A radioautographic analysis of the migration and fate of cells derived from the occipital somites in the chick embryo with specific reference to the development of the hypoglossal musculature

By R. D. HAZELTON

From the Research Division, Faculty of Dentistry, University of Toronto

SUMMARY

The migration pattern and fate of cells of the occipital somites and overlying ectoderm have been described for the chick embryo with particular reference to the development of the hypoglossal musculature.

Tritium-labelled thymidine (0.5–10 μCi per egg) was used as a cell-specific marker. Occipital somites (2–5) with overlying ectoderm were transplanted orthotopically from labelled donor embryos to unlabelled host embryos (Hamburger & Hamilton, stage 9–10). The embryos were incubated for varying lengths of time (24 h–5 days), sacrificed, sectioned and the migration pattern and fate of the labelled cells determined radioautographically.

It appears that the hypoglossal as well as other hypopharyngeal musculature originates from the occipital somites.

The mesodermal migration pattern extended from the occipital somite region in a ventro-posterior direction to the dorsal surface of the pericardial cavity posterior to the expanded portion of the pharynx. At this position a so-called hypoglossal cord formed on each side which ran anteriorly to the level of the second pharyngeal pouch where it turned medially and together with the cord from the other side entered the pharyngeal area of the embryo. This material apparently forms the intrinsic musculature of the tongue. The mesodermal movements are attributed to differential growth movements of the areas concerned as well as to active cell multiplication and migration.

Selective embryonic neuronal staining was undertaken to study the relationship between the migrating hypoglossal cord and nerve. The cord preceded the nerve in its migration.

There is an occipital somitic contribution to the primitive meninx, to the endothelial walls of developing blood vessels, possibly to microglial cells and to the cartilage surrounding the notocord.

The occipital ectoderm expands dorso-anteriorly and ventro-laterally. In the ventro-lateral position as contact is made with the pharyngeal endoderm a placode is formed which contributes cells to the nodose ganglion of the tenth cranial nerve. There is no other contribution of the ectoderm to the underlying tissues.

1 Author's address: Apartment 4, 101 Hazelton Avenue, Toronto 185, Ontario, Canada.
INTRODUCTION

Morphological approaches used in distinguishing similar embryonic cell groups have led to difficulties in determining the origin of the hypoglossal musculature from either somitic or local mesoderm. Experiments using carbon particle marking techniques by Deuchar (1958) indicated a somitic origin, but the introduction of cell-specific markers such as tritiated thymidine, has made a more critical and extensive experimental analysis possible, using the techniques developed by Weston (1962, 1963), Weston & Butler (1966), Chibon (1962, 1964, 1967), Johnston (1966), Hazelton & Johnston (1968) and Langman & Nelson (1968). In the present series of experiments the occipital somites and overlying ectoderm were transplanted from embryos labelled with tritiated thymidine to unlabelled host embryos in order to determine the migration pattern and fate of the labelled cells.

MATERIALS AND METHODS

Handling of eggs

The eggs employed in this study were from the white leghorn strain of fowl, Gallus domesticus. They were incubated first on their side for approximately 30 h by which time most of the embryos had reached stages of development immediately preceding or just following the onset of somite formation (Hamburger & Hamilton, stage 6–8). They were then prepared for labelling with tritiated thymidine. After transferring the air space to a position overlying the embryo, a piece of shell with underlying shell membrane approximately 1 cm square was removed to provide a 'window' for access to the embryo.

Tritiated thymidine, in saline solution, was deposited on to the vitelline membrane over the embryo. The window was then covered with plastic tape and the egg returned to the incubator. At least 3–4 h elapsed before transplantations of the occipital somites and overlying ectoderm to unlabelled host embryos were carried out. The dose of thymidine used varied between 0.1 and 10 µCi.

Operative technique

The second to fourth or fifth occipital somites plus the overlying ectoderm were removed as a single entity from stage 8–9 unlabelled host embryos and replaced with comparable segments from the donor embryos labelled with tritiated thymidine (Figs. 1, 2). The ectoderm was included in the grafts to hold the somites together and facilitate their orientation. The access hole in the vitelline membrane was kept as small as possible. The grafts were cut out with the use of glass needles and transferred by pipette from the donor to the host embryos.
Fig. 1. Stage 9 chick embryo (Hamburger & Hamilton, 1951) to show the transplanted area of occipital somites and overlying ectoderm (cross-hatch).

Fig. 2. Section of Fig. 1 to illustrate the various parts of somite as well as transplanted segment of somite and overlying ectoderm (cross-hatch).

**Fixation of embryos and preparation of radioautographs**

Postoperative incubation periods lasted from 15 min until 5 days, with the oldest embryos reaching approximately stage 30 at the time of fixation. A total of 200 experiments were carried out, of which 30 were successful. The embryos
were fixed in either Bouin's fixative (young embryos) or in 10% buffered formalin (old embryos). Sections were cut at 8 μ and radioautographs were prepared using essentially the procedure of W. G. Aldridge (see Gerber, Aldridge, Koszalka & Gerber, 1962).

Since the original series of transplants involving both the occipital somites and overlying ectoderm were completed, further experiments involving the transplant of placodal ectoderm alone have been undertaken. By transplanting only the ectoderm any contamination from the ectoderm to underlying tissues was determined.

**Fig. 3.** A stage 38 chick embryo (Hamburger & Hamilton, 1951) to illustrate the migration pattern of the occipital somites (cross-hatch).

**RESULTS**

One of the main problems of this type of experimental approach was the difficulty in keeping the embryos in a viable state following the transplanting procedures. In order to reduce the fatality rate it is recommended that good quality eggs be obtained and a high relative humidity (70–80%) maintained throughout all operative and incubatory procedures. There were seasonal variations in the success of operations, with the spring and fall being the most productive and the summer and winter least productive.
Fate of occipital somites

Figure 4

(A) Photomicrograph of a section through the pharyngeal region of a stage 25 chick embryo. Hypoglossal cord is illustrated in box. ×100.

(B) Photomicrograph using reflected light of the hypoglossal cord designated in box of Fig. A. Because of the use of reflected light the labelled cells are white and are indicated by arrows. ×400.

(C) Photomicrograph through the oral cavity of a stage 30 chick embryo. The hypoglossal cords of each side have now fused and form a V (arrow). ×100.

(D) Higher-power photomicrograph of combined hypoglossal cords seen in C. Labelled cells are indicated by arrows. ×400.
Migration pattern of the occipital somites

The overall migration pattern of the occipital somites and overlying ectoderm is illustrated in Fig. 3. An active proliferation and ventro-lateral migration of cells brings the myotomic portion of the somite to the dorso-lateral surface of the pericardial cavity posterior to the expanded portion of the pharynx. At this position a so-called hypoglossal cord forms (Fig. 3, Fig. 4A, B) which runs forward to the level of the second pharyngeal pouch where it turns medially into the floor of the pharynx and after further forward movement unites with the cord of the opposite side to form a V (Fig. 4C, D) and it is in this fused state

![Figure 5](image)

(A) Photomicrograph passing through the posterior pharyngeal (P) region of a stage 30 chick embryo. Labelled cells can be observed lateral to the laryngeal-tracheal groove (L) and are illustrated by a box. × 100.

(B) Photomicrograph of labelled somite cells (arrows) seen in box of A. These cells will develop into myoblasts of the larynx. × 400.

(C) High-power photomicrograph of a section through the neural tube (N) and primitive meninx (P) of a stage 25 chick embryo. Labelled cells may be seen in the primitive meninx (arrows). × 400.

(D) Photomicrograph of labelled cells (arrows) in the endothelial lining of the anterior cardinal vein (AV). × 400.
Fate of occipital somites

that the final differentiation into the intrinsic musculature of the tongue occurs. The migration of the occipital material is accompanied by the hypoglossal nerve.

As the myotomic material sweeps ventrally to form the hypoglossal cords, some myotomic cells migrate to a ventro-lateral pharyngeal position in the region of the laryngo-tracheal tube to form laryngeal musculature (Fig. 5A, B).

![Figure 6](image)

(A) Photomicrograph of a section through the developing notochord (N) of a stage 32 chick embryo. Labelled cell within the cartilage matrix (M) is indicated by an arrow. × 400.

(B) Photomicrograph taken through the posterior part of the pharynx (P) of a stage 22 chick embryo to indicate relationship of the hypoglossal nerve (N) to the hypoglossal cord (H). The embryo had been stained with silver. × 100.

(C) Photomicrograph of a section through the developing nodose ganglion (N) of a stage 21 chick embryo. Placodal cells (P) are streaming in toward the developing ganglion. × 400.

The contribution of the occipital somites to the primitive meninx (Fig. 5C) adds to the confusion regarding a mesodermal or neural crest origin of this tissue (Harvey & Burr, 1926; Harvey, Burr & van Campenhout, 1933; Raven, 1936; Flexner, 1929; Sensenig, 1951; Triplett, 1958; Johnston, 1966).

Labelled cells were observed entering the substance of the brain just posterior
to the otic vesicle. These could be microglial cells (Penfield, 1932; Kershmann, 1939) or simply endothelial cells of developing blood vessels. Labelled cells were found in the endothelium of the internal carotid artery, aortic arches, the basilar artery and the anterior cardinal vein (Fig. 5D).

The sclerotomic portion of the somites contributed to the cartilage matrix surrounding the notocord (Fig. 6A).

The occipital ectoderm undergoes an antero-dorsal and postero-ventral expansion and in the postero-ventral region, as contact is made with the pharyngeal endoderm, placodes form which eventually contribute to the nodose ganglion of the tenth cranial nerve (Fig. 6C). This is the only ectodermal contribution to the underlying tissues.

Silver staining procedures were used as an additional technique to study the migratory relationship between the hypoglossal nerve and cord by selective neuronal staining and it was established that the cord precedes the nerve (Fig. 6B).

**DISCUSSION**

The somites are among the earliest morphological units to be formed (Hinsch & Hamilton, 1956) and appear in a regular sequential manner from the otocyst to the tip of the tail. A central space divides the somite into an inner and outer region (Fig. 2). Different fates have been attributed to these two regions with the outer region forming the connective tissue layer of the skin (dermatome) and the inner region forming both skelatogenous (sclerotome) and voluntary muscle tissue (myotome). However, the absolute origin of the somite parts is in dispute with the myotomic portion being the main contributor to the confusion. Although muscle protein has been demonstrated in the myotome by both immunofluorescent studies (Holtzer, Marshall & Finck, 1957; Ozawa, 1962; Ikeda, Abbott & Langman, 1968) and by electron-microscopic identification of myofilaments (Przybylski & Blumberg, 1966; Allen & Pepe, 1965; Dessouky & Hibbs, 1965; Obinata, Yamamoto & Maruyama, 1966), the definitive origin of the myotome has not been established: Williams (1910) and Hamilton, Boyd & Mossman (1962) state that the myotome originates by a multiplication of cells at the dorso-medial angle of the somite while Remak (1855), His (1888), Bardeen (1900) and Langman & Nelson (1968) state that the myotome develops by proliferation and differentiation of the overlying dermatome.

By using the electron microscope the myotomic portion of early chick embryos can be identified from the other somite parts by the larger size and lighter colour of its cells (Hay, 1968).

The first post-otic somite is rudimentary (Patterson, 1907; Hinsch & Hamilton, 1956; Arey, 1938) with the second to fifth making up the occipital series (Kingsbury, 1915; Hunter, 1935; Goodrich, 1936).

The majority of evidence for either a somitic or local mesodermal origin of the hypoglossal musculature has been of a descriptive nature. Platt (1891), Neal (1897), Kallius (1905), Hunter (1935) and Bates (1948) stated that the hypoglossal
musculature developed by a ventral and anterior migration of the occipital somites whereas Lewis (1910) concluded that the hypoglossal musculature developed locally from the mesoderm of the floor of the mouth.

The few experimental studies undertaken have not been entirely conclusive. By extirpating the occipital somites in Amblystoma Detwiler (1929, 1937) observed an absence of the hypoglossal musculature. In a series of carbon marking experiments in the chick Deuchar (1958) concluded the hypoglossal musculature originated from the occipital somites. Although Deuchar’s results tended to confirm earlier descriptive studies, some objections may be made to the technique: the carbon particles could have been carried to the hypoglossal musculature by the movements of associated parts or the carbon could have been engulfed by phagocytic cells which in turn migrated to the hypoglossal area (Straus & Rawles, 1953; Seno, 1961). However, the present experiments confirm and extend Deuchar’s findings.

In order to determine the migration and fate of similar embryonic cell groups one must first establish their identity. This has been done in the present series of experiments by specifically marking groups of cells (e.g. occipital somites) with tritiated thymidine. These tissue segments were then transplanted to unlabelled host embryos and the migration and fate of the labelled cells determined radioautographically.

The organization of the occipital somites into so-called hypoglossal cords and the migration of these cords into the hypopharyngeal region has been described by several investigators (Hunter, 1935; Bates, 1948; Detwiler, 1955; Kallius, 1905). Although differential growth movements of involved and associated areas are important in the movement of this material, other factors such as cell proliferation and migration must be considered. If no further cell proliferation occurs once the myotome has formed (Langman & Nelson, 1968) other processes must account for the final adult population of cells. This could be attributed to the hypoglossal cord containing undifferentiated cells which form myotubes only when a final destination is established or the hypoglossal material could induce the differentiation of myotubes from local mesoderm.

The role of the hypoglossal nerve in the development of the hypoglossal cord must also be considered. By selectively staining embryonic tissue for neural elements the relationship between the nerve and cord was studied, and it was shown that the muscle cord precedes the nerve along its migratory path. The same relationship was observed between the sensory nerves of the visceral arches and their muscle cords.

The expansive movements of the ectoderm in the chick were similar to those found in Amblystoma by Wilens (1959) and are due to the morphogenetic movements of embryonic parts. The most significant ectodermal finding was its contribution to the nodose ganglion of the vagus nerve. This finding led to an investigation of the entire sensory cranial ganglion system, the results of which will be published in the future.
RÉSUMÉ

Analyse radioautographique de la migration et du devenir des cellules dérivées des somites occipitaux chez l'embryon de Poulet en se référant au développement de la musculature hypoglosse

Le schéma de la migration et le devenir des cellules des somites occipitaux et de l'ectoderme susjacent sont décrits chez l'embryon de Poulet en se référant au développement de la musculature hypoglosse.

On utilise la thimidine tritiée (0,5-10 µCi par œuf) comme marqueur spécifique des cellules. Les somites occipitaux (2-5) et l'ectoderme susjacent sont transplantés, de façon orthotopique, des embryons donneurs marqués aux embryons hôtes non marqués (Hamburger & Hamilton, stade 9-10). Les embryons sont incubés pendant des périodes de temps variables (24 h à 5 jours), sacrifiés et sectionnés. On détermine de façon radioautographique la migration et le devenir des cellules marquées.

Il apparaît que la musculature hypoglosse, comme toute musculature hypopharyngienne, a pour origine les somites occipitaux.

La migration mésoédermique s'étend depuis la région somitique occipitale en direction postéro-ventrale jusqu'à la surface dorsale de la cavité péricardique postérieure à la portion renflée du pharynx. À cet endroit un cordon, appelé cordon hypoglosse, se forme de chaque côté; ce cordon s'étend antérieurement au niveau de la seconde poche pharyngienne; là, avec le cordon de l'autre côté, il pénètre dans l'aire pharyngienne de l'embryon. Ce matériel forme apparemment la musculature intrinsèque de la langue. Les mouvements mésoédermiques sont aussi bien attribués aux mouvements de croissance différentielle des aires concernées qu'à l'active multiplication cellulaire et à la migration.

On utilise la coloration neuronale embryonnaire sélective pour étudier le rapport entre la migration du cordon et du nerf hypoglosse. Le cordon précède le nerf dans sa migration.

Il y a une contribution somitique occipitale à la méninge primaire, aux parois endothéliales des vaisseaux sanguins en développement, et, peut-être, aux cellules microgliales et au cartilage entourant la notocorde.

The author wishes to thank Dr M. C. Johnston for his invaluable guidance both in the experimental procedures and in the preparation of the manuscript.

REFERENCES


(Manuscript received 11 December 1969)