Mesenchymal derivatives of
the neural crest: analysis of chimaeric quail and
chick embryos

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SUMMARY

Interspecific grafts of neural tube and associated neural crest (NC) have been made between quail and chick embryos. Structural differences of the interphase nucleus in the two species make it possible to identify quail from chick cells in the chimaeras after Feulgen-Rossenbeck’s staining and at the electron microscope level. Owing to the stability of the natural quail nuclear marker labelling, migration pattern and developmental fate of the grafted NC cells could be followed in the host embryo. In previous work it has been demonstrated that the visceral skeleton derives entirely from NC mesenchyme and the various levels of the neural axis from which visceral cartilages and bones originate have been established. In the present work, the contribution to the lower jaw and pharynx of NC mesenchymal derivatives other than bones and cartilages has been studied. It is shown that the dermis in the face and ventrolateral side of the neck has a neural origin. The wall of the large arteries deriving from the branchial arches (systemic aorta, pulmonary arteries, brachiocephalic trunks and common carotid arteries) are entirely made up of mesectodermal cells except for the endothelial epithelium which is mesodermal in origin. The presence in the wall of the common carotid arteries of fluorogenic monoamines-containing cells is demonstrated using the formol-induced-fluorescence technique. Like the secretory cells of the carotid body, the fluorescent cells of the carotid artery wall originate from the rhombencephalic NC.

Connective tissue of the lower jaw, tongue and ventrolateral part of the neck originate from the neural crest. Mesectoderm participate in the formation of the glands associated with the tongue and pharynx (lingual gland, thymus, thyroid, parathyroids) giving their mesenchymal component. On the other hand, as demonstrated previously by our group, NC cells are the main cellular component of the UB since they give rise to the calcitonin-producing cells. The wall of the esophagus and trachea is of mesodermal origin, but adipose tissue around the trachea and parasympathetic enteric ganglia of the digestive tube derives from NC. NC cells participate in the formation of striated muscles of the branchial arches and differentiate there into connective and muscle cells.

It appears from this study that the differentiating capabilities are similar in mesenchymal and mesectodermal cells with the exception of blood vessel endothelia which in our experiments are always of host origin in mesectoderm-derived tissues.

The capacity of the NC to give rise to mesenchymal derivatives is restricted to the cephalic neural axis down to the level of the 5th somite in both chick and quail embryos.

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INTRODUCTION

Katschenko (1888) was the first to suggest that some of the mesenchyme of the head originates from the neural crest. He drew this assumption from the observation of selacian development. Later, Goronowitsch (1892, 1893) stated that in teleosts and birds also the neural crest contributes to the formation of mesenchyme. At the same time Platt (1893) found that in Necturus embryos the cartilage of the visceral arches as well as the dentine of the teeth derive from ectoderm, but she considered the main source of the mesenchyme that gives rise to these structures to be the lateral ectoderm of the head. She proposed the term of mesectoderm for the mesenchyme originating from the ectoderm and mesendoderm for the mesodermal mesenchyme. The conclusions of Platt were based on the fact that the yolk platelets of ectodermal cells were conspicuously smaller than those of either mesoderm or endoderm, making possible the histological distinction of migrating cells of the neural crest and placodal origin from other mesenchymal cells.

Experimental studies on the fate of neural crest cells were later undertaken by several investigators, mainly utilizing amphibian embryos. By extirpations and transplantations Stone (1922, 1926, 1929) was able to demonstrate that the skeletal connective tissues of the upper face and visceral arches were largely of neural crest origin.

Similar conclusions have been drawn by several authors using interspecific and intergeneric transplantation techniques (Raven, 1931; Holtfreter, 1933, 1935a, b; Harrison, 1935, 1938; Andres, 1946, 1949; Wagner, 1949, 1955, 1959; and others: see reviews of Hörstadius, 1950 and Weston 1970 for bibliography). Artificial cell marking techniques such as vital dyes (Stone, 1932; Hörstadius & Sellman, 1946) and tritiated thymidine (Chibon, 1962, 1964) were also applied to this problem in amphibians.

Less numerous are the studies dealing with the contribution of the neural crest to mesenchymal structures in higher vertebrates. After extirpation experiments in chick embryo, Hammond & Yntema (1953, 1964) observed severe deficiencies of the nasal septum and visceral skeleton. Johnston (1966) using the experimental technique of isotopic labelling of nuclei previously developed by Weston (1962, 1963) to study the migration of spinal neural crest cells in the chick embryo, carried out an autoradiographic study of the evolution of the crest at the cranial level. He clearly showed that crest cells contribute extensively to the mesenchyme of upper facial regions and visceral arches and consequently that their behaviour is remarkably similar to that of their amphibian counterparts. He was able to demonstrate that labelled crest cells participate in the formation of cartilage, but due to the instability of the labelling which becomes diluted through the rapid proliferation of embryonic cells, the isotopic technique cannot provide comprehensive data on the ectodermal mesenchyme capabilities.
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The present report deals with the application of a biological cell marking technique, which has the advantage of being stable (Le Douarin 1969, 1971b, 1973a), to the problem of neural crest cell migration and differentiation. Thus it is possible to follow the migrating crest cells until they have reached a fully differentiated state. Cell identification is based on structural differences of the interphase nucleus in two closely related species of birds, the Japanese quail (*Coturnix coturnix japonica*) and the chick (*Gallus gallus*). In the quail, the nucleus contains one or several large heterochromatic masses associated with the nucleolar RNA, making the nucleolus considerably enlarged in all embryonic and adult cell types. In the chick the chromatin is evenly distributed in the nucleoplasm forming a fine network with some small dispersed chromocenters and the amount of nucleolar-associated chromatin is small. As a result of this different disposition of the chromatin material quail and chick cells can easily be distinguished after the application of the Feulgen–Rossenbeck’s specific staining procedure for DNA. They can also be recognized at the electron microscope level after routine uranyl acetate–lead citrate staining or by means of the EDTA preferential staining procedure for RNA according to Bernhard (1968), in which RNA is stained while DNA and most of the proteins are unstained (Le Douarin 1971a, 1973a, 1973b).

The 'quail-chick marking system' has already been applied to the problem of migration and differentiation of neural crest cells by our group (Le Douarin & Le Lièvre, 1970; Le Douarin & Teillet, 1970; Teillet & Le Douarin, 1970; Le Douarin, 1971a, b; Le Douarin & Le Lièvre, 1971; Le Douarin & Teillet, 1971b; Le Lièvre, 1971a, b; Teillet, 1971a, b; Le Douarin & Le Lièvre, 1972; Le Douarin, Le Lièvre & Fontaine, 1972; Le Douarin, 1973a; Le Douarin & Teillet, 1973a, b; Le Lièvre & Le Douarin, 1973; Pearse et al. 1973; Le Douarin, Fontaine & Le Lièvre, 1974; Le Lièvre, 1974; Le Lièvre & Le Douarin, 1974; Polak et al. 1974; Teillet & Le Douarin, 1974) and others (Johnston, Bhakdinaronk & Reid, 1973; Saxod, 1973). In previous papers it was not only shown that the entire visceral skeleton originates from mesectodermal cells, but it was also established from what levels of the neural axis the different bones and cartilages are derived (Le Lièvre, 1971b, 1974).

The observations reported here concern the capability of neural crest to give rise, in addition to bone and cartilage, to other mesenchymal derivatives. Due to the stability of the labelling provided by quail cells, it was possible to show that the neural crest mesenchyme contributes to various tissues of face and neck.

**MATERIAL AND METHODS**

The eggs employed throughout this study were from the White Leghorn strain of the fowl *Gallus gallus* and the Japanese quail *Coturnix coturnix japonica*. They were incubated in a humidified atmosphere at 38 ± 1 °C. The developmental stage was determined by the number of somites for the early stages until 2½ days.
of incubation. After this, staging was done by the days of incubation. For the chick it was useful in some cases to use the staging series of Hamburger & Hamilton (1951).

Isotopic and isochronic grafts of sections of quail neural primordium into the chick have been carried out according to the following technique.

In a first step, a piece of neural tube and associated neural crest is surgically removed at a prescribed transverse level of a chick embryo, between the anterior limit of mesencephalon to the level of the 9th somite inclusive. The length and location of the excision varies according to the experiment. The more cranial the intervention the younger the embryo had to be, since the neural crest becomes progressively established soon after the closure of the neural tube and disperses in a cranio-caudal sequence.

In a second step the corresponding transverse part is taken from a quail embryo at the same developmental stage and immersed in a 0.1% solution of trypsin in Ca$^{2+}$, Mg$^{2+}$ free Tyrode solution (10 min at 2 °C) (Moscona & Moscona, 1952). All tissues adhering to the neural tube are manually removed with fine dissecting needles. Thus the isolated quail neural rudiment is completely devoid of contamination by non-neurectodermal cells (Fig. 1). The tissue is then rinsed in Tyrode containing horse serum for 10 min and then in Tyrode without horse serum for an additional 10 min. After the final rinse the neural tube is orthotopically grafted into the chick in the space resulting from the previously excised neural tube.

Four experimental series were made:

1. In 5- to 7-somite embryos the graft consisted of the mesencephalic primordium (Fig. 2, Expt. 1a) or the level of the presumptive mesencephalon + anterior rhombencephalon back to the level of the 1st somite (Fig. 2, Expt. 1b).

2. In 6- to 10-somite embryos the intervention consisted of either the anterior rhombencephalon (from the mesencephalo-rhombencephalic constriction to the 1st somite (Fig. 2, Expt. 2a), the posterior rhombencephalon from the 1st to the 5th somite (Fig. 2, Expt. 2b), or the whole rhombencephalon (Fig. 2, Expt. 2c).

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**Figure 1**

Experimental procedure for isotopic and isochronic transplantations of quail neural primordium into chick embryo. Ch, Notochord; NC, neural crest.

(A) Transverse section of a 10-somite control embryo at the level of anterior rhombencephalon. ×180.

(B) Ten-somite chick embryo after removal of the rhombencephalic primordium. ×250.

(C) Isolated rhombencephalic neural tube and neural folds of a 10-somite quail embryo. ×380.

(D) Transverse section at the rhombencephalic level in a chick embryo which has received, 6 h before fixation, the graft of a quail neural primordium. ×250.

The same procedure is used for the reverse graft of chick neural primordium into quail.
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Fig. 2. Levels of heterospecific transplantations of neural rudiment.

Expt. 1. The operations are carried out at 5- to 7-somite stage of host and donor embryos at the level of mesencephalon (a) or mesencephalon + anterior rhombencephalon down to the 1st somite (b).

Expt. 2. The transplantations are carried out at 6- to 10-somite stage at the level of anterior rhombencephalon (between mesencephalo-rhombencephalic constriction and 1st somite) (a), posterior rhombencephalon (from the 1st to the 5th somites) (b) or concern the whole rhombencephalon (c).

Expt. 3. Isotopic and isochronic transplantations of short fragments of quail neural tube into chick in order to determine the posterior limit of NC capability to give rise to mesenchymal derivatives. Stage of intervention: 7—11 somite; graft of a fragment of neural tube corresponding to the length of 3 somites from the 1st to the 9th somites.

3) In a third series of experiments short fragments of the chick neural primordium were removed and replaced by their quail counterparts at the level of the first somite pairs in order to find out the location of the posterior limit to the capabilities of the neural crest cells to give rise to mesenchymal derivatives. The embryos employed in these latter experiments were at 7- to 11-somite stages and the length of the neural axis involved in the experiment corresponded to the extent of three somites (Fig. 2, Expt. 3).

4) In order to control the validity of the results obtained by grafting quail neural tube into chick, the reverse operation was carried out in an experimental series, i.e. heterospecific transplantation of rhombencephalic primordium.

**Histological procedures**

The operated embryos are sacrificed between 3 and 19 days of incubation. Up to the 11th day the anterior half of the body (head, neck and thorax down to and including the anterior limbs) is fixed in Zenker's fluid and cut in serial 5 μm thick transverse sections.
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Fig. 3. Fixed regions in 11- to 19-day-old chick embryos.
(A) Common carotid artery (c.c.a), vagus nerve (x), carotid body (cb) and pharyngeal glandular derivatives: parathyroids (pt), thymus (tm), thyroids (thy) and ultimobranchial bodies (r.ub., l.ub.). tr, trachea; es, oesophagus.
(B) Large vessels, superior part of the ventricles, bulbus and truncus arteriosus. ao, Systemic artery; lpa, left pulmonary artery; ra, right atrium; rbca, right brachiocephalic artery; rda, right ductus arteriosus; dao, dorsal aorta.

In the older embryos the following pieces are taken and treated in the way described above:
(a) The region containing the following tissues: common carotid artery and carotid body, jugular vein, gastric nerve, nodosum ganglion, thyroid, parathyroids, ultimobranchial body, and the caudal portion of the thymus (Fig. 3 A).
(b) The heart and large arteries according to the scheme of Fig. 3 B.
(c) The head and the anterior half of the neck.

Sections are first treated according to Feulgen–Rossenbeck’s technique (1924) and then lightly stained with picroindigocarmine. This staining enabled us to identify quail and chick cells (Fig. 4).

The formol-induced-fluorescence technique (FIF) which detects histochemically fluorogenic monoamines (Falck, 1962) has been applied to certain pieces of experimental embryos. After having been observed in u.v. light, sections are post-fixed in Zenker’s fluid and stained by the Feulgen–Rossenbeck procedure which makes it possible to determine which species, quail or chick, the fluorescent cells represent. The FIF treatment is applied as follows:

The blocks are freeze-dried in a thermoelectric tissue dryer at −40 °C for 18 h. They are then exposed to formaldehyde vapour at 80 °C for 2 h and directly embedded in vacuo in Epon–Araldite. In each case, part of the freeze-dried
FIGURES 4 AND 6

Fig. 4. Quail and chick cells stained with Feulgen–Rossenbeck's reaction. Mesenchyme of the 1st branchial arch of 4-day-old chick (A) and quail (B) embryos. In chick nuclei the chromatin is evenly dispersed in the nucleoplasm while quail nuclei show one or several heterochromatic condensations. ×1440.

Fig. 6. Transverse section of the right maxillary process of a chick host embryo at 4½ days of incubation which has received the graft of a quail mesencephalon and anterior rhombencephalon at 7-somite stage (Expt. 1b of Fig. 2). The mesenchyme originating from the neural crest is made up of quail cells and lined by the chick host ectoderm (E). (A) General view, ×80. (B) Detail, ×440. Feulgen–Rossenbeck's staining.

Material is embedded without formaldehyde vapour treatment. Those samples form the control series. Serial sections from all blocks are cut at 5 μm, placed in a drop of distilled water on glass slides, attached through rapid water evaporation, and observed directly without a coverslip. All sections are examined by fluorescence microscopy using a Leitz Orthoplan microscope fitted with an HBO 200 W mercury arc lamp. Filters used are BG 12/5 mm, BG 12/3 mm, BG 12/1.5 mm for excitation with a K 510 barrier filter. Photomicrographs were taken on Tri-X, Pan film or Rayoscope film.
Fig. 5. Diagrammatic representation of the extension of mesencephalic and rhombencephalic NC cells in facial and pharyngeal structures of 4-day-old embryos. (A) Lateral view. 1–4: 1st, 2nd, 3rd and 4th branchial arches. 6, 7: Level of the cross-sections illustrated in Figs. 6 and 7. mp, Maxillary process; h, heart. (B) Cross-section through the 2nd branchial arch. ao, Dorsal aorta and 2nd aortic arch; ch, notochord; j, anterior cardinal vein; m, muscle plate; ph, pharynx. NC cells are located ventrally (stippled area) and extend dorsally to the level of anterior cardinal vein. The muscle plate is essentially of host origin.

Electron microscopy

For ultrastructural observation tissues are fixed in 6% glutaraldehyde in 0.1 M phosphate buffer, at pH 7.4 for 20 min at 4°C, and postfixed in 1% osmium tetroxide in phosphate buffer for 1 h. The blocks are embedded in Epon, sectioned, stained by lead citrate and uranyl acetate, and observed in an Hitachi HS 8 electron microscope.

RESULTS

I. Observation of the host embryos at 3rd and 4th days of incubation

Transverse sections (5 μm) of the anterior part of the embryos were stained with the Feulgen–Rossenbeck's technique. The neural crest [NC] cells, which contribute to visceral arch morphogenesis, could be recognized as a result of the quail nuclear marker.

The results obtained after the graft of mesencephalic and rhombencephalic primordia (experimental series represented Fig. 2, Expts. 1 and 2) are summarized in Table 1.

It appears that the mesenchyme of maxillary buds and branchial arches is composed primarily of mesectodermal cells (Figs. 5–7). Chick cells were found...
Table 1. Participation of mesectodermal cells in the genesis of facial and pharyngeal structures in a 4-day-old chick embryo

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<tr>
<th>Level of heterospecific graft</th>
<th>Appearance of heterospecific mesectodermal cells in the host</th>
<th>Maxillary process</th>
<th>1st branchial arch</th>
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forming the endothelium of the aortic arch and the muscle plate, in which, however, some quail cells were dispersed.

Similar observations were done whatever the sense of heterospecific combinations was: quail neural tube grafted into chick or inversely. Figs. 7 and 8 show the distribution of host and donor cells in the 2nd visceral arch in the two kinds of grafts at rhombencephalic level.

II. Further localization of differentiated mesectodermal cells in facial and pharyngeal structures

Due to the stability of the nuclear marker provided by quail cells, the ultimate localization of neurectodermal cells in the various structures of the head and neck could be determined. From the 6th day onwards, the contribution of quail cells to the visceral arch derivatives has been studied in chick embryos which had received various grafts as indicated in Fig. 2.

Mesectodermal cells which colonize the branchial arches give rise to the entire visceral skeleton as previously reported by Le Lièvre (1974). In addition quail NC cells have been found to differentiate into many other kinds of cells.

A. Contribution of NC cells to the dermis

In the face and neck, mesectodermal cells differentiate into dermis. This results in the formation of chimaera feather buds in which the papilla is made up of quail cells while the epidermis belongs to the host chick (Fig. 9 A). Quail melanocytes spread dorso-ventrally and cranio-caudally from the grafted neural tube in the host skin (Teillet & Le Douarin 1970; Teillet 1971a). They are numerous inside the chick epidermis of the chimaeric feathers (Fig. 9 B). Quail mesectodermal cells form the smooth arrector muscles associated with feathers (Fig. 9 C) and the subcutaneous adipose tissue.

B. Connective tissues deriving from the NC

The connective tissue of the lower jaw, the tongue and the ventral part of the neck derives from NC mesenchyme. The dorsal limits of the mesectodermal area reach the level of the auditory pit and the internal carotid arteries. Mixed with chick host cells, mesectoderm participates in the histogenesis of the wall of these vessels. No quail cells are encountered dorsally to the notochord.

The loose connective tissue in the tongue and the floor of the mouth is mostly derived from the NC. Such is the mesenchymal component of salivary glands (Fig. 10) and the connective stroma in the tongue and lower jaw muscles.

The mesenchymal components of the glandular pharyngeal derivatives are of NC origin. Quail cells are found in the interlobular spaces and in the medulla of thymic lobes, forming the parafollicular cells of thyroid gland and the connective tissue located between the cords of parathyroid glandular cells (Fig. 11). They are the main cellular component of the ultimobranchial and carotid bodies as previously described by our group (Le Douarin & Le Lièvre, 1970, 1971, 1972;
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The walls of the oesophagus and trachea are primarily derived from chick host cells with the exception of the enteric parasympathetic ganglia which arise from the grafted NC when the operation involves the hind brain level, as reported previously (Le Douarin & Teillet, 1971b, 1973a). During the second half of the incubation period, mesectodermal cells differentiate into adipose tissue in the vicinity of trachea (Fig. 12).

C. Participation of NC cells in striated muscle differentiation

Mesectodermal cells participate to a certain extent in the histogenesis of the striated muscles of the tongue, the face and the neck though they mainly derive from anterior somite mesoderm (Johnston, personal communication). However, as mentioned above, some quail cells are found in the muscle plates of the branchial arches and are incorporated afterwards in the muscles (Fig. 13).

Striated muscles, which differentiate in the second branchial arch, have been observed at the electron microscope level and were seen to be a mixture of quail and chick striated muscle cells (Fig. 14) with a prevalence of host cells. On the other hand, as mentioned above, the muscle connective tissue derives from NC mesenchyme.

D. Mesectodermal origin of the arteries deriving from aortic arches

Due to the elongation of the neck and the anteroposterior movement of the heart during the first half of embryogenesis, the blood vessels which derive from aortic arches are drawn caudad and incorporated into the thoracic cavity. Except for the endothelium, the wall of the large arteries deriving from the aortic arches originates entirely from mesectoderm.

When the graft of a quail neural primordium into a chick embryo involves

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**Figures 7 and 8**

Fig. 7. Graft of anterior rhombencephalon of a quail into a chick embryo at 7-somite stage (Expt. 2a). Cross-section through the 2nd branchial arch at 4½ days of incubation. Feulgen–Rossenbeck’s staining.

(A) General view. × 90.

(B) Detail showing that the mesenchyme originates from the grafted quail neural tissue (Q). The muscle plate (M) is essentially of host origin but contains some dispersed quail cells. Ectoderm (E) and pharyngeal endoderm (En) are made up of chick cells. × 280.

Fig. 8. Similar experiment as in Fig. 7: chick rhombencephalon is grafted into a 7-somite quail embryo. As in Fig. 7, most of branchial arch mesenchyme is derived from the graft while the muscle plate and the lining ectodermal and endodermal epithelia are of host origin. Feulgen–Rossenbeck’s staining.

(A) General view. × 220.

(B) Detail. × 580.
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the whole rhombencephalic primordium (Fig. 2, Expt. 2c) the wall of the arteries which arise from the 3rd (brachiocephalic trunks and common carotid arteries), the 4th (systemic aorta) and the 6th (pulmonary arteries) aortic arches, are entirely made up of quail cells (Figs. 15, 16). In a transition zone between the bulbus arteriosus and the aortic trunks arising from the heart, the vessel wall is formed by a mixture of quail and host cells. The same observation is made in the distal part of the arch of systemic aorta and in ductus arteriosus corresponding to the distal portion of the 6th arches. After the 6th day of incubation, quail mesenchymal cells become distinctly arranged as smooth muscle around the tube formed by chick endothelial cells lining the aortic arch primordium. Thus the NC cells undergo smooth muscle cell differentiation and elaborate elastin fibrils. The chromatin pattern of quail nuclei is modified during this differentiative process. The initial single heterochromatic mass of the nucleus becomes fragmented in two or three smaller Feulgen-positive patches attached to the nuclear membrane (Fig. 16A). The same disposition is observed in vessel walls of control quail embryos.

The 3rd visceral arch is the site of differentiation of the carotid body in the close vicinity of the common carotid artery wall as described by Fontaine (1973) in both quail and chick embryos. The common carotid artery of experimental embryos (i.e. chick embryos which had received the graft of quail neural primordium) was sectioned and treated by FIF technique during the second half of the incubation time. Numerous single or grouped fluorescent cells were observed randomly distributed throughout its wall (Fig. 16B). They show the same greenish fluorescence as do quail carotid body cells, which has been demonstrated to be due to dopamine (Pearse et al. 1973). If the sections first observed in u.v. light are then post-stained by Feulgen–Rossenbeck’s technique, fluorescent cells are identified as belonging to quail species, as are the connective wall of the artery and the carotid body (Le Douarin et al. 1972).

E. Do NC cells participate in endothelial wall of blood vessels?

Various pharyngeal structures in which the connective tissue derive from the mesectoderm were observed at the electron microscope level in order to find out whether the endothelial cells of the blood capillaries belonged to mesodermal or neurectodermal mesenchyme. In all the cases observed (dermis, thymus,
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thyroid) none of the endothelial cells have been found to exhibit the quail nuclear marker. The pericytes, however, which line the external side of the capillaries, are evidently of quail origin (Fig. 17). Therefore, it must be assumed that the organs deriving from mesectodermal rudiments are invaded by mesodermal capillary buds during their histogenesis.

Table 2 and Fig. 18 summarize the observations described above, i.e. the distribution of NC cells in the various structures of the face and neck according to the level at which the graft of the quail primordium has been made.

III. Posterior limit of mesenchymal potentialities of the NC

Grafts of short fragments of quail neural primordium have been made in chick according to the schema of Fig. 2, Expt. 3. Ninety embryos have been operated and 39 survived and could be observed at 6–12 days of incubation.

When the graft is made behind the level of the 5th somite, no mesenchymal cells are found in the host embryo. Some dispersed quail cells are encountered in the 6th aortic arches following a graft involving the levels of 3rd and 4th somites. In the latter case no quail cells are found in connective tissue of the host, neither in the dermis. Cells originating from the level of somite 1–3 give rise to the various kinds of connective cells described above. Thus one can consider that the posterior limit of the mesenchymal capabilities of the crest corresponds roughly to the level of the 5th somite.

In the kind of experiments involving the graft of short fragments of quail neural tube into chick, quail cells are always found mixed to host cells. That shows that the same transverse level of the pharynx receives NC cells from a large portion of the neural axis. Thus it appears necessary to perform extensive

Figures 10–13

Fig. 10. Experiment 1b. Graft of a quail mesencephalon + anterior rhombencephalon into a 7-somite chick embryo. Section through the body of the tongue of the 14-day-old chick embryo. All mesenchymal derivatives (Q) originate from the grafted neural quail primordium: loose connective tissue (l.c.); pulp of horny papilla (h.p.); lingual gland mesenchyme (l.g.); keratinized epithelium (E) and lingual glandular cells are of host origin. × 300. Feulgen–Rossenbeck’s staining.

Fig. 11. Experiment 2c. Graft of a quail rhombencephalon into a 10-somite chick embryo. Parathyroid of the 8-day-old chick host embryo. Numerous quail mesenchymal cells (Q) are present between the chick glandular cords (pt). × 510. Feulgen–Rossenbeck’s staining.

Fig. 12. Experiment 2c. Graft of a quail rhombencephalon into a 7-somite chick embryo. In the neck of the 12-day-old chick host, quail mesectodermal cells have differentiated into adipose cells (A). × 920. Feulgen–Rossenbeck’s staining.

Fig. 13. Experiment 2c. Graft of a quail rhombencephalon into a 10-somite chick embryo. Branchiомandibularis muscle (3rd branchial arch) of the 10-day-old host chick embryo. The muscle is made up of a mixture of host and quail cells. × 920. Feulgen–Rossenbeck’s staining.
heterospecific transplantation of the neural tube to get a clear view of the normal distribution and developmental capabilities of crest cells.

**DISCUSSION**

Interspecific combinations between quail and chick embryos have made it possible to investigate the normal process of NC cell migration and to follow the NC derivatives until their completely differentiated state. Unknown derivatives of this transitory embryonic structure have thus been identified and the extent of its contribution to cephalic and cervical morphogenesis has been recognized.

1. **Reliability of the experimental technique**

Experimental techniques devised to investigate embryogenic mapping must disturb as little as possible the normal developmental conditions of the rudiments. Transplantations of early primordia on the chorioallantois or into the coelomic cavity have often been used (Willier & Rawles, 1931, Rudnick 1932, 1938; Andrew, 1964, 1969, 1970) but they disturb the micro-environment of the embryonic tissues and therefore cannot provide really reliable information on their normal developmental fate during embryogenesis. That is especially true when the cells concerned undergo extensive migrations in the embryo before differentiating. As demonstrated for NC cells (Weston, 1963; Weston & Butler,
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Fig. 15. Aortic-arch-derived arteries. The vessel wall is entirely made up of mesectodermal cells originating from mesencephalic and rhombencephalic NC, except for the endothelium which is of mesodermal origin. Mesectodermal and mesodermal cells are mixed. The vessel wall is made up of mesodermal cells in entirety. 3-6, Arteries deriving from the 3rd, 4th and 6th aortic arches; CCA, common carotid artery; DA, dorsal aorta; PA, pulmonary artery; SCA, subclavian artery; TA, aortic trunk; TP, pulmonary trunk.

1966; Le Douarin & Teillet, 1974), migration pathways are indeed highly dependent on the structures and substrates encountered.

Isotopic and isochronic grafts of neural primordium between two species of birds closely related in taxonomy provide almost normal developmental conditions for both host and grafted structures and result in normally developed embryos, when the operation has been properly done. Compared to extirpation or electrocauterization of the neural tube (see Hörstadius, 1950, and Weston, 1970, for review), this grafting technique has the advantage of precluding regulatory mechanisms due to crest cells from anterior and posterior levels.

The developmental rate being a little faster in the quail than in the chick embryo, it was important to perform neural tube exchanges between the two species in both directions, i.e. quail into chick and chick into quail and to compare the distribution of NC cells in the different combinations. When the quail neural tube is grafted into the chick embryo, the rapidly growing quail cells might indeed have an advantage on the chick cells and expand more in the host structures than in normal conditions. However, comparison of the embryos show a similar pattern of NC cell migration in both kinds of grafts.

In fact, the difference of growth rate is rather slight in the early developmental
stages when the most significant morphogenetic events occur and becomes significant only during the second half of the incubation period (Hamburger & Hamilton 1951; Zacchei 1961). Since chick embryos are more resistant than quail to surgical interventions, most of the results reported in this study have been obtained on chick hosts grafted with quail NC.

It can be assumed that, in experimental embryos, the structures composed of cells with the nuclear characteristics of the grafted primordium are of neural origin. Numerous histological controls of the neural tube before the graft and after trypsination have shown that the neural rudiment is easily isolated from neighbouring tissues at the early developmental stages and is, at grafting, completely devoid of mesenchymal cell contamination.

2. Differentiating capabilities of mesectodermal cells, and role of inductive influences

Studies on chimaeric quail-chick embryos lead to the conclusion that the neural primordium contributes extensively to structures of the facial and pharyngeal regions. Though the interspecific grafts included the whole neural tube rather than the neural folds alone, it is reasonable to assume that most if not all mesectodermal derivatives observed in the experimental embryos originate from the NC. Some results obtained previously by Johnston (1966), using tritiated thymidine-labelled transplants of the NC alone, are in complete agreement with our findings.

Thus it appears that most of the lateroventral part of head and neck is edified by material originating from the mediodorsal embryonic region, which undergoes extensive migration before reaching its definitive site in the face and branchial arches.

The results obtained here demonstrate that ectodermal mesenchyme has a large range of developmental capabilities and appears able to give rise to the

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**Figures 16 and 17**

Fig. 16. Experiment 2c. Graft of a quail rhombencephalic primordium on a 10-somite chick embryo.
(A) Arterial trunk. The endothelium (E) belongs to the host while the musculo-connective tissue of the vessel wall is derived from the quail grafted tissue. × 780. Feulgen–Rossenbeck's staining.
(B) FIF technique applied to the area represented in Fig. 3 A of a 11-day-old embryo. Fluorogenic monoamine-containing cells in the wall of the common carotid artery, as carotid body cells, are derived from rhombencephalic NC. × 140.

Fig. 17. Capillaries in the dermis, connective cells are of NC origin, endothelial (E) cells belong to the host.
(A) Experiment 1b. Graft of quail mesencephalon + anterior rhombencephalon into a 6-somite chick embryo. Chick host was fixed after 12 days of incubation. × 1280. Feulgen–Rossenbeck’s staining.
(B) Experiment 2c. Graft of a quail rhombencephalic primordium into a 7-somite chick embryo. Chick host was fixed after 16 days of incubation. P, Pericyte. × 11600. Uranyl acetate–lead citrate staining.
Table 2. Differentiating capabilities of mesectodermal cells deriving from various levels of mesencephalic and rhombencephalic neural crest
same differentiated cell types as mesodermal mesenchyme: general and differentiated (elastic and fibrous) connective tissues, adipose cells, dermis, smooth and striated muscles, bone and cartilage. In addition mesectodermal cells are able to differentiate into odontoblasts (Hörstadius & Sellman, 1946; Sellman, 1946; De Beer, 1947; Hörstadius, 1950; Wagner, 1955; Signoret, 1960; Koch, 1965; Chibon, 1966). However, the only developmental competency which neurectodermal mesenchyme does not seem to possess is the formation of endothelium of the blood vessels. In the branchial and facial regions of experimental embryos, the vascular endothelia are always host in type, and are thus of mesodermal origin, NC cells participating only in the formation of the vessel wall: i.e. pericytes of capillaries and musculo-connective sheath of large vessels.

During their migration and in their final localization close relationships are established between NC cells and various mesodermal, endodermal and ectodermal rudiments. The extent to which intertissular inductive reactions between neurectodermal cells and those tissues control their subsequent differentiation is not clear. Until now, the normal fate of the NC in the cephalocervical region being only partially known, this question could not be fully investigated.

Owing to the stability of the quail nuclear marker labelling, the exclusively neurectodermal origin of the visceral arch cartilages and membrane bones of the lower jaw is demonstrated. A number of investigators (Hörstadius, 1950; Hörstadius & Sellman, 1946; Newth, 1954; Holtfreter, 1968) have shown that chondrogenesis is induced in NC mesenchyme by pharyngeal endoderm. The heterotopic transplantations of cephalic neural tube into the trunk, recently carried out in our laboratory (Le Douarin & Teillet, 1974) show that, in avian embryos, inductive influences from other sources can play the same role: cephalic NC cells grafted into the trunk and thus submitted to the rachis morphogenetic field contribute to vertebral chondrogenesis.

Intimate relationships are established between mesectodermal and mesodermal cells in striated muscle differentiation in branchial arches. The mesodermal cores of each visceral arch derive from somitic mesenchyme (Johnston & Listgarten, 1973). From the earliest stages following completion of their migration, crest cells invade this mesodermal core and become intermingled with the somite deriving cells essentially concerned with the formation of skeletal muscle. The ultrastructural study shows that crest cells give rise not only to the connective tissue elements of muscles but also to striated muscle cells. Mauro (1961), Moss & Leblond (1971) and others have suggested that the growth process of muscle fibres involves the addition to the primary myotube of satellite cells. Whether NC cells play the role of satellite cells in muscles is a hypothesis which would be worth studying.

NC cell differentiating capabilities include secretory elements such as adrenomedullary (Le Douarin et Teillet, 1971 a), calcitonin producing cells (Le Douarin & Le Lièvre, 1970) and carotid body glandular cells.
Fig. 18. For legend see opposite.
Mesenchymal derivatives of avian neural crest

In previous work indeed we have demonstrated the neural crest origin of the carotid body (CB) the cells of which are characterized by a high content of fluorogenic monoamine (Le Douarin et al. 1972; Pearse et al. 1973). This glandular structure differentiates in the 3rd branchial arch, in close association with the common carotid artery. Among the connective cells which form the vessel wall, some fluorogenic cells of neural origin are present. The nature of the monoamine they contain seems to be the same as that of CB cells: in the chick it is serotonin, with a yellow-greenish fluorescence; in the quail, dopamine, with a greenish fluorescence (Pearse et al. 1973). In chick embryos grafted with a quail rhombencephalon the monoamine containing cells of CB and common carotid artery which exhibit the quail nuclear marker after Feulgen–Rossenbeck’s staining have a greenish fluorescence, like the homologous structures of control quail embryos.

3. Extension of NC cell migration in head and neck

In higher vertebrates the competency of neurectoderm to give rise to mesenchyme is restricted to the cephalic region of the neural axis, while in lower forms mesectoderm also derives from trunk NC (cf. Hörstadius, 1950). From our observations and those of Johnston et al. (1973) based on the use of the quail chick marker system, it becomes evident that the mesectoderm is quantitatively a more extensive component of cephalocervical morphogenesis than has pre-

![Figure 18](image-url)

**Figure 18**

Distribution of NC mesenchymal derivatives in the lower jaw and neck of 9-day-old chick embryo following the transplantation of quail mesencephalon and rhombencephalon into the chick host.

(I) Distribution of quail cells in the dermis and inner organs of the chick shown in lateral and ventral views. **,** Mesencephalic NC derivatives. ••••, Rhombencephalic NC derivatives. A, B, C, Levels of the sections which are represented in figures IIA, B and C. cub, ultimobranchial body; hy, hyoid; thym, thymus; thy, thyroid.

(II) Transverse sections at three different levels of the host showing the distribution of NC cells.

(A) Lower jaw and tongue. Cells of mesencephalic origin **,** form (me) Meckel’s cartilage and membrane bones (mb) (angular, supra-angular dentary and opercular); cells of rhombencephalic origin •••• form the hyoid apparatus (bb, basibranchial; bh, basihyal; cb, ceratobranchials; eb, epibranchials; and entoglossum) and both differentiate into loose connective tissue, dermis and muscle cells; ch, notochord; es, oesophagus; ic, internal carotid. (B) Neck. (C) Level of carotid bodies.

[:] Tissues entirely made up of quail cells: d, dermis; c, wall of carotid arteries; ge, enteric ganglia; cb, carotid body. ••••, Tissues made up of a mixture of NC and mesodermal cells: cj, loose connective tissues; x, Schwann cells of the vagus nerve; gn, glial cells of the nodosum ganglion; connective tissue of thyroid; p, parathyroid; tm, thymus.

j, Jugular vein; m, tracheohyoideus and tracheolateralis muscles; tr, trachea.
viously been estimated. Due to the elongation of the neck, cells originating from encephalic NC are carried down into the thorax with the heart and the glandular pharyngeal derivatives. During these morphogenetic movements, mesectodermal rudiments receive mesoderm-derived cells, making the neck a very composite structure. Most of its ventral part originates from initially anterior mediodorsal ectodermal material which migrates ventrad during the early stages of embryogenesis and caudad during later morphogenesis.

Although these data result from studies on the avian embryo, it is possible to infer the general picture of NC migration pattern in mammalian embryos. Thus, a number of spontaneous malformations of the face and brain in the human can be explained by defective crest cell migration and differentiation.

RÉSUMÉ

Des greffes interspécifiques d’ébauches neurales rhombencéphaliques et mésencéphaliques ont été réalisées entre embryons de Caille et de Poulet. Les caractères particuliers du noyau chez la Caille permettent de reconnaître les cellules de l’hôte de celles du greffon dans les embryons chimères, et d’étudier les voies de migration et les capacités de différenciation des cellules issues de la crête neurale (CN) greffée. Le ‘marquage’ fourni par les cellules de Caille est en effet stable et permet de suivre les cellules migrantes jusqu’à ce qu’elles aient atteint un stade de complète différenciation. Dans un travail précédent basé sur une expérimentation similaire on avait montré que le squelette viscéral dérive dans sa totalité des crêtes neurales rhombencéphaliques et mésencéphaliques. Les recherches rapportées ici concernent les dérivés mésenchymateux de la CN autres que les tissus osseux et cartilagineux.

Après la greffe isotopique et isochronique du rhombencéphale et du mésencéphale, le derme de la face et de la partie ventrolatérale du cou de l’hôte est formé de cellules issues du greffon et par conséquent d’origine mésectodermique. Il en est de même de la paroi musculo-conjonctive des gros troncs artériels issus du cœur et dérivant des arcs aortiques (troncs brachiocéphaliques, carotides communes, crosse aortique, artères pulmonaires). Le conjonctif lâche du plancher buccal, de la langue, de la face latéroventrale du cou, ainsi que la trame conjonctive des glandes dérivées du pharynx et de la bouche (glandes linguales, thyroïde, parathyroïdes, thymus) sont formés par du mésectoderme neural.

Les cellules mésenchymateuses originaires des CN ont la potentialité de se différencier en cellules musculaires lisses et participent aussi à l’histogénèse des muscles striés des arcs branchiaux. Elles fournissent du tissu adipeux dans le derme profond et au voisinage de la trachée. Comme cela a été précédemment démontré par notre groupe, les CN rhombencéphaliques sont à l’origine des cellules à calcitonine du corps ultimobranchial et des cellules glandulaires du corps carotidien. Ces dernières sont caractérisées par une forte teneur en monoamines fluorogènes. Des cellules similaires ont été détectées dans la paroi de l’artère carotide commune par la méthode de fluorescence induite par les vapeurs de formol et nous avons pu montrer qu’elles dérivent également de la CN.

Il apparaît donc que les potentialités de différenciation des cellules du mésectoderme sont variées. Cependant il ne semble pas qu’elles soient à l’origine de l’endothélium des vaisseaux sanguins qui, dans nos expériences, dérivent toujours de cellules mésodermiques appartenant à l’embryon hôte. La capacité de la crête neurale à fournir des dérivés mésenchymateux est limitée à la région du névraxe antérieure au niveau du 5ème somite.
REFERENCES


Mesenchymal derivatives of avian neural crest


*(Received 13 December 1974)*