Experimental Demonstration of Tongue Muscle Origin in Chick Embryos

by E. M. Deuchar

From the Department of Anatomy and Embryology, University College London

WITH ONE PLATE

Since in all classes of vertebrates the tongue muscles are innervated by nerve XII, a segmental nerve of the occipital region, it is usually argued on this criterion alone that they originate from occipital myotome tissue. Descriptive evidence in support of this generalization is, however, far from adequate. The most complete accounts that exist refer to one amphibian and two reptile species. In the amphibian Necturus, Platt (1897) observed that ventral outgrowths of the 3rd and 4th occipital myotomes became tongue muscles, and Edgeworth (1935) has described the development of tongue muscles in the reptiles Sphenodon and Lacerta, from ventral parts of two occipital and two cervical myotomes, all innervated by nerve XII. In avian and mammalian embryos, however, early muscle rudiments are extremely difficult to recognize with any certainty histologically. Although Butcher (1929) and Bates (1948) have observed ventral outgrowths of occipital myotomes in rat and cat embryos, and Bates claimed that these are recognizable as tongue muscle primordia in later stages, both these authors drew their conclusions from relatively few fixed specimens, and in Bates's photographs the rudiments are not nearly as distinct as his diagrams suggest they are. The same criticism applies to Hunter's description (1935) of occipital myotome rudiments that apparently develop into tongue muscles in the chick. The only other detailed description of tongue muscle development in birds is that of Kallius (1905) who dealt with advanced stages when the muscle primordia are already in situ.

In the work to be described here, an experimental approach to the problem has been adopted, and occipital somites of early chick embryos have been marked with carbon particles in order to trace their later fate. Forty-five-hour embryos (stages 10–12, Hamburger & Hamilton, 1951) were operated on in ovo via a window in the shell. Carbon particles, moistened slightly with Pannett-Compton saline, were pushed into one of the first three post-otic somites on the
right-hand side of the embryo with a fine tungsten needle. In all cases, jabs with
the needle were continued until it was quite certain that carbon had firmly pen-
etrated the somite; then excess carbon particles lying on the vitelline membrane
were removed with a micropipette. After replacing the shell ‘window’ the embryo
was reincubated for a further 3–4 days, then fixed in Bouin’s fluid, imbedded by
Peterfi’s celloidin-paraffin technique, sectioned usually sagittally, and stained
with Weigert’s haematoxylin and eosin or with Delafield’s haematoxylin and
Congo red.

Mortality was high in these experiments: out of 98 operated embryos only 25
survived long enough to be suitable for histological examination, and only 6 of
these were actually alive at the end of the experiment. The chief causes of death
seemed to be adherence of the vitelline membrane to the shell window, or split-
ting of the vitelline membrane when the original hole made with the needle had
been somewhat large. There was also in some embryos an extensive vasodilata-
tion, which may have been due to blockage of the blood-vessels with carbon
particles, since particles were sometimes seen to escape into the vitelline arteries
at the time of operation.

However, despite the low yield, the results were very consistent. In 21 out of
the 25 embryos examined histologically, carbon had reached the tongue region
on the right-hand side and was specifically localized here, hardly any occurring
in other parts of the pharynx. In the 4 embryos where this was not so, death had
occurred at an early stage (3 days) and in 2 of them carbon was scattered
throughout the somewhat macerated tissues, making it impossible to say that it
was localized in any particular region. The 2 others which had also met an early
death showed carbon still at the ventral borders of the 2nd and 3rd post-otic
somites.

The 6 embryos that were living at the time of fixation (stages 25–28, Ham-
burger & Hamilton) had well-developed tongue rudiments with muscle already
evident, and in 5 of these carbon was found in, and adjacent to, the geniohyoid
muscle on the right-hand side (Plate, figs. A, B). There were 4 more embryos
(stages 20–22) where the tongue rudiment had as yet no clear muscle, and 3 of
these showed carbon particles in the tongue mesoderm; the 4th had carbon in
near-by mesoderm of the mandibular arch.

There remain to be considered 11 embryos which had died too early to show
a tongue rudiment. In 8 of these carbon had reached the mandibular arch meso-
derm of the right side, in 2 others carbon lay near the base of the right mandib-
ular arch, and in the remaining one there were scattered carbon particles in the
mesoderm of the pharyngeal floor.

The conclusion may be drawn from these experiments that in chick embryos
cells from the occipital somite region do indeed migrate ventralwards to the
lower jaw and participate in the formation of tongue muscles. Hence the inner-
vation of these muscles by nerve XII is understandable, and the generalization
on which previous statements about tongue muscle origin have been based—
A

Developing tongue

B

Tongue rudiment

E. M. DEUCHAR
namely, that when muscles migrate during development they carry their nerve-supply with them—seems fully justified for this particular case.

SUMMARY

Carbon marks placed in the occipital somites of 45-hour chick embryos have been observed in mandibular arch tissue at later stages, and within the tongue muscles when these have developed. Thus the occipital origin of tongue muscles in the chick seems clearly established.

REFERENCES


EXPLANATION OF PLATE

Fig. A. Carbon particles (ringed) in and near the geniohyoid muscle. Sagittal section. × 73.
Fig. B. Transverse section through mouth region of a 5½-day chick embryo. Carbon mass (ringed) in geniohyoid muscle. × 83.

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