Brachial muscles in the chick embryo: the fate of individual somites

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SUMMARY

The wing and wing-associated muscles of the shoulder and thorax in the bird all cleave from common myogenic masses in the developing wing bud and are referred to collectively as brachial muscles. In this study the precise embryonic origin of the brachial muscles was determined using chick-quail chimaeras. Such chimaeras consisted of a graft of one somite taken from a 2-day quail donor embryo transplanted to the equivalent location in a 2-day chick host embryo. The chimaeras were analysed at 9.5-10.0 days in ovo to determine the location of the grafted cells and therefore the structures that were derived from the transplanted somite. The somites that were studied in this manner were somites 13 to 23 inclusive. The results show that only somites 16 to 21 inclusive contribute cells to the brachial musculature; moreover, the cells from a given somite are not distributed randomly among the brachial muscles but populate specific muscles only: thus it has been possible to map the somitic origin of individual brachial muscles. Moreover, there is an indication that each somite plays a unique role in the development of the brachial muscles.

INTRODUCTION

Since the discovery by Le Douarin (1969, 1973) that the nuclei of quail cells could be distinguished from nuclei of chick cells by appropriate staining methods, the embryonic origin of avian skeletal muscles has been extensively investigated. Interspecific chimaeras made by grafting somites or somatopleure from quail to chick embryos or vice versa have provided consistent evidence that all limb and trunk muscles in birds are derived from somites. Christ Jacob & Jacob (1914a, b; 1976, 1977, 1978) first showed that muscle cells in the brachial, thoracic and abdominal area, including the pectoral musculature, were derived from somites but that the connective tissue and tendons of these same muscles were derived from the adjacent somatopleure. This finding has since been abundantly confirmed (Chevallier, Kieny & Mauger, 1977a; Chevallier, Kieny Mauger & Sengel, 1977b; Chevallier, Kieny, & Mauger 1978; Chevallier, 1979; Beresford, LeLievre & Rathbone, 1978).

More recently, attempts have been made to define precisely which somites give rise to the brachial musculature, i.e., the intrinsic wing muscles and the

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wing-associated muscles of the shoulder and thorax. These muscles can be considered as a group, since they all develop from myogenic masses that form in the wing bud and subsequently divide into individual muscle primordia (Sullivan, 1962). The grafting of multiple somites from quail to chick embryos early in development suggested that the brachial musculature was derived from somites 16–20 (Beresford et al. 1978). The present study is a follow up of that report. Single somites between the levels of somites 13 and 23 inclusive were grafted from quail to chick embryos at 2 days in ovo. The resulting chimaeras were subsequently analysed to determine whether the grafted somite contributed to the brachial muscles, and whether the cells from a single somite populated all the brachial muscles or were restricted to a few muscles only.

The results show clearly that all of the brachial muscles are derived from somites 16 through 21 inclusive, but that each individual muscle is derived from three or four adjacent somites within this range. Thus it has been possible to map the embryonic origin not only of the brachial muscles as a group but also of individual brachial muscles.

In this report, the muscles of the shoulder, back, and upper arm are considered. The muscles of the forearm and hand will be the subject of a future publication.

A preliminary report of this study has appeared elsewhere (Beresford, 1979).

MATERIALS AND METHODS

Fertile eggs of white leghorn (Gallus domesticus) and Japanese quail (Coturnix coturnix japonica) were acquired from local suppliers and incubated at 100°F (37.5 °C) until the embryos had reached stages 12 to 15, according to the morphological criteria established for staging of chick embryos by Hamburger & Hamilton (1951). Chick embryos took approximately 48 to 52 h to reach stages 12 to 15; quail embryos took approximately 44 to 48 h to reach the equivalent stages of development. Therefore, quail eggs were routinely placed in the incubator 4 h later than chicken eggs.

Surgical Procedure

The surgical procedure is represented diagrammatically in Fig. 1. Quail embryos were taken out of the eggs and placed in Hanks’ balanced salt solution, where the appropriate region of somitic mesoderm was removed using an electrolytically sharpened tungsten needle. The somites were placed in 0.25 % trypsin to remove surrounding tissue, rinsed in a trypsin inhibitor solution, and the somite to be transplanted was separated from the others and transferred to the host embryo.

A hole was cut in the shell of the host egg, exposing the embryo directly beneath. The appropriate somite was separated from surrounding tissue with a finely ground microscalpel and removed with a suction pipette. The neural tube,
endoderm, and somatic mesoderm surrounding the somite were left intact, as were the somites rostral and caudal to it. The donor somite was transferred to the host embryo and manoeuvred into the site of the operation. The original orientation of the donor somite with respect to the anteroposterior, dorso-ventral, and mediolateral axes was maintained. The hole in the egg was sealed with cellophane tape and the egg was replaced in the incubator to develop further.

**Counting somites**

Since this study was designed to investigate the behaviour of individual identified somites, the method of counting somites is obviously crucial to the outcome. Between stages 12 and 15, when these experiments were performed, the most anterior pair of somites is beginning to disperse and therefore has no clear anterior boundary. Nevertheless, it was always included in the counts as somite pair number one. The first pair of full somites immediately caudal to this was counted as somite pair number two. Every fully formed pair of somites was counted. If the last pair of somites was visible but not fully segmented, it was not counted, nor was it transplanted. In no case was a somite either removed from a host embryo or transplanted from a donor embryo unless it was fully segmented; it was felt that removing or transplanting a somite whose boundaries are only vaguely defined is contrary to the aim of the study, *i.e.*, precision, and could lead to more variability. If for any reason it was not possible to establish precisely the number of somites in either a donor or a host embryo, that embryo was not used.
This method was strictly followed, and it resulted in highly consistent placement of the grafted cells in the vertebral column of the 10-day chimaera, i.e., somite 19 consistently formed the first floating rib, somite 20 formed the second floating rib, and somite 21 formed the first true rib. These observations conform to that of de Castro (1963), who used a similar method of counting somites. He consistently observed that the 13th spinal root emerges between somites 17 and 18. In the present study, his observation was confirmed.

The donor and host embryos were matched as closely as possible with respect to developmental age. Three pairs of somites is the interval between successive developmental stages of Hamburger & Hamilton (1951) at this period of incubation. The quail donor was therefore never more than two somite pairs younger or older than the host embryo, i.e., the difference in developmental age between donor and host was less than the interval between stages. The purpose of this limitation was to ensure that the grafted somite was developmentally equivalent to the host environment.

Chimaera analysis

By 9·5 to 10·0 days in ovo, the brachial muscles have attained their adult locations (Sullivan, 1962). Therefore chimaeras were analysed at stage 35 or 36 (9·5–10·0 days, Hamburger & Hamilton, 1951). They were fixed in Zenker fixative, embedded in Paraplast, and cut transversely or longitudinally in 7 μm serial sections. The sections were stained using the Feulgen method for DNA and counterstained in picro-indigo-carmine (0·4% indigo carmine in saturated aqueous picric acid).

Each chimaera was examined initially to determine the location of the grafted cells in the vertebral column. Subsequently, the entire embryo was examined to determine which of the brachial muscles contained quail cells.

The identification and nomenclature of the muscles in this investigation are taken from the study by Sullivan (1962).

RESULTS

This study is based on observations made on 41 morphologically normal chimaeras. They are listed in Table 1 according to the graft received.

As stated in the Introduction, the brachial muscles consist of all of the skeletal muscles that derive from myogenic masses in the wing bud. The derivations and nomenclature of these muscles have been exhaustively studied by Sullivan (1962), who identified those muscles that cleave from the dorsal myogenic mass, those from the ventral myogenic mass, and a third category of muscles belonging to the axial system. A thorough examination of all the chimaeras used in the present study showed that all of the brachial muscles are derived from somites 16 through 21. This result is highly consistent: in no instance did a somite grafted anterior to 16 or posterior to 21 contribute cells to any brachial muscle.
Table 1.

<table>
<thead>
<tr>
<th>Grafted Somite</th>
<th>No. of Chimaeras</th>
</tr>
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<tbody>
<tr>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td>14</td>
<td>2</td>
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<td>22</td>
<td>3</td>
</tr>
<tr>
<td>23</td>
<td>2</td>
</tr>
</tbody>
</table>

The somites that were grafted are listed in the left column, and the number of chimaeras analysed in this study that received a graft of a particular somite is listed in the right column.

Of greater interest was the observation that the cells from a given somite were distributed neither universally nor randomly among the shoulder muscles, but were restricted to certain muscles only, and in a predictable fashion. For example, all four of the chimaeras that received a graft of quail somite 20 subsequently had quails cells in the same six muscles, while all other muscles were entirely derived from the host. Similarly, the cells from each of the other somites were restricted to a few muscles, rather than scattered throughout the shoulder musculature, and their distribution was consistent for any given somite. Thus it was possible to determine the specific contribution of each somite to the brachial musculature. When the results were tabulated, the embryonic origin of each individual muscle could be determined.

In the paragraphs that follow, I describe each of the shoulder muscles considered in this study, the somites from which it is derived are named, and any variability in the results is noted. Fig. 2 shows cross-sections from four levels of the brachial region of a 10-day embryo to indicate the locations of the muscles described below.

Tensor propatagium

Sullivan (1962) found only a single belly for this muscle but referred to a description by Kaupp (1918) who described two bellies. In the present study two bellies were seen. Possibly this discrepancy is due to genuine variation among strains of domestic fowl. Both muscle slips originate at the shoulder joint (from the clavicle, according to Sullivan; from the coracoid, according to Nickel, Schummer, Seiferle & Siller (1977)) and spread over the shoulder and into the propatagium (wing web). Both bellies receive contributions from somites 16, 17
and 18. However, quail cells were also seen in this muscle in two out of five chimaeras that received a graft of somite 19, suggesting a variable contribution from this somite.

**Coracobrachialis anterior**

There are two parts to this muscle. It originates on the coracoid and inserts on the humerus, but it constricts as it passes through the foramen triosseum (the space formed by the juncture of the humerus, coracoid, and clavicle). The area of constriction is tendonous and contains no myotubes. Both parts of the muscle receive cells from somites 16, 17 and 18.
Coracobrachialis

This is a small muscle located on the ventral surface of the proximal end of the humerus. It is derived from somites, 16, 17, 18 and 19.

Deltoid

This muscle originates on the coracoid and passes over the shoulder to insert on the dorsal surface of the humerus. About 75% of this muscle is derived from somites 17 and 18, with the remainder derived from somite 19. However, quail cells were seen in this muscle in one out of five chimaeras that received a graft of somite 16. In chimaeras that received grafts of somite 17 or of somite 18, the quail cells in the deltoid were concentrated in the lateral region of the muscle. In chimaeras that received grafts of somite 19, the quail cells in the deltoid were found more medially.

Pectoralis major

This is the largest brachial muscle in the chicken. It has an extensive origin on the sternum and clavicle and inserts on the humerus, covering the supracoracoideus and the coracobrachialis posterior. It consists of two parts separated by a band of connective tissue (Fig. 2), and the two parts have different embryonic origins. The medial region arises primarily from somites 16, 17, and 18, with small but consistent contributions from 19 and 20. The cells from somites 16 and 17 are found mostly in the anterior end of this medial part of the muscle, and cells from somites 18 and 19 are usually found in more posterior areas. The lateral region is derived from somites 19, 20 and 21. If the two parts together are considered as one muscle, the pectoralis major is the only muscle that is derived from all six brachial somites.

Scapulohumeralis anterior

This muscle originates on the anterior end of the scapula and inserts on the humerus. It lies anterior to the anterior latissimus dorsi (ALD), and at the insertion end it passes ventral to the deltoid and triceps. It is derived primarily from somites 18 and 19; however, in two out of seven chimaeras that received a graft of somite 17, quail cells were seen in this muscle.

Biceps

As the name implies, this muscle has two parts, although the division between them is not obvious. According to Sullivan (1962), it originates from the coracoid and from the humerus at the shoulder joint, and it has two tendons of insertion, one on the radius and one on the ulna at the elbow joint. There is no sheet of connective tissue separating the two parts, as in the pectoralis major. The biceps is derived largely from somites 17 and 18, with small but consistent contributions
from somites 16 and 19 (approximately 10% each). In some chimaeras the graft-derived cells were found mostly in one or the other division, but this was not a consistent enough finding to assign separate embryonic origins to the two parts.

**Supracoracoideus**

Sometimes called the pectoralis minor, this muscle lies beneath the pectoralis major along the length of the sternum. Its tendon passes through the foramen triosseum and inserts on the dorsal part of the head of the humerus. Its function is to raise the wing, the opposite of the pectoralis major. Like the pectoralis major, it consists of a medial and a lateral region separated longitudinally by a band of connective tissue (Fig. 2). Unlike the pectoralis major, both regions have the same embryonic origins: somites 16, 17 and 18.

**Coracobrachialis posterior**

This muscle originates on the coracoid and inserts on the humerus. For much of its length it is situated between the coracoid bone and the lateral region of the pectoralis major (Fig. 2). It is derived from somites 17, 18 and 19, although in two out of five chimaeras that received a graft of somite 16, substantial numbers of quail cells were seen in this muscle.

**Subscapularis and subcoracoideus**

Like Sullivan (1962), I have treated these two muscles together because at their insertion end they merge into one muscle. The subscapularis originates from the ventral region of the scapula; the subcoracoideus originates from the dorsal region of the coracoid. The two muscles merge distally and attach to the humerus (Fig. 2). Both muscles are derived from somites 19, 20, and 21.

**Latissimus dorsi**

Sullivan (1962) treated the anterior and posterior latissimus dorsi muscles as one muscle with an anterior and posterior part, because the two parts cleave from a single primordium in the limb bud. Grim (1971), however, favoured the view that they are two distinct muscles, since they separate early in ontogenesis and develop separate tendons. The results of the present study show that they also differ with respect to embryonic origin. Therefore I have treated them as separate muscles:

**Anterior latissimus dorsi (ALD)**

This muscle has its origins on the first two thoracic vertebrae (Nickel *et al.* 1977). In cross sections of embryos it is seen as a thin strap just beneath the skin of the back, extending from the backbone toward the wing (Fig. 2). At the insertion end it travels ventral to the scapular head of the triceps and attaches to the humerus between the scapular and humeral heads of the triceps. Embryonically
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It is derived almost entirely from somites 19 and 20; however, in four out of six chimaeras that received grafts of somite 18, quail cells were seen in this muscle.

Posterior latissimus dorsi (PLD)

This muscle originates on the last thoracic vertebra (Nickel et al. 1977) and travels anteriorly and laterally to attach to the humerus by a long tendon. It derives from somites 19, 20 and 21.

Grim (1971) describes two additional latissimus dorsi muscles, both of which are long and narrow, originate near the origin of the PLD, and insert in the skin of the back. The dorsocutaneous latissimus dorsi extends forward from its anatomical origin and inserts into the skin anterior to the ALD; the metapatagial latissimus dorsi runs dorsal and nearly parallel to the PLD and inserts into the skin posterior to the ALD. Both of these smaller muscles have the same embryonic origin as the PLD, i.e. somites 19, 20 and 21.

Triceps

There are three heads to this muscle. The scapular head originates on the scapula and is the most dorsal head. The more ventral humeralis part has two origins, one on either side of the pneumatic fossa at the proximal end of the humerus. More distally all three heads merge into one muscle, much of which is attached ventrally to the posterior edge of the humerus. It inserts on the ulna at the elbow. It arises from somites 19, 20 and 21, the majority of the muscle being provided by somites 20 and 21. In one chimaera that received a graft of somite 18, a very small number of quail cells were seen in the triceps.

Scapulohumeralis posterior

This is a large muscle that originates along the lateral edge of the scapula in the thoracic region and travels anteriorly and laterally to insert on the humerus. It arises primarily from somites 18 and 19 but receives a small but consistent contribution from somite 17.

Brachialis

This small muscle originates from the distal end of the humerus and inserts on the proximal end of the ulna, spanning the inside of the elbow joint. Somites 16, 17, 18 and 19 all contribute equally to this muscle.

The results described above are summarized in Table 2, where the muscles are grouped according to the divisions and myogenic masses from which they cleave (Sullivan, 1962). If the table is read horizontally, the embryonic origin of individual muscles is shown. Each muscle is derived from two or more adjacent somites, and the number of somites that contribute to a particular muscle bears little relation to the size of the muscle; the coracobrachialis, a small muscle, is derived from four somites, while the scapulohumeralis posterior, a large muscle, is derived from three.
Table 2.

<table>
<thead>
<tr>
<th>Muscle Mass</th>
<th>Muscle Division</th>
<th>Muscle</th>
<th>16</th>
<th>17</th>
<th>18</th>
<th>19</th>
<th>20</th>
<th>21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dorsal</td>
<td>Tensor propatagium</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td></td>
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<tr>
<td>Deltoid</td>
<td>Deltoid</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Subscapular</td>
<td>Scapulohumeralus anterior</td>
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<td>+</td>
<td>+</td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Scapulohumeralis posterior</td>
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<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Coracobrachialis posterior</td>
<td>±</td>
<td>+</td>
<td>+</td>
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<tr>
<td></td>
<td>Subscapularis</td>
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<td>+</td>
<td>+</td>
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</tr>
<tr>
<td></td>
<td>Subcoracoideus</td>
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<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triceps</td>
<td>±</td>
<td>+</td>
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<td>+</td>
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<tr>
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<tr>
<td></td>
<td>Biceps</td>
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<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>±</td>
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<td>Pectoralis major lateral</td>
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<tr>
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</table>

Contributions of the six brachial somites to individual brachial muscles. The + indicates a consistent contribution of the somite to the muscle in all chimaeras that received a graft of the somite. The ± indicates a variable contribution of the somite to the muscle, i.e. not all of the chimaeras that received a graft of the somite had quail cells in the muscle (see text for details).

The table shows that there is no correlation between a particular muscle mass and a particular group of somites. Muscles that cleave from either the dorsal or the ventral muscle mass may be derived from any of the six somites. With respect to the muscle divisions, all of the muscles within the deltoid, ventral brachial, and supracoracoid divisions originate from the anterior somites. The muscles within the other divisions, however, have dissimilar origins.

If the table is read vertically, the contribution of the individual somites can be seen. Although in some cases two adjacent somites make a very similar contribution (e.g. compare somites 17 and 18; also 20 and 21), no two somites perform identical functions. This phenomenon is even more evident when the forearm muscles are taken into consideration (Beresford, manuscript in preparation). Thus each somite plays a distinctive role in the formation of brachial muscles. Fig. 3 is a comparison of muscles in two chimaeras. The muscles that are compared are the deltoid, the ALD, and the supracoracoideus. The three muscles on the left side of the plate are from a chimaera that received a graft of somite 17: in this chimaera quail cells are seen in the deltoid and the supracoracoideus, but not the ALD. On the right side of the plate are seen the same three muscles from a chimaera that received a graft of somite 20: in this case, quail cells are seen in the ALD, but not in the deltoid and the supracoracoideus.

A more dramatic comparison is shown in Fig. 4. Both photographs show a
Fig 3. Comparison of three muscles in two chimaeras that received grafts of a different somite. A, C and E are the deltoid, ALD, and supracoracoideus muscles (respectively) from chimaera 1133, which received a graft of somite 17. Quail cells from somite 17 (arrows) are found in the deltoid and in the supracoracoideus, but not in the ALD. B, D and F are the same muscles from chimaera 1050, which received a graft of somite 20. In this case, quail cells from somite 20 (asterisk) are found in the ALD, but not in the deltoid or the supracoracoideus. Scale bar: 20 μm.
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similar view that contains the biceps and triceps muscles of the chimaera. In the top photograph is a chimaera that received a graft of quail somite 17: the biceps contain quail cells, but the triceps does not. In the bottom photograph is a similar view from a chimaera that received a graft of quail somite 21: in this case, the triceps contain quail cells, but the biceps does not. In chimaeras with grafts of somite 19, quail cells were seen in both the biceps and the triceps muscles (not shown).

In summary, the results show a contribution to the shoulder muscles from somites 16 through 21 only, with a distinctively different pattern of distribution of the cells from each somite.

Other muscles

Somites also give rise to the intervertebral or axial muscles. In single somite chimaeras, the axial muscles derived from the grafted somite were those muscles most closely associated with the vertebrae derived from that somite. An exception to this involved the most ventral axial muscles. These muscles run longitudinally beneath the vertebral column, in the brachial region, and quail cells from a single grafted somite were consistently seen in ventral axial musculature as far as three segments (vertebrae) posterior to the level of the graft.

In the chimaeras used in the present study, and in chimaeras used in previous studies that received grafts of multiple somites (Beresford et al., 1978) there were some muscles that were not derived from somites. A few very small muscles that were found in the dermis of the skin are derived from lateral plate mesoderm (Beresford, unpublished observations). This may be the source of the striated muscle seen by some investigators, who claim that lateral plate mesoderm is capable of differentiating into muscle (Chevallier et al. 1977b, 1978; McLachlan & Hornbruch, 1979). Although these small muscles appear to be striated muscle, they do not attach to bone or cartilage and are not responsible for locomotion in the bird; therefore, they are not true skeletal muscles. It appears that in the brachial area of the bird, all skeletal muscles are derived from somites (Christ et al. 1974a,b 1977; Beresford et al. 1978).

One final observation on the muscles in the chimera relates to the ability of chick and quail myoblasts to co-operate to form myotubes. It was not uncommon to see chick nuclei and quail nuclei in the same muscle fibre (Fig. 5). This phenomenon has been seen previously in vivo (Kieny, 1978) and in vitro (Filogamo, Peirone & Comoglio 1980) and provides visual confirmation of the
evidence presented by Mintz & Baker (1967) that nuclei from two different strains can fuse together into the same myotube.

**Cartilage derivatives**

Somites form vertebral cartilages (Remak, 1855; Williams, 1910). But there is not a one-to-one correspondence between somite and vertebra; descriptive studies have indicated that each individual vertebra is derived from the posterior halves of one pair of somites and the anterior halves of the next (Remak, 1855; Trelstad, 1977). This has been confirmed in the present study. In chimaeras that received a graft of a single quail somite on the right side, graft-derived cells were found in the right posterior quadrant of one vertebra and the right anterior quadrant of the next vertebra. Ribs are also derived from somites (see also Christ et al., 1974a, b).

It has recently been reported that the scapula is formed by somites. Chevallier (1977) has stated that somites 15 to 24 are responsible for this structure. In the present study, somites 15, 16 and 17 did not contribute to the scapula. Each of somites 18 to 23 formed a band of quail cells in the scapula, and the location of this band was approximately one segment posterior to the level of the graft; e.g., in a chimaera with a graft of somite 18, quail cells were found in the scapula at a level corresponding to the location of somite 19. Thus the scapular cells from
each somite migrated not only laterally, but also in a posterior direction. The posterior displacement could be caused by the rotation of the base of the wing, which occurs between stages 23 and 27 (Yander & Searls, 1980).

**Dermal derivatives**

In single somite chimaeras, quail cells were found in the dermis somewhat anterior to the graft level and did not extend as far in a posterior direction as the quail cells in the vertebral cartilage. Quail cells were sometimes seen in the dermis across the midline on the unoperated side of the animal, but their numbers were small. Laterally, quail cells populated the dermis as far as the scapula.

**DISCUSSION**

The data presented here reveals two pieces of information about brachial muscle development:– (1) all brachial muscles are derived from somites 16 through 21 inclusive; and (2) there is a correlation between the position of a somite and the muscles to which it contributes, i.e. the myogenic cells that migrate from a somite into the periphery do not move in a random fashion but contribute to a specific group of muscles.

The positions of somite 16 to 21 correspond closely to the level of the neural tube from which the brachial plexus arises. The developing brachial plexus contains nerves from spinal roots 12 through 17 (Roncali, 1970). According to de Castro (1963), the 13th spinal root lies between somites 17 and 18. Therefore, the six roots 12 through 17, which contribute to the brachial plexus, are found between somites 16 and 22. At least in the brachial area, therefore, muscles appear to receive their innervation from the spinal nerves most closely associated with the somites from which the muscles were derived.

The results presented here show that there is a correlation between the position of a somite and the muscles to which it contributes. This association is not intrinsic to the somite itself, but rather to its position along the anteroposterior axis, since myogenic cells from somites at the lumbar level can participate in the formation of brachial muscles if the lumbar somites are grafted to the brachial level early in development (Chevallier et al., 1977a,b). In other words, myogenic cells from somites will migrate into the periphery and form muscles in positions that are appropriate for the level in which the somites are placed rather than the level at which they originally developed.

Another group of investigators, using similar methods, have presented results which conflict with both of the major findings discussed above. Chevallier (1979) grafted groups of three or more somites from quail to chick embryos and concluded that the shoulder muscles were derived from somites 12 through 22. While it is true that the origin of the shoulder muscles is within the range of somites 12 through 22, the precise origin cannot be determined unless experiments are designed to test the contribution of individual somites. Kieny & Chevallier
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(1980) studied the origin of the intrinsic wing musculature using the more accurate method of grafting groups of two somites, and they concluded that the wing muscles are derived from somites 13 to 21. These investigators found no evidence of a specific role for individual somites; moreover, one series of single somite grafts failed to show a correlation between the grafted somite and specific muscles. Although it is true that Kieny & Chevalier (1980) studied the intrinsic wing muscles, while the present study deals with the shoulder muscles, both studies included the biceps and triceps muscles. Kieny & Chevalier report that neither of these muscles is derived from a particular group of somites and that, at best, there appears to be a weak correlation between the anterior somites (13 through 17) and the most dorsal muscles, including the triceps. In contrast, my results show that somites 13 to 15 do not participate at all in the formation of brachial muscles, that there is a definite correlation between the position of a somite and the muscles to which it contributes, and that the triceps in particular is derived from the posterior somites (18 to 21). Although I do not include my observations on the forearm muscles in this publication, the same principles hold true for these muscles also (Beresford, manuscript in preparation).

The reason for the discrepancy between the two sets of results is not obvious, and any explanation for it must be based on conjecture. One possibility is that the method of counting somites is different. As I stated in the Materials and Methods, the first partial pair of somites was always counted as somite pair number one. Kieny & Chevallier (1980) do not describe their method of counting somites. If they did not consistently count the first partial pair of somites, their identification of a given somite (e.g. somite number 16) would not be consistent, and therefore the results of grafting that somite would not be consistent.

One other factor that may contribute to the discrepancy is that on occasion, Kieny & Chevallier grafted somitic tissue that was not fully segmented (see their Materials and Methods, fig. 1). In the interests of precision, I did not transplant a somite unless it had fully segmented; until a somite has fully segmented, its boundaries are only vaguely defined, and it may still contain cells that will ultimately move into the next segment posterior to it. If this cell movement is completed after the somite is transferred to the host embryo, the graft-derived cells will migrate into the limb bud from two somites rather than one, obscuring the role played by each.

The use of white Leghorns in the present study, as opposed to the Wyandotte × Rhode Island Red embryos used by Kieny & Chevallier, is a factor whose contribution to the differences in the two investigations cannot be entirely ruled out without appropriate control studies.

Given that there is a correlation between a somite and a group of muscles, how is it that the cells from each somite arrive at the correct sites without mixing randomly with myogenic cells from other somites? A clue to this mechanism might be found in the results of a study by Meier (1980). His observations of 2-day chick embryos with the scanning electron microscope showed a ribbing
Individual somites and the limb musculature

pattern in the lateral plate mesoderm; this ribbing runs perpendicular to the axis of the embryos and corresponds exactly to the somites, thus extending the segmental pattern of the somites laterally into the periphery in the form of channels. Meier speculates that these channels could provide initial pathways for neural crest cells. It is obvious, however, that they could also be utilized by migrating myoblasts as pathways to the appropriate sites in the developing limb bud. The cells from each somite would migrate along the channel that corresponded to that somite and thus arrive at a unique location within the wing, and at a unique time in development. Such a mechanism would suggest a high degree of order during early development that is not evident at the level of the light microscope nor with the manipulation of multiple somites, but which reveals itself if we ask sufficiently precise questions.

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REFERENCES


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