Experimental studies on the columella-capsular interrelationship in the turtle *Chelydra serpentina*

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The reciprocal effect of two neighbouring structures of different origins on each other during development is of considerable interest to the embryologist. Such a relationship exists between the columella auris and the auditory capsule in the vicinity of the fenestra ovalis. This particular relationship, in addition, embraces an aspect of fundamental importance to the morphologist viz. the possible derivation of part of the columella from the capsule.

The vast literature pertaining to this latter aspect and particularly in regard to the development of the human stapes, was recently reviewed in great detail by Strickland, Hanson & Anson (1962). References to literature related to other vertebrates are found in e.g. van der Klaauw (1924), Versluys (1936), and Werner (1960). An earlier paper (Toerien, 1963) deals with the rather specialized problem of Amphibian stapes development.

The results of certain extirpation procedures on early embryos of the turtle (*Chelydra serpentina*) throw interesting light on the columella-capsule interrelationship. The planning of the operations was based on the following earlier observations:

1. The visceral skeleton is almost entirely derived from neural crest cells and consequently the removal of the cranial neural folds will lead to the absence of the visceral arch skeleton (Hörstadius, 1950; Mangold, 1961; Hammond & Yntema, 1964). Since the stapes is part of the visceral skeleton, its absence was also anticipated.

2. Removal of the otic placode or otocyst inhibits the development of the auditory capsule in most vertebrates (see Yntema, 1955; Benoit, 1957; Toerien, 1965).

**MATERIAL AND METHODS**

The eggs were obtained from turtles collected in the vicinity of Syracuse N.Y. and from the Lemberger Co. of Oshkosh, Wisconsin. The recovery of the eggs, their preparation for the operation and the subsequent treatment of the embryos are described by Yntema (1964).

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The following operations were carried out by means of glass needles and sharpened watchmaker's forceps:

2. Bilateral removal of the neural folds between the second somite and the optic enlargement (somite stages 4–6).
3. Bilateral removal of the neural folds anterior to the optic enlargement but excluding the anterior (transverse) fold (somite Stages 4–6).

The eggs were incubated at 30°C for a period of 30 days. At this temperature, 30 days constitutes about half the total incubation time for normal eggs.

The average shell lengths of the specimens when sacrificed are given in Table 1. Serial sections, 16 μ in thickness, were prepared of all the heads and graphic reconstructions made of the chondrocrania.

### Table 1

<table>
<thead>
<tr>
<th>Extirpation of otic placode</th>
<th>Extirpation of anterior cranial neural fold</th>
<th>Extirpation of posterior cranial neural fold</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of specimens</td>
<td>14</td>
<td>7</td>
</tr>
<tr>
<td>Specimens selected for study</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Average shell length when sacrificed</td>
<td>13.7 mm.</td>
<td>12.7</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

The following results bear on the present problem.

(a) Absence of almost the entire auditory capsule and a deficient footplate of the columella follow the removal of the placode on that side (Toerien, 1965).

(b) One of the most conspicuous results of the removal of the anterior cranial neural folds is the absence of the cartilaginous elements of the mandibular visceral arch. In addition to Meckel's cartilage and the quadrate, the development of the tympanic membrane, which is supported by the latter structure, is also inhibited.

(c) Extirpation of the posterior cranial neural folds lead to the suppression of the development of the cartilaginous derivatives of the hyoid visceral arch, including the columella auris.

Although the changes occurring in the chondrocrania of the operated animals are largely consistent for the different experiments, minor variations are found. Cartilaginous structures representing remnants of the ear capsule (Toerien, 1965) or parts of the visceral arch skeleton are not always completely suppressed. Only those specimens in which the structures operated for are completely or
almost completely absent from otherwise fairly normal skulls, were selected for
detailed study. The results reported above derive from these specimens (Table 1).
They allow the study of the following aspects of cranial development:

1. The configuration of the stapes in the absence of the two structures with
which it is most intimately connected viz. the tympanic membrane and the
auditory capsule;
2. The development of the fenestra ovalis in the absence of the columella and
3. The possible contribution of capsular material to the formation of the
columella footplate.

The Columella Auris

In the normal embryo of 14 mm. shell length, the columella is entirely carti-
laginous. It is dumb-bell shaped with a slender, gently curved rod, connecting
the oval footplate with a lateral disc. (Dohrer, 1919). A ventral process (inter-
hyal process, Bender, 1911, Kunkel, 1912b) of varying length extends ventrally,
posteriorly and medially from the ventral rim of the lateral plate. Posterior to
this process the plate is excavated from behind (Text-fig. 1).

TEXT-FIG. 1. Graphic reconstruction of lateral view of part of normal auditory capsule of
embryo of Chelydra serpentina Columella auris incompletely drawn. Aud. cap., auditory
capsule; Fen. oval., fenestra ovalis; Col. ftp!., columella auris footplate; Col. latpl., colum-
ella auris lateral plate; Proc. interh., interhyal process; Proc. metot., metotic process
(parotic crest).

Medial aspect.

The development of the columella was studied in several chelonians and the
possible capsular origin of its footplate received much attention. According to
Smith (1914) and Shaner (1926) the columella in Chrysemys marginata is
chondrified from two centres. The precartilaginous mesenchyme is, however, laid down in one piece which is also continuous with that of the interhyal and hyoid cornu (Smith, 1914). At a later stage the interhyal process (remnant of the interhyal) joins Meckel's cartilage or becomes connected to it by means of a dense connective tissue string, \((Emys lutaria;\) Kunkel, 1912b).

The view of these authors as to the non capsular origin of the entire columella is in contrast to that of earlier workers, Parker \((1880, Chelone virides),\) Noack \((1907)\) and Fuchs \((1907, 1915 Emys europaea)\) who claimed a capsular origin for (at least) the footplate of the columella. At first glance the experimental evidence seems to support this earlier point of view since the medial end—footplate—is always deficient after suppression of the cartilaginous ear capsule.

**Text-fig. 2.** Graphic reconstruction of ventral view of auditory capsule and columella auris of *Chelydra serpentina.* Both structures are virtually unaffected by the absence of a quadrate and tympanic membrane which was induced experimentally. Abbreviations as in Text-fig. 1.

This is in conformity with the results of similar experiments on other tetrapods. (Luther, 1927, *Rana, Bombinator*; Violette, 1930, *Rana*; Reagan, 1915, 1917 chicken and Toerien, 1963, *Ambystoma*).

The columellar rod in the experimental animals is complete. Since the medial half of the columella, i.e. the footplate and half the rod, is developed from one chondrification centre it seems clear that it is the footplate of the columella that is affected by the absence of the ear capsule and not the entire medial chondrification. A variable number of footplate cells tend to be present around the medial end of the columella. These two observations, together with the fact that a separate chondrification centre in the capsular wall representing the footplate was not found by any investigator, indicate that the footplate as such cannot be regarded as part of the cartilaginous ear capsule.

The process whereby cartilage cells surrounding the fenestra ovalis can ‘dedifferentiate’ and then join the rudimentary stapes footplate as described for man
by Bast & Anson (1949) and Hanson et al. (1962) cannot be verified by the present experiments and it is also possible that the capsule merely induces the full development of the footplate as in the amphibians that were investigated experimentally (Toerien, 1963).

**Lateral Aspect**

The absence of a tympanic membrane and quadrate, following extirpation of the anterior neural folds, does not seem to have much effect on the development of the lateral plate of the columella. The ventral (interhyal) process, however, appears to be displaced more dorsally (Text-fig. 2).

**The Auditory Capsule**

The fenestra ovalis is found between the capsular cartilage superiorly and anteriorly, the basal plate ventrally and the ‘stapes inferior’ ventro-posteriorly. It was shown elsewhere (Toerien, 1965) that the ‘stapes inferior’ of Kunkel (1912a) is not of capsular origin and therefore not homologous with the amphibian operculum. The fenestra is oval shaped with its long axis directed antero-posteriorly. The footplate of the columella fits closely into this opening (Text-fig. 1).

**TEXT-FIG. 3.** Graphic reconstruction of lateral view of auditory capsule in embryo of *Chelydra serpentina* in which the development of a columella auris is experimentally suppressed. *H. cart. rem.*, cartilaginous remnants of hyoid branchial arch. Other abbreviations as in Text-fig. 1.
Fairly normal auditory capsules were found in a number of specimens of which the posterior neural folds were removed. This was also found in *Ambystoma* by Hörstadius & Sellman (1946). In the absence of a columella the fenestra ovalis has the appearance of an irregular fissure (Text-fig. 3). A separate (loose) footplate was not found in the absence of a columella. The irregularity of the fenestra ovalis is apparently not caused by the effect of the removal of the neural fold on the development of the capsule. In some specimens, where the capsule is less complete but a columella present, the fenestra is normal. However, the fenestra is sometimes entirely absent in the small vesicular capsules.

**SUMMARY**

1. As in the chicken and amphibia the presence of the auditory capsule in the turtle is necessary for the full development of the medial end of the columella auris.

2. The experimental evidence supports the more recent views obtained from the study of normal series of chelonians that the footplate is not developed as a separate entity from capsular cartilage.

3. The columella is virtually unaffected by the absence of the quadrate and tympanic membrane.

4. The development of a normal fenestra ovalis appears to be dependent on the presence of a columella.

**RÉSUMÉ**

*Recherches expérimentales sur les relations mutuelles entre la capsule auditive et la columelle chez la tortue Chelydra serpentina.*

1. Comme chez le poulet et les Amphibiens, la présence de la capsule auditive est nécessaire, chez la tortue, au plein développement de l’extrémité médiane de la columelle.

2. Les résultats expérimentaux appuient les considérations les plus récentes tirées de l’étude de séries de Chéloniens, selon lesquelles la plaque stapédiale ne se développe pas en tant qu’entité séparée à partir du cartilage capsulaire.

3. L’absence du carré et de la membrane tympanique n’affecte virtuellement pas la columelle.

4. Le développement d’une fenêtre ovale normale apparaît comme dépendant de la présence de la columelle.

**ACKNOWLEDGEMENTS**

The experiments on which these results are based were carried out in the Department of Anatomy, State University of New York, Upstate Medical College at Syracuse. I am most grateful to Professor Chester L. Yntema who not only suggested the problem to me but supervised and assisted in its execution while I was a guest in his laboratory as International Post-doctoral Research Fellow of the National Institutes of Health.
Columella-capsular interrelationship 271

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