Participation of neural crest-derived cells in the genesis of the skull in birds

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SUMMARY

The differentiation of cephalic neural crest cells into skeletal tissue in birds has been observed using the quail-chick nuclear marking system, which is based on specific differences in the distribution of the nuclear DNA. Chimaeras were formed by replacing a fragment of cephalic neural primordium of a 2- to 12-somite chicken embryo by the corresponding fragment isolated from an equivalent quail embryo. The participation of the graft-derived cells in the formation of the skull of these embryos was studied on histological sections after Feulgen and Rossenbeck staining.

Cells from the prosencephalic neural crest migrate into the frontal nasal process and mix with the mesencephalic neural crest cells in the lateral nasal processes, around the optic cupule and beneath the diencephalon. In addition, the mesencephalic neural crest cells form the bulk of the mesenchyme of the maxillary processes and mandibular arch, whereas the rhombencephalic neural crest cells become located in the branchial arches.

The origin of cartilages of the chondrocranium and bones of the neurocranium and viscerocranium has been shown in the chimaeric embryos: the basal plate cartilages, occipital bones, sphenoid bones and the cranial vault are mainly of mesodermal origin. However some parts have a dual origin: rhombo-mesencephalic neural crest cells are found in the otic capsule, and the frontal bone, the rostrum of parasphenoid and the orbital cartilages contain diverse amounts of prosencephalo-mesencephalic neural crest cells. The squamosals and the columella auris are formed from mesectodermic cells as are the nasal skeleton, the palatines and the maxillary bones. The mesectodermal origin of mandibular and hyoid bones and cartilages was already known.

From these results it appears that the cephalic neural crest is particularly important in the formation of the facial part of the skull, while the vault and dorsal part are mesodermal and cartilages and bones found in the intermediary region are of mixed origin. The presence of mixed structures implies that the mesoderm and the mesectoderm are equally competent towards the specific inducers of these bones and cartilages. This correlates with the equivalence in differentiation capacities already shown for cephalic mesodermal and mesectodermal mesenchymes.

INTRODUCTION

The hypothesis that neural crest-derived cells participate in the morphogenesis of various parts of the skull was first expressed by Platt (1893), Goronowitsch (1893), at the time when the neurectodermal origin of a part of the mesenchyme was also discovered. Since that time numerous attempts have been made to verify this hypothesis either by descriptive studies of the evolution of the

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neural crest cells (cf. review by Hörstadius) or by applying experimental techniques. These latter consisted of excisions of neural primordium or pieces of mesectoderm and the use of various marking systems (cf. Hörstadius, 1950; Weston, 1970). The following techniques were used: vital dyes, natural marking system, in amphibians, related to specific differences in aspect and size of the cells and nuclei and tritiated thymidine radioactive marker.

In amphibians, it has been found that the greatest part of the viscerocranium (Meckel’s cartilage, palatoquadrat and hyobranchial skeleton, except for the second basibranchium) as well as part of the neurocranium (the anterior part of the trabeculae) have, at least partially, a neururectodermal origin. According to some authors, the mesectoderm might also be included in the formation of some membrane bones like the mandibular bones. In birds, the presence of neural crest cells has been experimentally demonstrated in the facial and branchial mesenchyme (Johnston, 1966; Noden, 1975; Le Lievre, 1974). By removing the rhombo-mesencephalic neural crest from the chick (Hammond & Yntema, 1964), it has been shown that mesectodermal cells take part in the formation of the hyoid (except the basibranchial), of the mandibular skeleton and the interorbital 

Recently, the use of a natural marking system in birds (Le Douarin, 1969) has made it possible to carry out a more precise study of the role of neural crest-derived cells in the genesis of cephalic and cervical skeleton (Le Lièvre, 1971, 1974). We have thus confirmed and completed the previous observations establishing that some of the mesectodermal cells which constitute the greatest part of the branchial mesenchyme will form the entire tongue skeleton (hyoid) and the mandibular skeleton (Meckel’s cartilage and membrane bones). Johnston, Bhakdinaronk & Reid (1973) using the same technique have observed the presence of quail donor cells in the skull of the chick host. Nevertheless it was still necessary for a detailed study of the participation of mesectodermal cells to be done. This paper discusses results obtained using the quail-chick nuclear marking system.

**METHODS**

**Marking system (Fig. 1)**

The method of Le Douarin (1969) was used as previously described (Le Lièvre, 1971, 1974; Le Lièvre & Le Douarin, 1975). The identification of quail and chick cells is allowed by structural differences of their interphase nuclei. These differences are revealed in light microscopy, particularly following Feulgen and Rossenbeck nuclear staining (1924), and are widely spread between embryonic and adult cell types. They can constitute a permanent marking system in quail-chick chimaeras. In the experiments to be described this was used to follow the neural crest-derived cells through their evolution and differentiation after grafting fragments of cephalic quail neural primordium into chicken embryos.
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Fig. 1. Sample showing quail (a) and chick (b) chondrocytes of the otic capsule of 8-day chimaeric embryos. After Feulgen-Rossenbeck staining, the chromatin is dispersed in the nucleoplasm of the chick whereas it is condensed in one or several masses in the quail nucleus. G × 1320.

Operations (Fig. 2)

These were performed as follows on stage 7–11 chicken embryos (stages of Hamburger & Hamilton, 1952) before the neural crest has left the neural axis and started to migrate, i.e. before the 7-, 10- or 13-somite stage respectively when the operation is done at the prosencephalo-mesencephalic level or at the anterior or median and posterior parts of the rhombencephalon.

1. Excision. The removal of a fragment of the cephalic primordium was first performed on the chick embryo. We used microscalpels to separate the neural tube and crest from the surrounding mesenchyme.

2. Preparation of the graft. An homologous fragment of neural primordium was isolated mechanically from a quail embryo at the same stage as the chick host, and incubated for 10–15 min at 0 °C in a 5% solution of trypsin in calcium- and magnesium-free Tyrode solution.

3. Graft. Isotopic and isochronic grafts of this isolated neural primordium were set in the groove left by the excision on the chick embryo and thus replaced the excised host neural tissue. The anteroposterior and dorsoventral
Fig. 2. Grafting technique: diagram of an orthotopic, heterospecific graft of prosencephalo-mesencephalic neural primordium. 1. Removal of the primordium from a 5-somite chick embryo. 2. Isolation of the corresponding transverse fragment of a 5-somite quail embryo. 3. Dissociation of the quail neural primordium. 4. Graft of this quail tissue into the chick, in the groove left by previous ablation.

orientations were maintained. Reverse grafts of chick neural primordium in the quail have also been done.

The embryos were killed between stage 19 and 40 (Hamburger & Hamilton, 1952). Their heads and necks were fixed in Zenker's fluid. They were cut in serial 5 μm thick sections and stained by the Feulgen & Rossenbeck (1924) nuclear reaction and lightly counterstained with picric-indigo-carmine.

Table 1 indicates the conditions of operation and fixation of the chimaeric embryos that have been used for this study.
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Table 1. Conditions of graft and fixation of the chimaeric embryos used for the study on the origin of the skull

<table>
<thead>
<tr>
<th>Graft</th>
<th>Fixation</th>
<th>Number of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Between stages 23 and 35 (4-10 days)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Between stages 36 and 41 (10-14 days)</td>
<td></td>
</tr>
<tr>
<td>Prosencephalon</td>
<td>1-6 somites</td>
<td>8</td>
</tr>
<tr>
<td>Mesencephalon</td>
<td>1-6 somites</td>
<td>20</td>
</tr>
<tr>
<td>Mesencephalon rhombencephalic</td>
<td>4-9 somites</td>
<td>21</td>
</tr>
<tr>
<td>Rhombencephalon</td>
<td>4-11 somites</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>6-12 somites</td>
<td>13</td>
</tr>
<tr>
<td>Number of cases</td>
<td>2 1 4 1 5 1 6 1 3 4 1 3 1 1</td>
<td>31 6</td>
</tr>
</tbody>
</table>

In birds, the anatomy of the bones of the skull as well as chondrocranium and neurocranium morphogenesis have been carefully described by several authors, most data concerning the skull embryology have been reported by Romanoff (1950).

The nomenclature used by Romanoff (1950) has been used here.

RESULTS

(A) Early localization of cephalic neural crest derived cells

The localization of mesectodermal cells in the branchial and facial regions of host embryos following grafts at the mesencephalon and rhombencephalon levels has been previously described (Le Lièvre, 1974; Le Lièvre & Le Douarin, 1975).

New grafting experiments have been done at the prosencephalon level in order to complete these observations on the superior part of the head and face. The prosencephalic neural crest cells mixed with mesencephalic ones; they surrounded the eye cups and spread along the lateroventral aspect of the diencephalon (Fig. 3). They also contributed some mesectoderm to the lateral nasal processes and provided almost the entire mesenchyme of the frontal nasal process. However, they became sparse towards the frontal region of the head.

Figure 4 indicates the localization of the neurectodermal mesenchyme between stage 19 and 23 of Hamburger & Hamilton (1952).
Fig. 3. At stages 19–25 of Hamburger & Hamilton, localization of the mesectoderm derived from the prosencephalic (■) and mesencephalic (□) neural crest. (a) Transverse section of a stage-19 chick embryo. (■): muscle plate (pm); max, maxillary process; olf, olfactory placode; ot, otocyst; Pros, prosencephalon; Rh, rhombencephalon. (b) Transverse section of a stage-23 chick embryo. Dienc, diencephalon; hyp, Rathke's pouch; oe, eye; Telenc, telencephalon.

(B) Origin of the skull

In a previous article we have shown that both the hyoid and mandibular skeleton are derived totally from mesencephalic and rhombencephalic neural crest (Le Lièvre, 1971, 1974).

The distribution of mesectodermal and mesodermal cells in the other regions of the skull of chimaeric embryos varies from one area to the other. We will successively consider, from posterior to anterior, the occipital and otic areas, the pituitary and orbital areas, the maxillary and palatal bones and the nasal region.
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Fig. 4. Localization of neural crest-derived cells in the superior part of the face of a grafted embryo before stage-25 of Hamburger & Hamilton (Feulgen and Rossenbeck staining). (a) Parafrontal section at the pituitary level (H) of a stage-17 embryo. The graft was performed at the prosencephalon and mesencephalon level. Note the presence of quail cells (→) in the vicinity of the grafted diencephalon (D) and the chick epithelium of Rathke’s pouch. G x 770. (b). Transverse section at the eye level of a grafted embryo at stage 21. The operation was performed at the mesencephalon level and at the anterior and median parts of the rhombencephalon. Ec, chick ectoderm; M, mesectoderm from the graft. G x 880.

1. **Otic and occipital skeleton (Fig. 5)**

Whatever be the level of the graft, the basal cartilages (acrochordal and parachordal) were always formed from chick host cells and had a pure mesodermal origin. However, when the grafts were performed at the anterior part of the rhombencephalon, quail chondrocytes were found in the otic capsules (6). These mesectodermic cells were mixed with chick mesodermal cells and took part in the formation of the metotic cartilage and, to a smaller extent, in that of the *pars cochlearis* and the *pars canalicularis* (Fig. 6).
The columella auris (7) chondrified during the 7th and 8th days of incubation and was entirely formed of mesectodermal cells derived from the mesencephalon and the anterior part of the rhombencephalon (Fig. 6) as was the squamosal (7), a lateral dermal bone, which appears after 7 days of incubation. Some observations on older chimaeric embryos, after 10 days of incubation, indicated that the other bones of this region, parietal (2) and occipital (3, 4, 5) have a mesodermal origin.
Fig. 6. Differentiation of neural crest-derived cells in the otic and occipital skeleton (Feulgen and Rossenbeck staining). (a) Transverse section in the otic region of a grafted embryo at stage 32. The graft was performed at the mesencephalic, anterior and median rhombencephalic level. co, columella; cp, otic capsule, E, pharyngeal chick host endoderm. G × 120. (b) Detail of (a). The columella and the surrounding mesenchyme derive from the graft. (c) Detail of (a). The otic capsule has a mixed origin: both quail cells (c) from the graft and chick host (p) cells are present. G × 690.
2. **Skeleton of pituitary and orbital regions** (Fig. 7A).

Before 6 days of incubation some mesectodermal cells were present in the mesenchyme which surrounded the pituitary (Rathke’s pouch). They came primarily from the mesencephalic neural crest. Later, in the pituitary pit region, the trabeculae only contained graft cells when the embryo had been operated at the mesencephalic level. The other cartilages, acrochordal and polar cartilages, were formed from host chicken chondrocytes, whatever the level of the graft, and had a mesodermal origin.

In the eye region orbital (12, 13, 14) and sclerotic (17) cartilages always contained a mixture of quail and chick cells whether the graft was at the mesencephalic or prosencephalic levels. However, quail cells from the prosencephalon were more numerous in the anteroventral part of the orbit and the sclerotic plates while the mesectodermal cells from the mesencephalic primordium were located mainly in the dorsal region of these cartilages (Fig. 8).

The beginning of the ossification in this part of the skull takes place after 8 days of incubation in the chick embryo. In this region, we found mesencephalic neural crest-derived cells in the rostrum parasphenoid (11). The orbitosphenoids (10) and the other bones of the sphenoid bone complex (8, 9) were mesodermal in origin. The bones of the orbital skeleton were always made up of a mixture of quail and chick cells as were the cartilages they replaced; some sparse mesectodermal cells may be seen in the frontal bone (15).

3. **The mandibular and palatal skeleton** (Fig. 7B)

When the graft was performed at the mesencephalic level, osteocytes of jugal (21), quadratojugal (22) and pterygoid (19) bones of the chimaeric embryos derived from the graft (Fig. 8). These bones were formed from the mesectoderm of the maxillary processes whilst the quadrates were differentiated from mesectodermal cells of the first branchial arch. Maxillaries (20) and palatines (18) also consisted of mesectodermal cells and were derived from the mesencephalic neural primordium except for their most anterior part. This region as well as the premaxillaries (24) was formed of quail cells when the graft had been performed at the prosencephalic level.

Fig. 7. Diagram of the skull of a 14-day-old chick embryo showing the localization of totally or partially mesectodermal bones and cartilages (§) in the orbital (A), maxillary and palatal (B), or nasal (C) regions. (a) lateral view; (b) ventral view. 8, Basisphenoid; 9, alisphenoid; 10, orbitosphenoid; 11, rostrum of parasephonid; 12, interorbital, inter-nasal septum; 13, supraorbital cartilage; 14, antorbital cartilage; 15, frontal; 16, lacrymal; 17 (*), sclerotic cartilage; 18, palatine, 19, pterygoid; 20, maxillary; 21, jugal; 22, quadratojugal; 23, quadrate; 24, premaxillary; 25, nasal; 26, concha nasalis.

* 17 indicates the situation of sclerotic cartilages which have not been represented on this drawing.
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4. The nasal skeleton (Fig. 7C)

Following grafting at the mesencephalic level, some quail cells were observed in the conchae nasalis (26) and in the nasal cartilaginous capsule (Fig. 8). These cartilages and the nasal septum (12) are composed primarily of cells from the prosencephalic neural primordium.

Nasal bone (25) and cartilage are differentiated in the nasal lateral and the nasal frontal processes. The mesenchyme in which the nasal bones, the premaxillaries (24) and the vomer form, has consequently a mesectodermal origin and these bones are derived from the prosencephalic neural crest.

DISCUSSION–CONCLUSIONS

The results obtained with the technique used appear to be reliable; the graft healed quickly and the distribution of the neural primordium-derived cells was not affected by the difference in species between host and donor. This has been confirmed by grafting chick neural primordium into the quail and vice versa. The results concerning the migration and the early localization of the graft cells are identical in both cases (Le Lièvre & Le Douarin, 1975). Also, observations on the migration of neural crest cells have been made using this technique (cf. Teillet, 1971; Le Lièvre, 1974) and these are in agreement with the results of other authors who have used different techniques such as tritiated thymidine marking (cf. Johnston, 1966; Weston, 1970). The marking system we used was stable enough for the study, in situ, of neural crest derivatives up to the time of the ossification of the skull. Johnston et al. (1973) used the same heterospecific graft technique and nuclear marker. They point out the presence of quail cells in the facial skeleton but they did not report systematic examination of the operated embryos nor specification of the respective location of mesodermal and mesectodermal bones and cartilages.

The experiments described have allowed us to determine the localization of neural crest-derived cells in the cranial and visceral skeleton. They also indicate

Fig. 8. Differentiation of neural crest-derived cells in the facial skull (Feulgen and Rossenbeck staining). (a) Transverse section at the eye level in a grafted embryo at stage 33 of Hamburger & Hamilton. The graft was performed at the mesencephalon level. The scleral cartilage (Sc) is formed from quail cells of the graft (p, endothelial and muscular chick-host cells). G × 470. (b) Transverse section at the maxillary level of the same embryo. The jugal (j) membrane bone is formed from quail cells of the graft as is the surrounding mesenchyme (Ec, chick host epidermis). G × 470. (c) Transverse section of the nasal region of a grafted embryo at stage 36 of Hamburger & Hamilton. This chick embryo was grafted at the level of the mesencephalon and the posterior part of the prosencephalon. N, Concha nasalis; S, nasal septum. G × 120. (d) Detail of (c). Note the presence of quail cells from the graft in the cartilages (N and S) and surrounding mesenchyme. The chick host cells form the nasal epithelium (Ep). G × 470.
the level of origin of the cells which will constitute the various bone and cartilages of these areas.

The prosencephalic neural crest cells, after a dorsoventral migration, reached the facial aspect of the head by passing in front of the eye cupule; they then migrated along the diencephalon as far as Rathke's pouch. Some mesencephalic neural crest cells also migrated forwards following the prosencephalic ones. Altogether they congregated in the face, in particular in the nasal processes, and constituted the main part of the bones and cartilages in the nasal skeleton, the anterior orbital skeleton and the rostral end of the maxillary skeleton. The mesencephalic neural crest cells primarily migrated into the maxillary processes and the first branchial arch as well as, to a smaller extent, into the second branchial arch. They formed the palate, the superior and inferior jaws and participated in the formation of some bones of the otic region and the entoglossum of the hyoid (Le Lièvre, 1974). The rhombencephalic neural crest cells migrated into the second, third and fourth branchial arches and into the posterior branchial regions. From this mesectoderm, the basihyal, the hyoid cornua and the brasibranchial differentiated (Le Lièvre, 1971), as well as the columella auris, and part of the otic capsules.

Although grafts were done at all levels, including the cephalic region and below the level of the third somite, no mesectodermal cells were ever found in the tracheal cartilages. Consequently these cartilages are mesodermal in origin.

Noting the general dorsoventral migration of neural crest cells at the cephalic level and their preferential localization in the face, three main areas can be observed in the skull according to the origin of chondroblasts and osteoblasts. The facial area (nasal, maxillary, palatal, mandibular and hyoid) is mainly, if not completely, of mesectodermal origin, while most of the vault of the skull and the cervical skeleton derive from the cordomesoblast. In the intermediary area (otic, orbital and frontal) some mesodermal and mesectodermal cells are mixed; cartilages and bones in this area have a dual origin (Fig. 9).

There is no clear-cut boundary between areas of the skull having a different origin. In this respect the composite nature of the trabeculae cranii had already been described in amphibia (cf. Hörstadius, 1950; Chibon, 1966). As far as skeletal tissues are concerned (cartilage, membrane and chondral bones) mesectodermal cells have the equivalent differentiation capabilities as prechordal mesoderm cells which constitute the rest of the skull.

Several studies have been done on the conditions of induction of the neurocranium and viscerocranium (cf. Hörstadius, 1950; Holtfreter, 1968; Tyler & Hall, 1977; Stewart & McCallion, 1975). The inducing influence of the encephalon in skull morphogenesis has been demonstrated. The rhombencephalon and the notochord induce the parachordal cartilage and the anterior part of the trabeculae from the cephalic mesenchyme (cf. Holtfreter, 1968) as well as the basal plate, the basioccipital and the basisphenoid (Schowing, 1968). This author also demonstrated the inductive role of rhombencephalon, mesencephalon
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and prosencephalon in the formation of various skull bones, squamosal, occipital and supra-occipital, parietal and frontal. In view of the mixed origin of the neurocranium, it appears that the origin of the mesenchyme on which the osteogenic induction is exerted does not matter; mesentoderm and mesectoderm in cephalic region would be equally competent towards the inducer which, in this case, is the encephalon.

This is also true for the skeleton of the otic region. Up to now the matter of the origin of the otic capsule has been controversial. Some authors derived it from the mesoderm (cf. Holtfreter, 1968). Some others, notably Yntema (1950), considered that these chondrocytes were, at least partially, from the ectodermal layer. Our observations confirmed the composite origin of the otic capsule. Two explanations may be valid for the presence of these ectodermal cells: they may come from the neighbouring mesectoderm which will differentiate into squamosals, quadrates and columella, and be contingently combined with the mesodermal capsule. The same explanation has been given for the presence of rhombencephalic neural crest cells in Meckel's cartilage articular region; the Meckel's cartilage itself being mainly derived from the mesencephalic neural crest (Le Lievre, 1974). On the other hand, and in agreement with Yntema's hypothesis, it is possible that marked mesectodermal cells have migrated from the placodal ectoderm, provided that a small piece of this structure is generally grafted with the neural primordium. Indeed the limit of the neural crest is not clear at early stages, when the graft is performed, and a small fringe of dorsolateral ectoderm is generally excised with the nervous tissue. Under joint actions of endomesoderm and rhombencephalon (Yntema, 1950; Holtfreter, 1968) the otocyst ectoderm forms vesicles, the epithelium of which will in turn induce the mesectodermic Columella and the cartilaginous otic capsule (cf. Yntema, 1950; Benoit, 1964) which is of mixed origin. The same chondrogenic effect of the same inducer is observed on mesenchymal cells with different origins.

As far as we know, considering the skeletal derivatives, the mesectoderm does not seem to be characterized by specific properties that it would owe to its origin, either in epithelio-mesenchymal relationship occurring during its development or in its differentiating capabilities. As to the other mesenchymal derivatives of the neural crest, we previously showed (Le Lievre & Le Douarin, 1975) that cephalic mesectodermal cells have the same developmental capabilities as mesodermal cells from the same transverse level except for the vascular endothelia. Indeed, the mesectoderm participates in the formation of facial striated muscles, feather smooth muscles and muscles and connective cells in the wall of arteries derived from aortic arches. The neural crest also provides a noticeable portion of ventrolateral connective tissues in the head and neck, adipose tissue, dermis and the connective components of muscles and various glands in this region (Fig. 10).

In Table 2 is presented a recapitulation of non-neural derivatives of the crest as known at present from the results of experimental studies performed on
Fig. 9. Summary of the results on the localization of skeletal ((bbox)[23]) derivatives of the cephalic neural crest. (a) Lateral view; (b) ventral view; (c) ventral view of mandibular and hyoid skeleton. 27, Articular; 28, angular; 29, supra angular; 30, dentary; 31, splenial; 32, mentomandibular; 33, Meckel's cartilage; 34, entoglossum; 35, basihyal; 36, ceratobranchial; 37, epibranchial; 38, basibranchial.
amphibia and birds. From a comparison of these data some observations can be made. Both bird chondrocranium and amphibian larval head skeleton have the same origin. The origin of cranial bones in adult amphibia has not been demonstrated clearly but it is possible that the greater part of the neurocranium and almost the whole viscerocranium derive from neural crest, as in birds. The first basibranchium in amphibia has a mesectodermal origin as does the basibranchial of birds. This is the only cartilage differentiating in the mesectoderm below the level of the third branchial pouches in birds; in amphibia cartilaginous bars are formed in the third, fourth, fifth and sixth branchial arches. The second basibranchium, on the other hand, is mesodermal in origin, as is, in Rana, the basihyal.

According to some authors (cf. Chibon, 1966) cephalic neural crest in urodeles and anurans gives some connective tissue. However, their technique did not allow them to specify the localization of this tissue. It might be assumed that, as in birds, it is localized in the ventral part of the head in the vicinity of the mesectodermal cartilages and bones. Some new neural crest derivatives have been found in birds; they are mesectodermal (Le Lièvre & Le Douarin, 1975) and glandular derivatives (Le Douarin, Le Lièvre & Fontaine, 1972; Pearse et al. 1973).
Table 2. Summary of non-neural derivatives of the neural crest

<table>
<thead>
<tr>
<th>Birds</th>
<th>Amphibia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cephalic mesectoderm</strong></td>
<td></td>
</tr>
<tr>
<td>Skeleton</td>
<td>Cephalic mesectoderm</td>
</tr>
<tr>
<td>Nasal</td>
<td>Larval skeleton</td>
</tr>
<tr>
<td>Orbital (partly)</td>
<td>Supra and infrarostral</td>
</tr>
<tr>
<td>Palatal and maxillary</td>
<td>Trabeculae (partly)</td>
</tr>
<tr>
<td>Mandibular</td>
<td>Palatoquadrate</td>
</tr>
<tr>
<td>Otic (partly)</td>
<td>Meckel's cartilage,</td>
</tr>
<tr>
<td>Hyoid</td>
<td>quadrate</td>
</tr>
<tr>
<td>Dermis, connective tissues (in</td>
<td>Hyoid and branchial</td>
</tr>
<tr>
<td>face and ventral part of the neck)</td>
<td>Connective tissue</td>
</tr>
<tr>
<td>Arterial wall (except endothelia)</td>
<td>Not studied</td>
</tr>
<tr>
<td>Some smooth and striated muscles (in head and neck)</td>
<td>Not studied</td>
</tr>
<tr>
<td>—</td>
<td></td>
</tr>
<tr>
<td><strong>Trunk mesectoderm</strong></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>Connective tissue (dorsal fin)</td>
</tr>
<tr>
<td><strong>Pigment cells</strong></td>
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</tr>
<tr>
<td>Adrenomedulla</td>
<td>Adrenomedulla</td>
</tr>
<tr>
<td>Calcitonin cells</td>
<td>Not studied</td>
</tr>
<tr>
<td>Type I cells of carotid body</td>
<td>Not studied</td>
</tr>
<tr>
<td><strong>Glandular cells</strong></td>
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</tbody>
</table>

On the whole, neural crest cells should have equivalent potential to develop in these two classes of vertebrates. However, in birds, as compared with amphibia, there is a caudo-cephalic restriction of mesenchymal potential which is reflected by the absence of neur ectodermal meninges and connective tissue at the trunk level and by a decrease in the most posterior skeletal derivatives in the branchial region.

The question arises whether neural and mesectodermal derivatives belong to two different population in the neural crest and if so, whether there is a competition between the two kinds of neur ectodermal cells. This question has to be studied further.

This work, using the quail–chick marking system has demonstrated the importance of the neural crest-derived mesenchymal cells in the genesis of cephalic structures, particularly the facial and cervical ones, in birds. The appearance of mesectodermal cells might correspond to a delayed gastrulation by which some complementary material would be provided for the derivatives of the mesoblast, which invaginated at the primitive streak level during previous stages. This complementary mesenchyme might be necessary at the cephalic
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level where the cordo-mesoblastic mesenchyme might not support the development of all head region derivatives as somitic and lateral plate mesoderm do at posterior levels.

The data in amphibia and birds have allowed us to emphasize and generalize to other vertebrates, particularly mammals, the diversity of cephalic neural crest potentialities and the importance of the participation of neurectodermal derivatives in the genesis of the face and neck.

RESUME

La différenciation des cellules issues des crêtes neurales chez les oiseaux a pu être étudiée grâce à l'utilisation du marquage caille-poulet basé sur des différences spécifiques de la répartition de l'ADN nucléaire. Des embryons chimères ont été obtenus en remplaçant, chez des embryons de poulet aux stades 7 à 11 de Hamburger et Hamilton, un fragment d'œuf neurale céphalique par le fragment correspondant prélevé sur un embryon de caille de même stade. La participation des cellules issues de l'œuf greffé à la constitution du squelette céphalique a été étudiée sur coupes histologiques colorées par la réaction nucléale de Feulgen et Rossenbeck.

Les cellules issues des crêtes neurales prosencéphaliques constituent le bourgeon nasal frontal et se mêlent aux cellules issues des crêtes neurales mésencéphaliques dans les bourgeons nasaux latéraux, autour des ébauches oculaires et le long des faces latéro-ventrales du diencéphale. On sait que les cellules des crêtes neurales constituent en outre l'essentiel du mésonécryphyme des bourgeons maxillaires et de l'arc mandibulaire alors que les cellules des crêtes neurales rhombencéphaliques migrent dans les autres arcs branchiaux.

L'origine des divers cartilages du chondrocrâne ainsi que des pièces osseuses du neurocrâne et du viscérocrâne a pu être mise en évidence chez les embryons porte-greffes. Les cartilages de la plaque basilaire, les os de la région occipitale, du complexe sphénoïde de la voûte crânienne sont principalement d'origine mésodermique. On note toutefois l'origine mixte des capsules otiques où se différencient des cellules issues des crêtes neurales rhombo-mésencéphaliques, du frontal, du rostre du parasphénoïde et des cartilages orbitaires à la formation desquels des cellules issues des crêtes neurales prosencéphalo-mésencéphaliques prennent part à des degrés divers. Le squameux et la columelle de l'oreille ont une origine mésectodermique ainsi que le squelette de la région nasale, les palatins et les os de la mâchoire supérieure. On connaissait déjà l'origine mésectodermique de la mâchoire inférieure et de l'hyoïde.

Ces résultats mettent en évidence l'importance des crêtes neurales dans la génése du squelette céphalique en particulier de sa partie faciale, la voûte et la région dorsale du crâne étant plutôt mésodermiques ; dans la région intermédiaire, on observe la présence d'os et de cartilages d'origine mixte. L'existence de ce type de structure implique que le mésoderme et le mésectoderme soient également compétents vis-à-vis des inducteurs spécifiques de ces pièces squelettiques. Ces constatations sont en faveur de l'équivalence des mésenchymes mésodermiques et mésectodermiques déjà démontrée en ce qui concerne leurs capacités de différenciation.

The author thanks Professor N. Le Douarin, Director of the Institut d'Embryologie du CNRS et du Collège de France, for his advice and encouragement with this work.

This work was supported by the Centre National de la Recherche Scientifique, by the Délégation Générale à la Recherche Scientifique et Technique and by a grant from the National Institute of Health, no. 1 RO 1 DE 04257-01 CBY.
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Neural crest-derived cells in the genesis of the avian skull


(Received 9 November 1977, revised 19 April 1978)