Anatomy, University of Glasgow, Glasgow, G12 8QQ, Scotland, U.K.

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INTRODUCTION

The fact that the avian skull is extensively pneumatised is well recognised (De Beer, 1937; Bellairs & Jenkin, 1960). Progressive spread of skull pneumatisation is in widespread use in ornithology as an index of age and maturity, where it is assessed by transillumination of either the head as a whole or the dried skull. While such data are unquestionably of value in determining age and maturity they were not found to be an accurate means of recognition of actual pneumatisation (Hogg, 1980).

Detailed anatomical work on the development of pneumatisation in the skull of the domestic fowl is limited to that of Bremer (1940). Much of his paper relates to the air-filled spaces between nasal conchae, the subocular sacs and the dilated nasolacrimal passages, although these are not in fact claimed to be examples of true pneumatisation, which is restricted to the invasion of bones by extensions from the tympanic cavity. Some details of this are given, particularly in relation to the early stages of invasion, and some bones, for example the quadrate, are dealt with in more detail. In other respects the account is only a generalised one indicating, for example, that all bones of the cranium become pneumatised in the manner generally described, as do many of those of the maxillary region, although not the mandible itself, facts not confirmed by Hogg (1984*a*) in an adult survey. A further difficulty results from the lack of agreed nomenclature when this was written, raising considerable doubts regarding the identities of certain bones in the description.

The comprehensive work of Jollie (1957) serves as the definitive account of skull ossification in the domestic fowl, resolving many of the inconsistencies and filling gaps in the previous reports of Schinz & Zangerl (1937) and Erdmann (1940). In the description of ossification of certain individual bones reference is made to the occurrence of pneumatisation, e.g. in the parietal, supraoccipital, exoccipital and quadrate. Sites of entry are specified quite precisely but no details of the timing of their invasion or indeed the exact routes by which extensions from the tympanic cavity invade particular bones.

This study aims to fill some of these existing gaps by detailing the routes of extensions from the tympanic cavity into various skull bones, the timing of their invasion, the relationship of invasion by the air sac to the stage of development of the bone and the further spread of pneumatisation within the skull subsequent to initial invasion. It follows the previous survey of pneumatisation in adults (Hogg, 1984*a*) and the development of pneumatisation in the postcranial skeleton (Hogg, 1984*b*).

Nomenclature is in accordance with *Nomina Anatomica Avium* (Baumel *et al.* 1979).

MATERIALS AND METHODS

Fertile eggs were obtained from a commercial producer (Ross Browns, Ross Poultry, Aberdeen). They were sampled in groups of three at daily intervals from 7–20 days of incubation and staged according to the criteria of Hamburger & Hamilton (1951) in which they represented Stages 29–46. The heads of two of each group were fixed in 10% buffered formalin after removal of skin and eyes from the larger specimens, decalcified in RDC (Histolab) for up to 12 hours and serially sectioned transversely to the long axis of the skull from the caudal extremity of the head to the rostral margin of the orbit. Sections were cut at a thickness of 10 μm and every tenth section was mounted and stained with haematoxylin and eosin. The remaining embryo in the group was cleared after staining with alizarin red S and alcian blue for differentiation of bone and cartilage according to the method of Dingerkus & Uhler (1977).

For the study of development of pneumatisation after hatching, two groups of specimens were employed. Firstly pairs of chicks of the same type as described above were killed by injection with sodium pentobarbitone at 7, 14 and 21 days after hatching and serial sections were prepared in a similar manner. Secondly, White Leghorn birds were sampled in pairs at hatching and at 7 day intervals and killed by the same method. The heads were fixed in 10% formalin, sectioned midsagittally with a fine bandsaw and the sectioned surface of the cranium examined with a stereomicroscope with magnifications up to × 40. Each half-head was then sectioned transversely, firstly just caudal to the orbit and secondly through the tympanic cavity at similar levels to those indicated by Hogg (1984*a*). These sectioned surfaces of the cranium were similarly examined with the stereomicroscope.

RESULTS

Pneumatisation during embryonic period

Squamosal

The onset of mineralisation was recognisable by alizarin red S staining and by histology at Stage 35. At Stage 38 a rostral extension of the dorsal region of the tympanic cavity was becoming surrounded by the ossifying squamosal (Fig. 1). By stage 40 other separate small extensions were seen and by Stage 46 numerous extensions of the tympanic cavity resulted in extensive pneumatisation of the squamosal although some regions still remained where bone spicules were separated by marrow spaces not invaded by air sac cavities (Fig. 2).

Parietal

The first indications of ossification were recognised at Stage 39. At Stage 42 small tongue-like processes of the dorsal extremity of the tympanic cavity protruded into the

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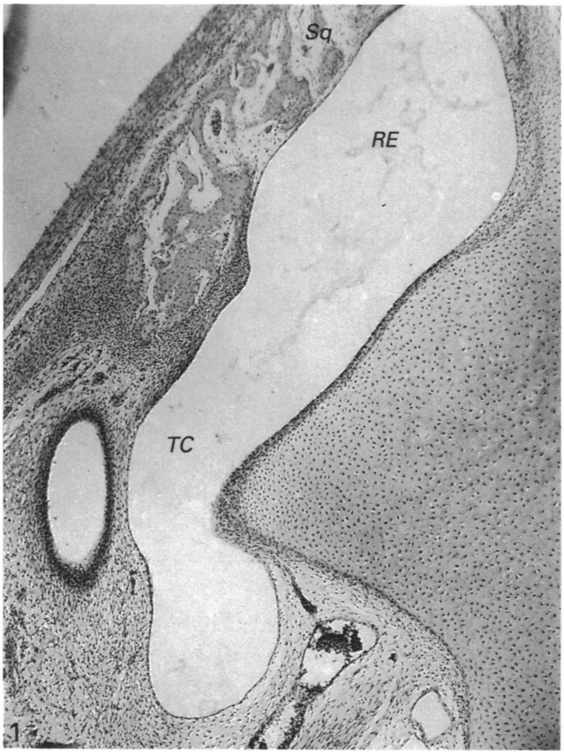
Fig. 1. Stage 38. The tympanic cavity (*TC*) has a rostral extension (*RE*) which is becoming surrounded by spicules of intramembranously forming bone of the squamosal (*Sq*). × 63.

Fig. 2. Stage 46. The squamosal (*Sq*) and the prootic (*Pr*) are now extensively pneumatised by interconnecting air cells subdivided by spicules of bone. External acoustic meatus, *EAM*; tympanic membrane, *TM*; tympanic cavity, *TC*. × 32.

Fig. 3. Stage 44. The dorsal extremity of the tympanic cavity (*TC*) has invaded the parietal (*Pa*) and extensions are continuing to spread between spicules of bone. × 32.

Fig. 4. Stage 43. The dorsal extremity of the tympanic cavity is invading the parietal (*Pa*), where spicules of intramembranously developing bone are surrounding it, and is starting to spread into the adjacent cartilage as this becomes resorbed in association with ossification of the supraoccipital (*SO*). × 32.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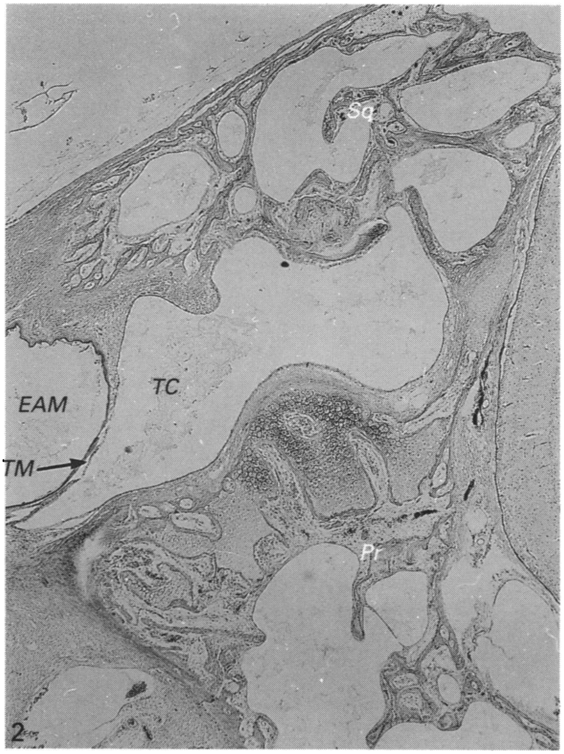
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overlying parietal and these had coalesced and become well established within the bone by Stage 44 (Fig. 3). By the time of hatching the extent of pneumatisation was still confined to the caudolateral region of the bone, the remainder still being in the form of a thin single plate of bone lacking in diploe.

Supraoccipital

Paired centres of ossification were present at Stage 40 and these rapidly fused into a single centre at Stage 41. The epiotic centres appeared at Stage 40 and rapidly fused with the supraoccipital. Invasion by a branch of the air sac to the parietal occurred at Stage 43 as the calcified cartilage was resorbed (Fig. 4). By the time of hatching pneumatisation had spread somewhat within the bone but was still present only in the lateral parts.

Exoccipital

The commencement of perichondral ossification occurred at Stage 38. The centre for the opisthotic was recognisable in the cleared specimen at Stage 42, although it appeared partially fused to the exoccipital from its inception. The first signs of development of pneumatisation were seen at Stage 41, when an expansion from the caudal region of the tympanic cavity spread into the developing exoccipital in association with the resorption of cartilage (Fig. 5). By Stage 46 pneumatisation extended throughout the exoccipital to the region of the occipital condyle. A caudal extension of the tympanic cavity, the caudal pneumatic antrum, now gave rise to various extensions within the exoccipital.

Prootic

The first indication of ossification was seen at Stage 40, when several small centres appeared in the otic capsule. At Stage 42 a small extension of the dorsal region of the tympanic cavity invaded its medial wall. This expanded slowly initially but by Stage 46 had spread to involve much of the prootic, closely surrounding parts of the osseous labyrinth and the facial canal (Fig. 2).

Parasphenoid and basisphenoid

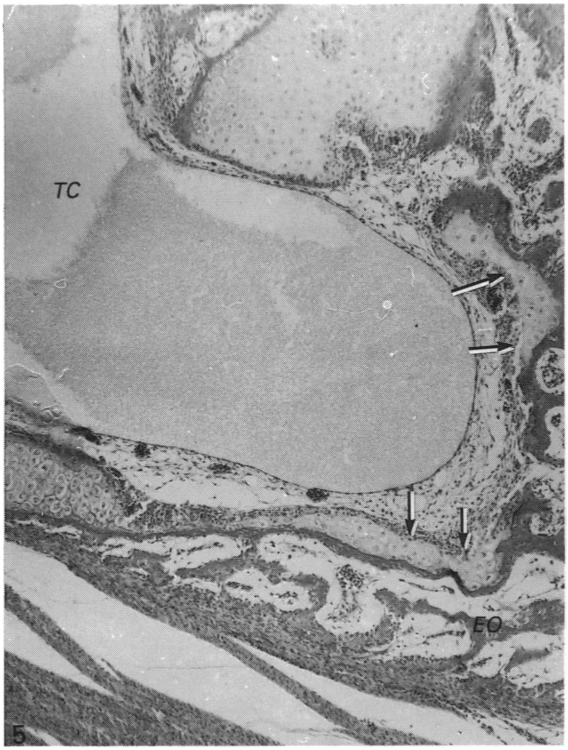
Ossification centres are named according to Jollie (1957). At stage 36 paired perichondral centres for the basisphenoid were seen and these were continuous with the intramembranously forming rostroparasphenoid centre rostrally and sellaparasphenoid centres slightly caudally. At Stage 38 the alaparasphenoid centres extended out on either side of the sella turcica and a continuous basiparasphenoid centre was situated more caudally. By Stage 39 some fusion had occurred between all centres to form a continuous complex bone referred to, from now on, as parasphenoid/basisphenoid.

Fig. 5. Stage 41. A caudal extension of the tympanic cavity (*TC*) is spreading into the ossifying exoccipital (*EO*) in association with calcification and resorption of cartilage (arrows). × 80.

Fig. 6. Stage 35. A small tympanic cavity (*TC*) has a rostral extension which is the future rostral pneumatic antrum (*RPA*). External acoustic meatus, *EAM*; tympanic membrane, *TM*: cartilage of the quadrate, *Q*; cartilage of the basis cranii, *BC*. × 80.

Fig. 7. Stage 39. The developing rostral pneumatic antrum (*RPA*) is surrounded by bone of the alaparasphenoid (*APs*) dorsally and the cartilage of the basis cranii (*BC*) medially. Small extensions are starting to invade the adjacent bone (arrows). × 32.

Fig. 8. Stage 43. Pneumatisation is extensive within the parasphenoid/basisphenoid. The systems on left and right have anastomosed. Pituitary gland, *PG*; cartilage of basis cranii, *BC*. × 32.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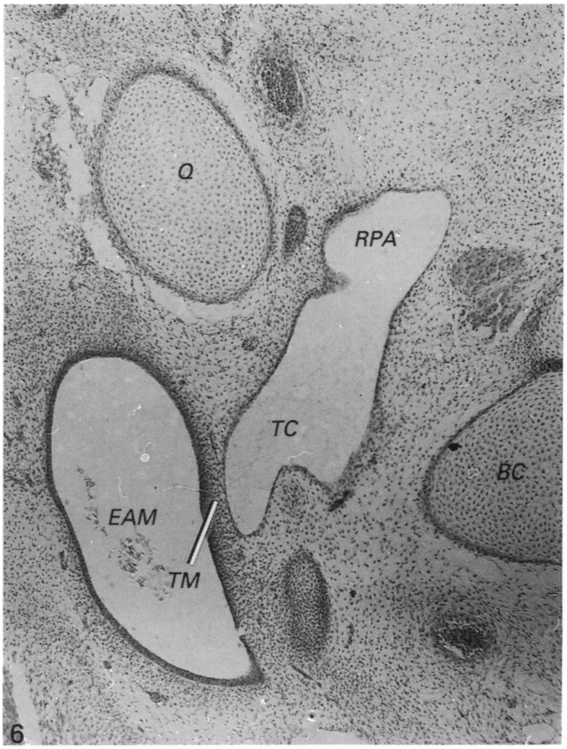
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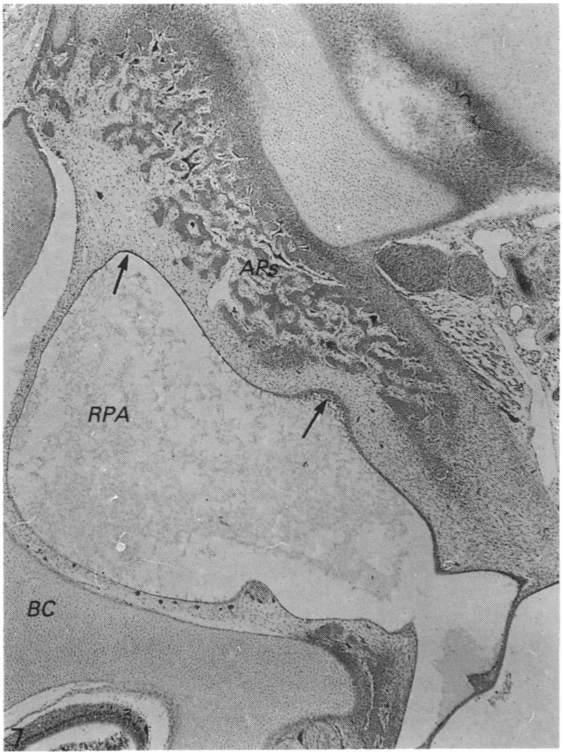
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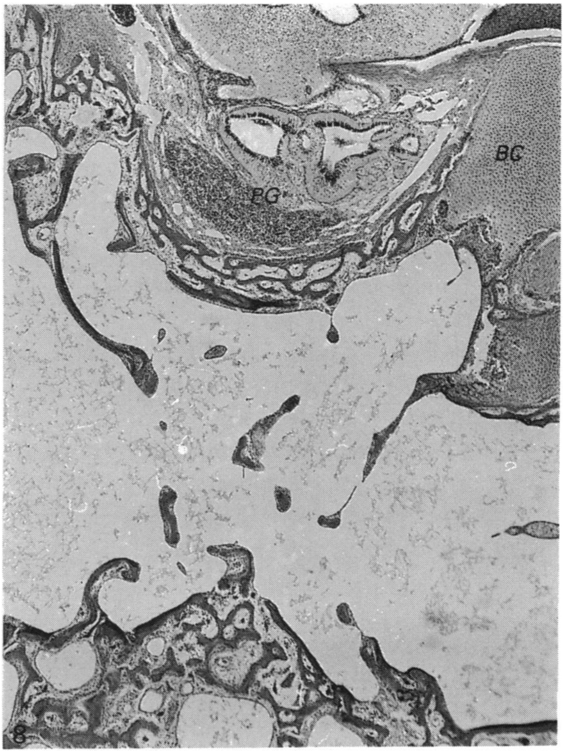
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Before any pneumatisation of skull bones had commenced the tympanic cavity was found to have a rostral expansion as shown at Stage 35 in Figure 6. Subsequently this enlarged considerably and by Stage 39 was interposed between the region of the alaparasphenoid dorsally and the cartilaginous basis cranii medially, in which further ossification had yet to occur (Fig. 7). Later as a result of fusion between alaparasphenoid and sellaparasphenoid the cavity becomes enclosed within the parasphenoid/basisphenoid secondarily and is termed the rostral pneumatic antrum. Small evaginations of this started to develop at Stage 39 and these proceeded to invade the adjacent bone surfaces. Pneumatisation became very extensive within all regions of the parasphenoid/basisphenoid by Stage 43, even reaching the vicinity of the tip of the rostrum of the parasphenoid. The process frequently crossed the midline and the systems on the left and right anastomosed (Fig. 8).

Quadrate

At Stage 36 the quadrate cartilage was showing the first indications of perichondral ossification and by Stage 38 an air sac diverticulum from the tympanic cavity lay close to the medial surface of the cartilage (Fig. 9) which was now showing signs of degeneration. At Stage 39 a diverticulum was found entering the medial surface of the ossifying cartilage in common with a vascular bud (Fig. 10). As ossification progressively spread, pneumatisation followed and soon involved a large proportion of the quadrate within the periosteum beyond the site of entry of the invading diverticulum. Further periosteal ossification caused this continuing diverticulum to become surrounded within the quadrate by a bony tube and to exit distally by a second pneumatic foramen and subsequently to penetrate the pterygoid (Fig. 11).

Pterygoid

Ossification was present at Stage 36 at which time a rostral diverticulum from the tympanic cavity was growing down the medial side of the quadrate cartilage. Intramembranous ossification of the pterygoid had advanced considerably by Stage 38 and was enclosing the terminal extremity of the diverticulum which had now reached it (Fig. 12). This diverticulum now branched to invade the quadrate and subsequently became enclosed within it as described above. Pneumatisation quickly came to involve the whole ossified length of the pterygoid but spread no further rostrally.

Mandible

Intramembranous ossification was identified in the angular, supra-angular, dentary and splenial elements at Stage 35 and in the prearticular at Stage 36. Perichondral ossification of the articular was first recognised in the region of the medial mandibular

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Fig. 9. Stage 38. A tubular diverticulum (*D*) from the tympanic cavity lies on the medial surface of the quadrate cartilage (*Q*) which is starting to show signs of degeneration. × 80.

Fig. 10. Stage 39. An extension from the diverticulum (*D*) is now invading the quadrate in association with resorption of calcified cartilage (*C*). Perichondral bone is developing (arrows) and surrounding the invading sac to form the pneumatic foramen. × 200.

Fig. 11. Stage 43. The distal extremity of the air sac diverticulum is escaping from the quadrate (*Q*) and is invading the pterygoid (*Pt*). More laterally the siphonium (*S*) lies on the medial surface of the cartilage of the mandible (*M*). × 32.

Fig. 12. Stage 38. The terminal part of the diverticulum (*D*) from the tympanic cavity is becoming surrounded by the intramembranously ossifying pterygoid (*Pt*). Cartilage of quadrate (*Q*).


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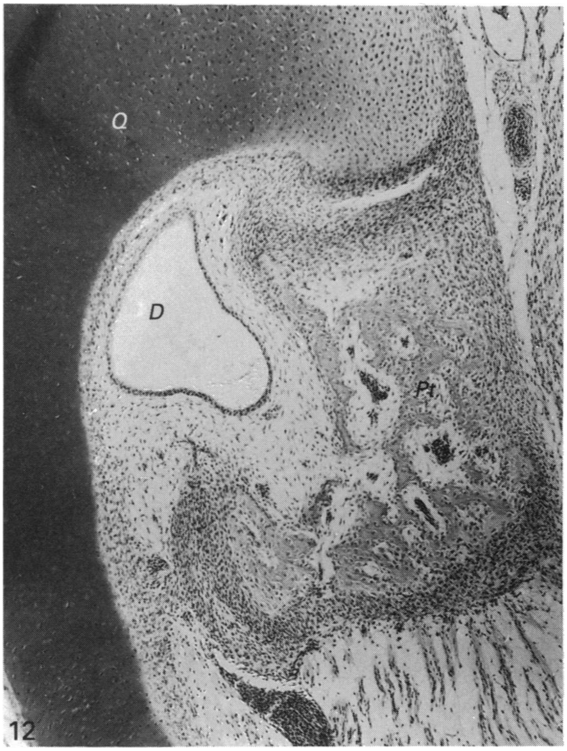
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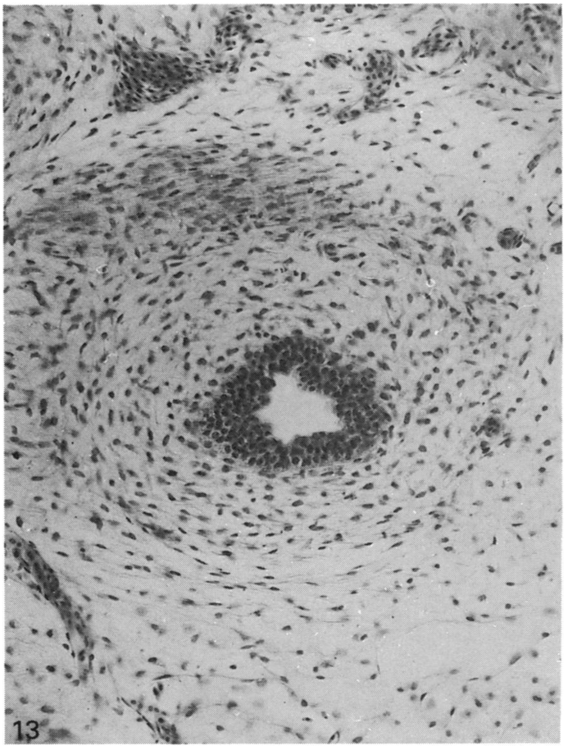

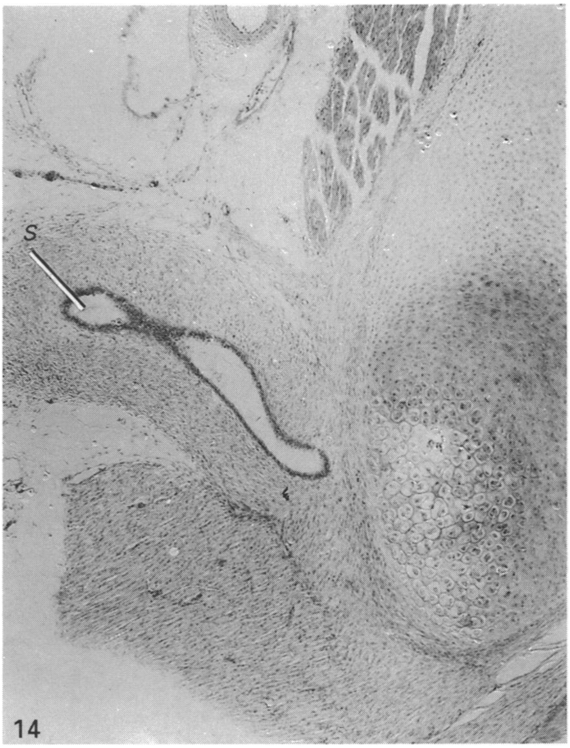

Fig. 13. Stage 38. The siphonium has a stratified columnar epithelium surrounded by concentrically arranged layers of mesenchymal cells. × 200.

Fig. 14. Stage 46. The distal extremity of the siphonium (*S*) turns laterally and lies close to the cartilage of the medial mandibular process which is now undergoing degeneration. The perichondrally ossifying region of the articular lies more distally. × 80.

process at Stage 43. This centre of ossification appeared to be continuous with that of the prearticular from its inception.

A rostral tubular diverticulum from the tympanic cavity termed the siphonium, and separate from that to the quadrate and pterygoid, was first seen at Stage 39. The relative locations of these are shown in Figure 11. The siphonium took origin close to the opening of the pharyngotympanic tube and projected rostrally towards the mandibular cartilage. Even at the time of its first appearance the structure of this diverticulum was somewhat different to the other, having a stratified columnar type of epithelium with a relatively narrow lumen (Fig. 13). When perichondral ossification of the articular commenced this was slightly distal to the point of termination of the diverticulum. By the time of hatching the situation was basically unchanged. The blind rostral tip of the diverticulum curved laterally and lay close to the perichondrium of the medial mandibular process where cartilage was now undergoing calcification and resorption (Fig. 14).

The times of ossification and of onset of pneumatisation of the bones which became pneumatised before hatching are summarised in Figure 15.

Pneumatisation during the posthatching period

Bones initially pneumatised prior to hatching

Pneumatisation was already extensive in most of this group at the time of hatching. The exception was the parietal where pneumatisation was confined to the caudolateral region, the only part yet containing diploe, the rest still being in the form of a single

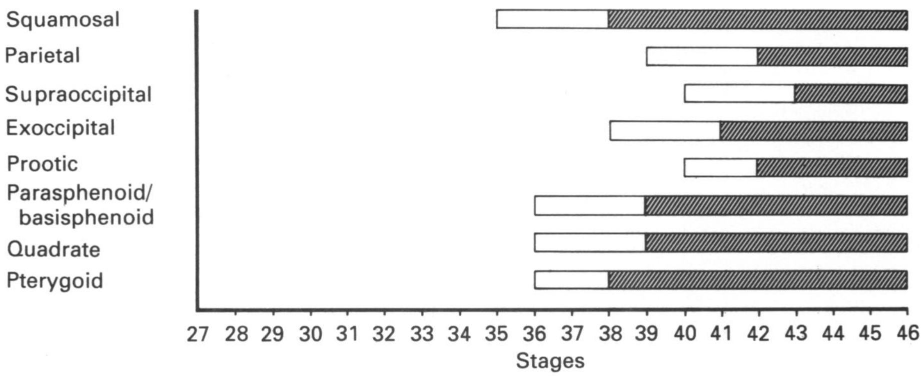

Fig. 15. Chart summarising the occurrence of ossification (clear areas) and pneumatisation (hatched areas) within these bones pneumatised during the embryonic period.

thin plate of compact bone. Diploe spread progressively and occupied more or less the whole bone by 42 days posthatching. A spread of pneumatisation followed but its final extent varied somewhat.

Bones initially pneumatised after hatching

Frontal

At hatching the frontal was entirely in the form of a single plate of compact bone. Diploe was first recognised at 35 days posthatching. From 42 days posthatching onwards pneumatisation could be identified and in general it spread progressively from caudal to rostral until it involved much of the bone although its final extent was somewhat variable.

Basioccipital

Pneumatisation was first identified at 42 days posthatching and in all birds thereafter. When it was first identified the wide synchondrosis between the basioccipital and the parasphenoid/basisphenoid was still present and the more likely route of spread would seem to be from the laterally placed exoccipital.

Pleurosphenoid (orbitosphenoid *N.A.A.* See Hogg, 1978)

Pneumatisation was first identified at 91 days posthatching.

Orbitosphenoid

This paired element was found to ossify between 70 and 84 days posthatching (Hogg, 1978). Subsequent to its fusion with the remainder of the cranium the region to which it contributed showed evidence of pneumatisation in the oldest birds in the series, i.e. 119 days and 126 days posthatching.

Mesethmoid

This showed pneumatisation at 119 days and 126 days posthatching.

Mandible

In most of the posthatching specimens examined by serial sectioning the situation was not significantly different to that encountered in the embryonic specimens and the distal extremity of the siphonium lay some distance short of the ossified collar of bone of the articular. Sometimes considerable disparity in this distance was noted on the opposite sides of the same specimen. In one case, at 14 days posthatching, the distal

Fig. 16. 14 days posthatching. The distal extremity of the siphonium (*S*) is now lodged within the perichondrally formed bone in the cortex of the articular bone of the mandible. Small blood vessels (arrows) are entering along with the air sac through the developing pneumatic foramen. ×250.

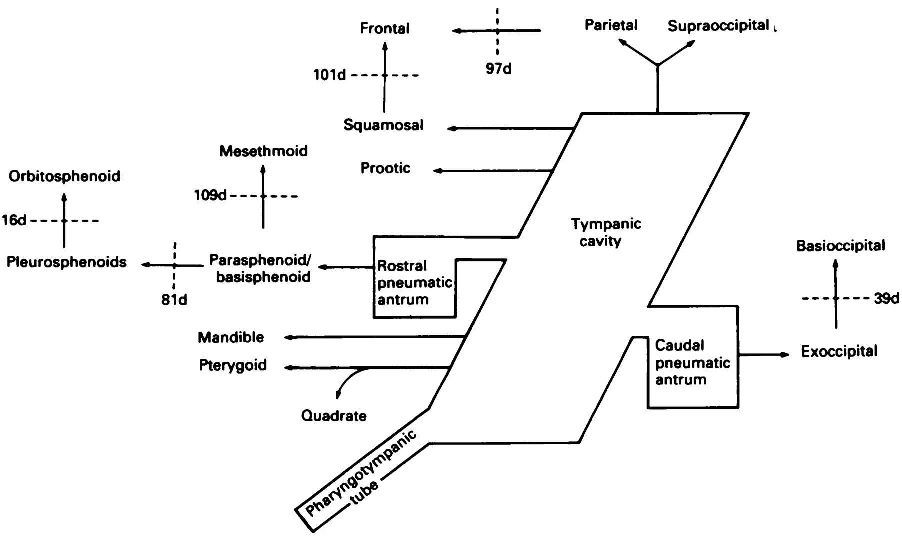

Fig. 17. Routes of pneumatisation from the tympanic cavity. With those bones pneumatised after hatching, the most likely routes of spread are shown, and fusion times of the intervening joints between adjacent bones are as reported by Hogg (1978).

extremity of the siphonium was found to have penetrated the developing bone on one side only (Fig. 16).

The direct routes of pneumatisation from the tympanic cavity and the likely routes of further spread after hatching are shown diagrammatically in Figure 17. In the case of spread of pneumatisation between bones the mean fusion times between these bones are indicated according to Hogg (1978).

DISCUSSION

In the account of skull development by Jollie (1957) the timing of ossification of individual bones is related only to incubation age. However, if these ages are converted to standard Hamburger–Hamilton stages almost complete agreement is found with the results in present study. In the case of these bones undergoing pneumatisation as the result of direct spread from the tympanic cavity, the relationship between the onset of ossification and air sac invasion was a remarkably constant interval of three stages in almost every case.

The caudal extension of the tympanic cavity produced as the result of cartilage resorption during the ossification of the exoccipital resulted in the formation of the cavity termed the caudal pneumatic antrum (*N.A.A.*) from which small separate extensions lead off into the exoccipital bone. This part of the system, therefore, bears a strong resemblance to the human mastoid antrum and mastoid air cells. The rostral extremity of the tympanic cavity, which becomes surrounded secondarily by the ossification of the parasphenoid/basisphenoid, forms the rostral tympanic antrum (*N.A.A.*) from which lead numerous extensions into the surrounding bone. A dorsal pneumatic foramen, consistently located between squamosal and prootic articular facets for the quadrate bone and leading to a dorsal pneumatic antrum, is also listed in *N.A.A.* In this study the cavity with extensions into the parietal and the supraoccipital had the appearance simply of the dorsal region of the tympanic cavity rather than a dorsal pneumatic antrum.

Pneumatisation was found to involve the whole cranium but not the face other than the quadrate and the pterygoid which represented the most rostral extent of pneumatisation. It was, therefore, rather less extensive than implied by Bremer (1940) but agreed with the adult survey by Hogg (1984*a*), except for the mandible in which pneumatisation was found variably in about 50% of adults. There was no evidence of this actually occurring in the embryo as Bremer (1940) maintains, but the one case observed of penetration of the articular bone region of the mandible at 14 days posthatching would seem to confirm the possible occurrence of mandibular pneumatisation in the domestic fowl. Although this specimen showed only this earliest stage of pneumatisation there seems no reason to suppose that any further barrier would have existed to its subsequent spread to reach the adult extent previously reported by Hogg (1984*a*). Indeed Bremer (1940) did describe a similar penetration of the outer layers of bone cortex in birds of 21 and 28 days posthatching but seems to have assumed that no further spread would have occurred. The variable occurrence of pneumatisation in the mandible and indeed in other bones in the skeleton is probably accounted for by the proximity of the air sac extension, in this case the siphonium, to the site of initial perichondral bone formation. If the air sac is not in the critical position at the critical time, the opportunity for the development of a pneumatic foramen for entry to the bone will be lost and the bone will remain unpneumatised.

The skull bones, other than the mandible, which develop pneumatisation by direct extensions of the tympanic cavity, i.e. by primary pneumatisation, are indeed exceptional within the skeleton in exhibiting apparently very little variability in the occurrence of pneumatisation, the timing of its onset or its final extent, unlike the bones of the postcranial skeleton (Hogg, 1984*a*,*b*). The remaining skull bones undergoing pneumatisation, i.e. the secondary pneumatisation group, do so by spread from the primary group and consequently are later in doing so. In the case of the bones of the calvaria the conversion of the bones from a thin unilaminar structure to a trilaminar one with enclosed diploe is a necessary precedent to the spread of pneumatisation. The variability in extent of this conversion, coupled with variability in spread of pneumatisation, accounts for the final variations seen in bones like the frontal and parietal (Hogg, 1980).

In bones belonging to the primary pneumatisation group which ossify intramembranously, e.g. squamosal, parietal and parasphenoid, initial invasion is multiple and indeed bone appears to be deposited around tongue-like extensions of the tympanic cavity or rostral tympanic antrum. In bones in the primary group undergoing perichondral ossification cartilage resorption is a necessary prerequisite to air sac penetration and frequently results in a single site of entry, the pneumatic foramen where the entry of the air sac is often accompanied initially by the vascular bud. The necessary cartilage resorption in advance of the spread of pneumatisation also appears to be a governing factor in its spread from bone to bone, thus delaying spread to the orbitosphenoid and mesethmoid for example until after these have undergone fusion to adjacent bones. On the other hand, spread to the frontal across adjacent sutures would appear to be possible at an earlier stage.

SUMMARY

The development of pneumatisation in the skull of the domestic fowl has been studied in a series of chick embryos from 7–20 days incubation (Hamburger & Hamilton Stages 29–46) and in birds from hatching to 126 days posthatching.

During the embryonic period primary pneumatisation developed by 3 routes. (i) The tympanic cavity directly invaded surrounding bones – squamosal, parietal, supraoccipital and prootic. (ii) Extensions of the tympanic cavity invaded the bones in which these occurred – the caudal pneumatic antrum in the exoccipital and the rostral pneumatic antrum in the parasphenoid/basisphenoid. (iii) A tubular diverticulum from the tympanic cavity grew rostrally and invaded the quadrate and pterygoid. A similar diverticulum grew rostrally towards the cartilaginous mandible but was only found to invade it in one case after the time of hatching.

In most instances onset of pneumatisation occurred three stages subsequent to the onset of ossification. In bones in which ossification is intramembranous bone tissue often formed around small air sac outgrowths, resulting in multiple sites of invasion while, in bones ossifying perichondrally, cartilage resorption was a necessary prerequisite and air sac invasion frequently occurred in common with a vascular bud resulting in a single pneumatic foramen.

After hatching secondary pneumatisation spread from the already pneumatised bones to involve the whole cranium. Spread throughout the parietal and frontal was preceded by the establishment of diploe within these bones and the final extent of pneumatisation was variable. Spread to the most distal parts of the cranium was only accomplished after the intervening sychondroses had fused.

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