EXPERIMENTAL ANALYSIS OF THE EMBRYONIC ORIGIN AND DEVELOPMENT OF THE PECTORALIS MAJOR MUSCLE OF THE CHICKEN

by

BONNIE JOAN BERESFORD, B.A.

A Thesis

Submitted to the School of Graduate Studies

in Partial Fulfilment of the Requirements

for the Degree

Doctor of Philosophy

McMaster University

September, 1979

DEVELOPMENT OF THE PECTORALIS MAJOR MUSCLE OF THE CHICKEN

DOCTOR OF PHILOSOPHY (1979)
(Medical Sciences)

McMASTER UNIVERSITY
Hamilton, Ontario

TITLE: Experimental Analysis of the Embryonic Origin and Development of the Pectoralis Major Muscle of the Chicken.

AUTHOR: Bonnie Joan Beresford, B.A. (Lawrence University, Appleton, Wisconsin).

SUPERVISOR: Doctor M.P. Rathbone

NUMBER OF PAGES: xi; 182

ABSTRACT

The purpose of the studies reported in this thesis was to obtain data concerning the embryonic origin and formation of the pectoralis major muscle of the chicken. This muscle is used extensively in studies on muscle development because it is large; readily available, and is composed almost entirely of one muscle fiber type. Moreover, it is the largest muscle to be affected by hereditary muscular dystrophy in the line of chickens afflicted with this disease. Information concerning its embryonic origin could be used for *in vivo* studies on the early development of both normal and dystrophic muscles.

Previous investigations into the embryonic origin of skeletal muscle in several classes of vertebrates have resulted in controversy. Some investigators have concluded that all skeletal muscles arise from the myotomal layer of the somites. Others have cited evidence to show that some muscles, including the pectoralis major muscle of the chick, are derived from the somatopleuric mesoderm adjacent to the somites.

In the present investigation, interspecific chimaeras have been used to study the problem. Whole somites, somite halves, or limb-buds were grafted from quail to chick embryos between 2 and 3 days *in ovo*. After further development, the chimaeras were fixed, embedded in paraffin, sectioned, and stained using the Feulgen reaction for chromatin. This procedure permitted the identification of those structures that were derived from the grafted quail tissue.

The observations in this study have led to the following conclusions:

The pectoralis major muscle arises from the dorsal halves of somites 16-21 of the 2-day *in ovo* chick embryo. These somites also give rise to all other wing and wing-associated muscles of the shoulder and thorax.

Each somite plays a specific role in the development of these muscles. The cells that ultimately form the pectoralis and other brachial muscles migrate from the somites into the lateral mesoderm between 2 and 2.5 days *in ovo*. The myotomal layers of the somites do not appear until 2.5 days *in ovo* and do not contribute to the formation of the brachial muscles.

ACKNOWLEDGEMENTS

This work was carried out under the supervision of Dr. M.P. Rathbone. I am grateful to Dr. Rathbone for giving me the opportunity to study embryology, and for his support and guidance during the course of this investigation.

I would also like to thank the members of my supervisory committee, Dr. David McCallion, Dr. Luis Branda and most especially Dr. Alan Peterson for the help that they extended during the course of my studies. In addition, I am deeply indebted to Dr. Ethel Cosmos and Jack Diamond for their unfailing interest and encouragement.

I wish to extend my gratitude to Dr. Christiane Le Lievre for her invaluable help with various technical aspects of this work and for many illuminating discussions of embryonic development.

I am grateful to Mr. C. Ikeson, Mr. M. Holmes, and Miss J. Brierley for technical advice and assistance during the course of this investigation, and to Dr. R. Butler for giving me free access to his histological and photographic equipment.

Finally, I would like to express my gratitude to Miss Carol Habinski for her tireless efforts in typing this thesis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

gm	gram
mg	milligram
ml	milliliter
μ	micron (10^{-3} millimeter)

INTRODUCTION

INTRODUCTION

For more than a century, embryologists have been debating the issue of the embryonic origin of skeletal muscles. There is no doubt that much of the dorsal musculature is derived from somites, the blocks of mesoderm that form adjacent to the embryonic spinal cord; the myotomal layer of the somites can be seen to differentiate into spindle-shaped myoblasts in situ. The controversial issue is whether the somites contribute cells to the more peripheral muscles, such as those of the limb, thorax, and abdomen, or whether these muscles are derived from the somatopleuric mesoderm, the lateral mesoderm that forms the body wall.

MUSCLE DEVELOPMENT (see Yaffe, 1969; Herrmann et al., 1970; Holtzer and Bischoff, 1970; Goldspink, 1974 for detailed reviews).

In the embryo, skeletal muscle develops from undifferentiated, proliferating cells that accumulate in regions corresponding to the sites that muscles will occupy in the adult, such as the peripheral regions of the limbs. Overt muscle differentiation begins with the appearance of bipolar spindle-shaped mononucleated myoblasts.

The existence of several classes of presumptive myoblasts has been postulated by Holtzer and Bischoff (1970). According to this hypothesis, many apparently undifferentiated cells in myogenic regions are committed to a myogenic lineage. These cells pass through a series of quantal mitoses, each of which advances the cell another step toward the fully differentiated state. This hypothesis is based on indirect evidence and is not universally accepted (Searls and Janners, 1969; Konigsberg and Buckley, 1974); nevertheless, it calls attention to the idea that cells may be covertly differentiated before they take on the morphological characteristics of the overtly differentiated state.

Once myoblasts appear, they continue to proliferate. At some later point, they begin to fuse with one another to form long multinucleated myotubes. It has been proposed by Holtzer (1972) that myoblasts withdraw from the cell cycle prior to fusion. His *in vitro* studies have indicated that only myoblasts in the G_1 phase of the cell cycle will fuse with one another or with myotubes. Other *in vitro* studies have demonstrated that nuclei contained within myotubes do not synthesize DNA or divide except under pathological conditions (Yaffe, 1969). A study of the *in ovo* development of skeletal muscles in the chick embryo (Marchok and Herrman, 1967) has shown that between 7 and 11 days of development, when the first wave of myotube formation occurs, the mitotic rate of myogenic cells decreases from 70% to 20%; yet at 11 days *in ovo*, almost 90% of all muscle nuclei are present in mononucleated cells; only about 12% are contained within myotubes. Thus it appears that myoblasts cease proliferating before they fuse. Other studies have shown, however, that withdrawal from the cell cycle is not obligatory prior to fusion (Konigsberg and Buckley, 1974). The issue of proliferation versus differentiation is a controversial one and has been reviewed by Lash (1974).

With the appearance of myotubes, bulk synthesis of muscle-specific proteins begins. Myosin synthesis within myotubes is readily detected, but there have been some reports of low levels of myosin synthesis in mononucleated myoblasts (Herrman *et al.*, 1970). It is not known whether this represents low levels of myosin synthesis by many cells or high levels of myosin synthesis by a few cells. However, it is apparent that large quantities of myosin, actin, tropomyosin, and troponin are synthesized only after fusion.

Myotubes first appear in the central regions of the presumptive muscle mass, and they grow in length by continued fusion with myoblasts; new myotubes appear in more peripheral regions of the muscle mass. New myotube formation may continue until hatching or birth, and even beyond in some species.

Thus, many stages of myogenesis are occurring within a given muscle throughout most of fetal development.

HISTORICAL INTRODUCTION

Since muscle differentiates in the sites that muscles will occupy in the adult, the migration of precursor cells from somites into the lateral plate mesoderm, if it occurs, will presumably begin prior to overt muscle differentiation. Therefore, investigations into the embryonic origin of skeletal muscles have necessarily focussed on stages of development prior to the appearance of myotubes.

Descriptive Studies

The studies done in the second half of the last century and the early part of this century were descriptive rather than experimental. In descriptive studies, serial sections of embryos at different stages in development are examined to try to determine the source of the skeletal musculature. During the early stages of development, the myotomal layers of the somites extend laterally toward the somatopleure (Fig. 1). Condensations of mesenchyme appear near these extensions in the limbs and body wall later in development; moreover, small groups of cells are sometimes seen very close to the distal ends of the myotomes and appear to migrate into the mesenchymal condensations at later stages. The condensations ultimately form skeletal muscles, leading some investigators to conclude that myoblasts migrate from the myotome, collect in myogenic masses, and form peripheral skeletal muscles. Descriptive studies by Balfour (1878) in elasmobranch fishes, Goodrich (1930) in amphibians, Mollier (1893) and Sewertzoff (1907) in reptiles, Remak (1855) and Lillie (1908) in the chick, and Zechel (1924), who studied human embryos, support a somitic origin for all skeletal muscles.

Figure 1

Stage 20 chick embryo (2.5 to 3 days of incubation; hatching occurs at 21 days in this species); lateral view.

Somites (s) are paired blocks of tissue that form on either side of the developing spinal cord. The peripheral, unsegmented mesoderm is called the lateral plate (lp). The limb buds (lb) arise in two specific regions of the lateral plate.

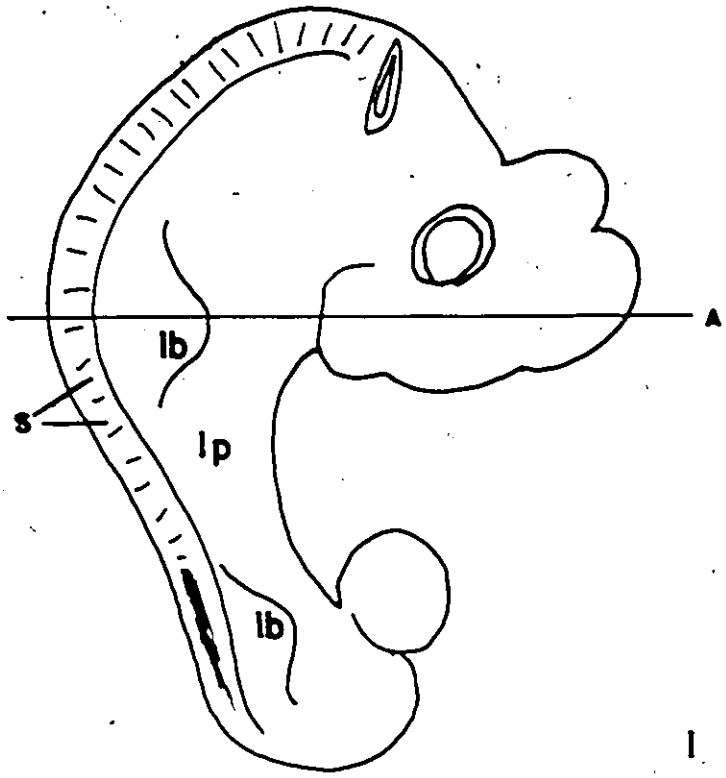
A. Cross-section through the level of the wing buds (the region of the section through the head is not shown).

In cross-section, somites at this stage consist of 3 layers: dermatome (d), myotome (m), and sclerotome (sc). The dermatome and myotome layers are epithelial (organized into sheets) and give rise to dermis and skeletal muscle respectively. The sclerotome is mesenchymal (loose and unorganized) and gives rise to cartilage.

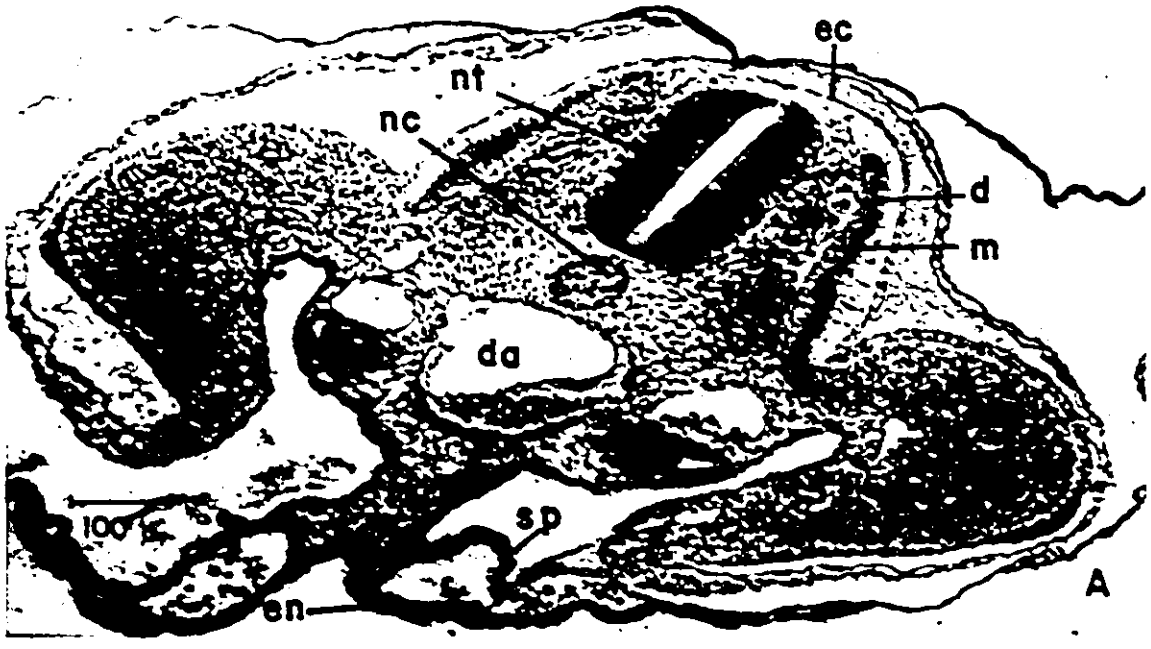
The lateral plate mesoderm consists of two layers: somatopleure (so) and splanchnopleure (sp). The limb buds arise in the somatopleure layer. The region of somatopleure between the wing and leg buds will form the body wall. The splanchnopleure layer will form the smooth muscle of the viscera.

The ectoderm (ec) will give rise to the skin and skin derivatives. The endoderm (en) will form the lining of the gut.

nt: neural tube nc: notochord da: dorsal aorta



I



Other investigators, however, could see no evidence of cell migration from the myotome into the somatopleure. Harrison (1895) stated that the muscles of the pectoral fins in teleosts were not of myotomal origin. Katznelson (1934) described the development of muscles in the limbs of urodeles and concluded that there was no contribution of the myotomal layer to these muscles. In studies of the chick, His (1868) found no evidence of somitic involvement in the formation of abdominal muscles; Paterson (1888) noted that the myotomes at the wing level do not extend as far into the mesenchyme as those at thoracic and abdominal levels, and concluded that limb muscles arise from somatopleural mesenchyme; Williams (1910) essentially confirmed Paterson's observations. Lewis (1901) favoured the view that in humans, the arm muscles arise independently of the somites.

The amount of information that can be derived from descriptive studies is limited, due to the fact that serial sections of fixed specimens reveal little about cell migration. A small number of cells migrating from one region to another may pass unnoticed if they cannot be distinguished from surrounding cells. Yet these few cells may make a significant contribution to the development of their new region. Similarly the proximity of two regions may suggest the possibility of migration between them when no such migration has occurred.

Experimental Studies

Experimental studies began in the early part of this century, as embryologists developed a variety of grafting and marking techniques to study the fate of specific regions of the embryo. Amphibian and bird embryos have been most commonly employed in such studies due to their relative accessibility.

1. Amphibians

Byrnes (1898) first demonstrated that limb muscles were not dependent on somites for development in amphibians. She destroyed the hind-limb somites of a frog embryo by burning them with a hot needle. Although there were subsequent deficiencies in the area of trauma, the limbs developed normally with no defects in the musculature. This experiment was repeated by Lewis (1910) on the urodele embryo, but instead of traumatizing the somites, he removed them entirely. As in Byrnes' experiments, abnormalities were found in the structures at the level of the operation, but the limb formed normally. Detwiler (1918, 1920, 1929, 1936) performed a variety of similar experiments in his extensive studies on amphibian development and observed similar results. He concluded that the limb muscles in amphibians must arise from the limb somatopleure.

These experiments showed that the absence of the limb somites does not prevent the development of a normal limb. However, other studies showed that limbs could sometimes develop normally even after the removal of prospective limb mesoderm (Harrison, 1917, 1918). These latter results, however, were interpreted as a demonstration of the remarkable regenerative capacity of the limb-forming region. Many embryologists considered the evidence to be strongly in favour of a somatopleuric origin for the limb musculature (Detwiler, 1936; Nicholas, 1955).

A variety of grafting techniques performed by Detwiler (1955) on urodele embryos showed that, unlike limb muscles, the muscles of the abdomen required a cellular contribution from the somites. Leidke (1958) noted that these observations contrasted with those of Straus and Rawles (1953) in the chick, where it was shown that abdominal muscles arose in the lateral mesoderm. Starting from the hypothesis that anurans might show an intermediate position between urodeles and birds, Leidke studied the problem in Rana pipiens.

Using extirpation and intra-coelomic grafting methods, she found that abdominal muscles showed an increasing independence from somitic tissue as development progressed. Earlier stages required the presence of somites for abdominal muscle development, while later stages did not. She concluded that somites do contribute to abdominal muscles, but the possibility of a somatopleuric involvement was not excluded.

2. Birds

Hamburger (1938) removed prospective wing somatopleure from chick embryos before the wing buds were visible and grafted them to flank regions of host embryos. Some of these grafts remained *in situ* and formed morphologically normal wings. Others dropped through the slit in the flank tissue and attached to the developing viscera. Hamburger thus inadvertantly performed the first intra-coelomic grafts. Many of these grafts also developed morphologically normal wings. Hamburger concluded that limb muscles could develop independently of somites, although parts of the somites were included in the graft.

Saunders (1948) marked the brachial somites of 2.5 - 4 day *in ovo* chick embryos with carbon particles. At subsequent stages, carbon particles were found in many dorsal tissues of the embryos, but no carbon particles were found in any tissues distal to the scapula (shoulder blade). Since there were no carbon particles in the wings, Saunders concluded that there had been no migration of somitic cells into the developing wing musculature and that all wing muscles arose from the somatopleuric mesoderm.

Straus and Rawles (1953) presented experimental evidence that the lateral mesoderm can give rise to skeletal muscle in the body wall. They used carbon particles to mark thoracic somites 21-25 or the corresponding region of somatopleure in 2.5 - 3.5 day *in ovo* chick embryos. In embryos whose somatopleure had been marked, carbon particles were subsequently found in the abdominal muscles only and in one case in the pectoralis. The authors concluded that in the

region between the wing and the leg, the somites formed the dorsal muscles and the lateral mesoderm formed the ventral muscles. The ability of the somatopleuric mesoderm to give rise to skeletal muscle was further tested by grafting the thoracic lateral plate (which comprised the somatopleuric and splanchnopleuric mesoderm and included the ectoderm and endoderm) into the coelom of host embryos. After 7 days of development or longer, more than half of the grafts contained skeletal muscle. These experiments demonstrate that thoracic somatopleure in 2.5 - 3 day *in ovo* chick embryos is capable of forming skeletal muscle.

In 1961 Seno published the results of similar studies. He used carbon-particle marking or intra-coelomic grafting of somites and somatopleure, but he performed his experiments on slightly younger embryos (2 - 2.5 days *in ovo*) and his studies included somites 19-28 and the corresponding region of lateral plate. His results differed from those of Straus and Rawles by demonstrating that somites do contribute to the abdominal musculature. When he marked the lateral plate, he subsequently found carbon particles in the pectoralis major muscle. He concluded that the abdominal musculature is somitic in origin but that the somatopleuric mesoderm forms other skeletal muscles, including the pectoralis major.

Pinot (1969) essentially confirmed Seno's results. She used intra-coelomic grafting or local X-irradiation of somites 21-25. When these somites were destroyed, the abdominal and intercostal musculature did not form, but the pectoral musculature was normal. She concluded that the abdominal and intercostal muscles arise from the somites, but that the pectoralis originates from thoracic lateral mesoderm.

3. Mammals

Experimental studies on post-gastrulation mammalian embryos are rarely attempted, due to the high mortality of manipulated embryos and the lack of adequate culturing methods for whole embryos. However, in a recent study, Agnish and Kochlar (1977) combined mouse limb buds with ^{3}H-thymidine labelled somites in culture. They found labelled cells in the humerus and in some muscles close to the somites, but there was no substantial labelling of cells in limb muscles.

4. Interspecific Chimaeras

The discovery by Le Douarin (1969, 1973) that nuclei of quail embryos can be distinguished from those of chick embryos has provided embryologists with a permanent and precise marker for distinguishing grafted and host cells. A difference in the distribution of DNA in the nuclei of chick and quail cells can be observed after staining for DNA with the Feulgen-Rossenbeck (1924) method.

Since the quail is phylogenetically closely related to the chicken, early embryonic stages are similar. It is therefore possible to make viable interspecific chimaeras by grafting regions of quail embryos to corresponding regions of chick embryos, and vice versa. At subsequent stages, the chimaeras can be fixed, sectioned, and stained to reveal the tissues and organs derived from the grafted tissues. The chick and quail cells retain their distinguishing characteristics regardless of the length of association between them.

This technique has been used extensively by Le Douarin and her colleagues to study derivatives of neural crest, a specialized group of cells in the dorsal portion of the neural tube (see Le Lievre and Le Douarin, 1975 for references). They have also applied the technique to the investigation of other tissues, such as the embryonic origins of thymus cells (Le Douarin and Jotereau, 1975) and haemopoietic and osteogenic cells (Jotereau and Le

Douarin, 1978). In all of these studies, quail tissues grafted into chick embryos form the same structures as the corresponding chick tissues grafted to quail embryos, indicating that this is an accurate and reliable method for studying the fate of specific regions of the embryo.

Other investigators have used this technique to study the embryonic origin of skeletal muscles in the chick. Christ, Jacob and Jacob (1974 a,b) grafted cervical and thoracic somites (or prospective somitic mesoderm) from quail to chick at the 15-somite stage. This is well before the limb bud appears and much earlier than the stage at which Straus and Rawles (1953), Seno (1961) and Pinot (1969) performed their experiments. When the chimaeras reached 9 days of incubation, quail nuclei were found in the myotubes of all the developing muscles of the wing and thorax, while the connective tissue of these muscles were of chick origin. Christ et al. (1974 a,b) concluded that in primary development, somites are the source of all myogenic cells of the wings, and the lateral mesoderm forms the connective tissue (See Appendix I, page 181).

AIMS OF THE PRESENT STUDY

The primary goal of the present study has been to define as precisely as possible the region of the two-day in ovo chick embryo that gives rise to the pectoralis major muscle. The development of the chick pectoralis has been extensively studied. It is the largest skeletal muscle in the chick, and it is therefore often used as a source of myoblasts for tissue culture studies. Moreover, unlike most muscles, which contain a mixture of metabolically diverse fiber types, the pectoralis is a nearly homogeneous muscle consisting of white fast-twitch glycolytic fibers (Cosmos, 1966; Cosmos and Butler, 1967). This makes it well suited for developmental studies.

Studies of muscle development have recently become increasingly focused on very early stages of differentiation, particularly the influence of the developing nervous system on muscle primordia. Bonner and Hauschka (1974; see also Bonner, 1978) have shown

that the neural tube can influence the number of myoblasts in the developing chick hindlimb. Bekoff and Betz (1976) have suggested that the neural tube may influence membrane characteristics in myoblasts. O'Hare (1972 a, b, c) has demonstrated that the ability of somites to differentiate into muscle in culture is dependent on the presence of the embryonic spinal cord at certain stages of development. All of these studies are consistent with the hypothesis (discussed in Cohen and Hay, 1971; Hay and Meier, 1974) that there is a critical time in early development when the spinal cord and notochord interact with somites in a developmentally significant manner.

This hypothesis may have significance for studies of muscular dystrophy in the chicken. In this inherited disease, it is mainly white fast-twitch glycolytic muscle fibers that are affected (Asmundson and Julian, 1956; Cosmos, 1966, 1970; Cosmos and Butler, 1967). The pectoralis major is the largest muscle to be affected. Although the muscles do not degenerate until after hatching, muscles from 14-day old in ovo dystrophic embryos cannot be rescued from their fate if they are transplanted into a normal environment (Cosmos, 1970). However, recent evidence indicates that the genotype of the embryonic spinal cord may affect the phenotype of the muscle, suggesting that at some period early in development, there is an abnormal interaction between spinal cord and somites in dystrophic embryos (Vetrano, 1974; Rathbone, Stewart and Vetrano, 1975; Rathbone and Stewart, 1978). It is this observation that provided the stimulus for the present study. If the primordium of the pectoralis major muscle could be precisely defined at two days in ovo (stages 12-14, Hamburger and Hamilton, 1951) the environmental factors that influence its development in both normal and dystrophic embryos might be experimentally manipulated.

In this study, I have shown that the pectoralis major muscle is derived from the dorsal halves of somites 16-21 of the stage 12-14 chick embryo.

These are also the somites that give rise to all other wing and wing-associated muscles. My results also indicate that the migration of myogenic cells of the somites into the lateral mesoderm occurs between 2 and 2.5 days *in ovo*; thus, myogenic cells are present in the lateral mesoderm from a very early stage of development. These results may explain and resolve the controversy in the literature regarding the embryonic origin of skeletal muscles in the chick.

MATERIALS AND METHODS

MATERIALS

Chemicals and Solutions

Standard laboratory chemicals were purchased from the Fisher Scientific Company, Toronto, unless otherwise indicated, and were of "certified" or "reagent" grade.

Hanks' balanced salt solution (Hanks and Wallace, 1949), hereafter referred to as HBSS, was prepared in the laboratory from its constituents (Table 1).

Trypsin was obtained from the Grand Island Biological Company (GIBCO). A 0.25% (w/v) trypsin solution was prepared in calcium- and magnesium-free HBSS obtained from GIBCO. It was brought to pH 7.2 with HCl, sterilized by filtration, and stored at -20°C in 5 ml portions. The trypsin inhibitor solution consisted of complete HBSS plus 5% fetal calf serum. It was brought to pH 7.2, sterilized by filtration, and stored at -20°C in 5 ml portions.

Eggs

Fertile white Leghorn chicken eggs were obtained from the Martindale Hatchery, Caledonia, Ontario.

Adult quail obtained from College Pets, Toronto were used to establish a local flock from which fertile eggs were collected daily. Quail eggs obtained from the University of British Columbia were hatched and the quail were raised and included in the local flock.

All fertile eggs were stored at 12°C until incubated.

Ink

India ink was layered beneath the host embryos to provide a black background against which the embryos could be clearly seen (this idea was provided by N. Le Douarin, personal communication). Not all brands of India ink are suitable for this purpose. Most of them are toxic to the embryo. Carbon (lamp black) or non-toxic paint powder mixed in HBSS did not stay

TABLE 1

HANKS' BALANCED SALT SOLUTION, 10 x FINAL CONCENTRATION

STOCK SOLUTIONS

Solution 1	NaCl	40 gm
	KCl	2 gm
	$Na_2HPO_4 \cdot 7H_2O$	0.45 gm
	K_2HPO_4	0.3 gm
	H_2O	500 ml
	Sterilize by autoclave	
Solution 2	glucose	5 gm
	H_2O	500 ml
	Sterilize by autoclave	
Solution 3	$NaHCO_3$	3.5 gm
	H_2O	100 ml
	Sterilize by filtering	
Solution 4	$MgSO_4 \cdot 7H_2O$	1.0 gm
	$CaCl_2$ (anhydrous)	0.2 gm
	Sterilize by autoclave	
Solution 5	Double distilled H_2O	2000 ml
	Sterilize by autoclave	

TO MAKE UP COMPLETE HBSS

Mix the following:-

Solution 1	200 ml
Solution 2	200 ml
Solution 3	7 ml
Solution 4	200 ml
Solution 5	1393 ml

STORE IN 50 ml PORTIONS

beneath the embryo but sank through the yolk. The best results were obtained with Pelikan (Gunther Wagner, Hanover, Germany), after bringing the pH to 7.2 with concentrated HCl. The beaker containing this ink was placed in boiling water for 20 minutes to sterilize the ink. The ink was divided into 2 ml portions. It was diluted approximately 1 to 1 (v:v) with HBSS before use.

Microscalpels

Two types of microscalpels were used. One type was made from an ordinary sewing needle. Opposite sides of the sharp end of the needle were ground flat for a length of approximately half a centimeter. The tip of this region was ground with a fine whetstone to form a small blade approximately 1 mm long, 100 microns wide and sharpened along one side.

The other type was made from strips of razor blade material. Wedge-shaped blades were cut from the sharp edge of the material with a wire cutter or scissors. The tips were approximately 100 - 130 microns wide.

In both cases, the blades were inserted into needle holders for use. Both types of microscalpels were satisfactory.

METHODS

Tissue Transplants

Chicken and quail eggs were incubated at 37°C until they reached the desired stage of development. Quail and chick embryos were staged according to the morphological criteria established for chick embryos by Hamburger and Hamilton (1951). Chick embryos usually reached stage 13-14 after 48 hours of incubation and stage 17-18 after 60 hours. Quail embryos hatch after 16 days of incubation and thus develop faster than chick embryos, which take 21 days to reach hatching. Therefore, quail eggs were placed in the incubator several hours after the chicken eggs, so that quail and chick embryos reached the desired stage of development at the same time.

Chicken eggs were incubated on their sides. Since the embryos are less dense than the yolk, they float to the most dorsal region of the yolk and are thus readily accessible. Before the operations were performed, the developmental stages of the chick embryos were determined. Under sterile conditions, a small hole was made in the pointed end of the egg and 1 ml of albumen was removed with a syringe. This caused the yolk to drop to a lower position in the egg, which increased the amount of space between the embryo and the overlying shell. Thus, the shell could be cut away without injuring the embryo. A pair of dissecting scissors was used to cut a hole approximately 1 cm in diameter in the shell overlying the embryo. Approximately 0.1 ml of India ink was injected beneath the embryo with a finely drawn glass pipet. The number of pairs of somites was counted, and recorded in pencil on the shell of the egg. The hole was covered with cellophane tape and the egg was replaced in the incubator. This procedure was repeated with all the chicken eggs. When the stage of development of the donor quail embryos was determined, a host of appropriate age could be selected from the pre-staged chick embryos. Every attempt was made to match a donor with a host that had precisely the same number of somites. In practice, however, this was not always possible. In approximately 90% of the chimaeras made at stage 12-14, the host was within two pairs of somites of the stage of the donor. For example, if the donor had 18 pairs of somites, the host chosen for it had at least 16 and no more than 20 pairs of somites. In the remaining 10% of the chimaeras, the host was within four pairs of somites of the stage of the donor. In all chimaeras made at stages 17-18, the donor and host were both at stage 17 or both at stage 18.

In all experiments, grafts were made orthotopically, i.e. the donor tissue was from the same level as the host tissue it replaced. In all cases, the grafts were taken from the right side of the donor embryo and grafted to the right side of the host embryo.

Somite Transplants

The method of somite transplants is represented diagrammatically in Figure 2 (page 22). When donor and host embryos had fewer than 21 pairs of somites, the positions of the prospective unformed somites were estimated. Obviously, in such chimaeras the exact number of somites or prospective somites transplanted could not be known at the time the operation was performed.

Preparation of Donor

At stage 12-13 (15-21 pairs of somites), the donor embryo was removed from the egg by cutting around it with a pair of scissors. It was placed in a dish of HBSS with a pair of fine forceps, briefly agitated to remove adhering yolk, and flattened on the bottom of the dish by removing all but a thin film of HBSS. The number of somite pairs was counted, and a portion of the embryo containing the somites to be transplanted was cut from the embryo with an etched tungsten needle. This tissue was placed in 0.25% trypsin at room temperature for approximately 10 minutes. Then it was transferred to trypsin inhibitor, where the somites (and/or unsegmented somitic mesoderm) were cleared of adhering tissue with fine forceps and a tungsten needle. Extraneous tissue was readily removed except for the tissue between adjacent somites; therefore, chains of clean somites were transplanted in one piece. Intermediate mesoderm (tissue which lies between the somites and the lateral mesoderm and will give rise to the excretory system) was left on the donor tissue whenever possible to identify the distal side.

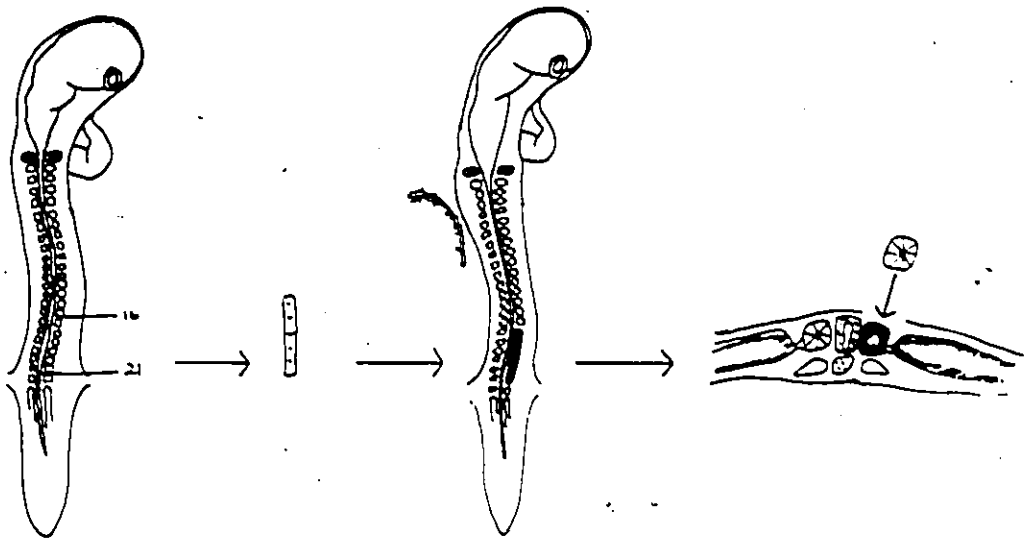
Occasionally, if the somites seemed particularly fragile, they were not thoroughly cleaned of adhering ectoderm. This practice produced no ill effects in the chimaeras unless the somites were inadvertently grafted

Figure 2

Diagrammatic representation of the procedure for transplanting the brachial somites. This operation is performed on embryos that have between 15 and 22 pairs of somites. If the embryos have less than 21 somites, the positions of the unformed brachial somites within the unsegmented somitic mesoderm are estimated. Donors and hosts are closely matched with respect to developmental age in this and all other operations performed throughout this study.

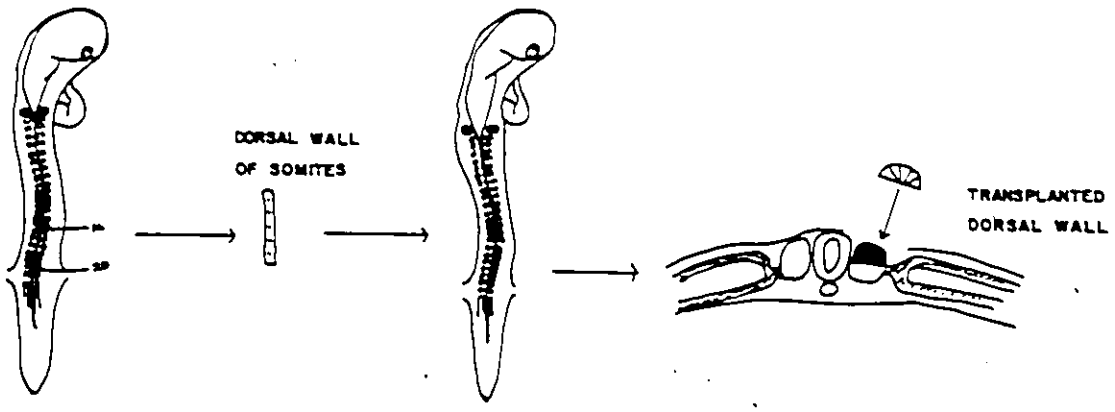
Figure 3

Diagrammatic representation of the procedure for transplanting the dorsal halves of the brachial somites. This operation is performed on embryos that have between 20 and 22 pairs of somites.



DONOR HOST CHIMAERA

2



QUAIL
DONOR

CHICK
HOST

CHIMAERA
CROSS SECTION

3

upside down or on their sides. In such cases, donor ectoderm formed a hollow pocket in the shoulder region of the chimaera. Migration of cells from the donor somites was reduced in some of these chimaeras. In all transplants, donor somites were cleaned of all traces of neural tube and notochord.

Preparation of Host

The host was selected from previously staged eggs. The host egg was re-opened and one drop of HBSS was dropped gently on to the embryo. This caused the vitelline membrane to float well above the embryo so that it could be cut without damaging the embryo. The somites to be removed were cut out with a microscalpel and teased away with a tungsten needle. Visible remnants of somites left on the endoderm or neural tube were removed by suction through a finely drawn glass pipet (tip size approximately 100 microns).

The donor tissue was transferred to the host with a drawn glass pipet (tip size 300-400 microns). It was manoevered into place in the host with a tungsten needle. No devices were needed to keep it in place. The egg was once again sealed with cellophane tape and placed back in the incubator to develop further.

Transplants of Somite Dorsal Wall

This procedure is represented diagrammatically in Figure 3 (page 22).

Preparation of Host

At stage 12-13, the vitelline membrane of the host was cut as before. A microscalpel was used to cut through the somites at the dorsomedial and dorsolateral aspects. The dorsal walls of the somites were removed by suction.

Preparation of Donor

The donor was removed from the egg and placed in a dish of HBSS. It was flattened in the dish with its ventral side facing upward. A suction pipet was used to remove the endoderm over somites 16-20 and the ventral walls of these somites. A tungsten needle was used to separate the dorsal walls from the surrounding tissue. The entire length of tissue containing the dorsal walls of the somites remained in one piece because it was transplanted with its overlying ectoderm. It was transferred to the host with a pipet, turned dorsal side up, and manoevered into place with a tungsten needle.

Dermomyotome Transplants

This procedure is represented diagrammatically in Figure 4.

Preparation of Host

At stage 17-18, the dorsal aspects of somites 16-20 (which included the dermomyotomes of these somites and undoubtedly a few cells of the sclerotome) of the host were removed by cutting through the dorsomedial and lateral walls of the somites and removing the separated tissue with a suction pipet.

Preparation of Donor

The donor embryo was removed from the egg and flattened in the dish with the dorsal side upward. As with the host, a microscalpel was used to make cuts in the dorsomedial and lateral walls of the somites. The tissue thus separated was gently teased loose with a tungsten needle and transferred to the host embryo with a pipet. It was manoevered into place with a needle.

Limb Bud Transplants

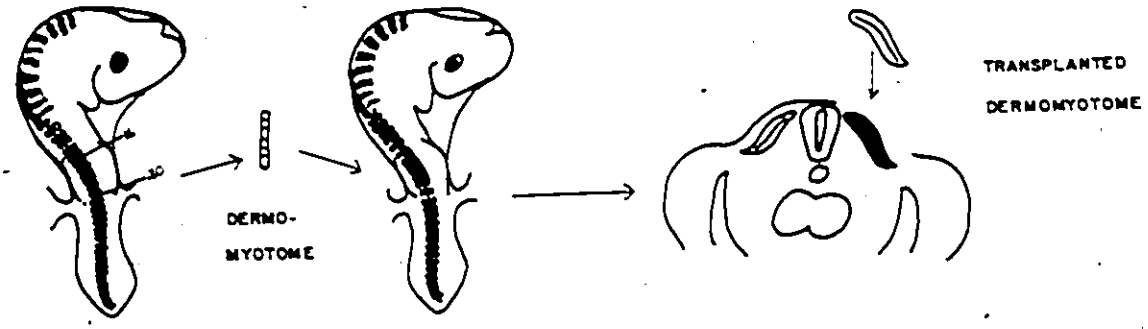
This procedure is represented diagrammatically in Figure 5.

Figure 4

Diagrammatic representation of the procedure for transplanting the dermomyotomes of the brachial somites. It is likely that small amounts of sclerotome are transplanted with the dermomyotome. This operation is performed on embryos of stages 17 - 18 that have between 28 and 36 pairs of somites.

Figure 5

Diagrammatic representation of the procedure for transplanting the wing bud. These experiments are performed on embryos at stages 17 - 18.



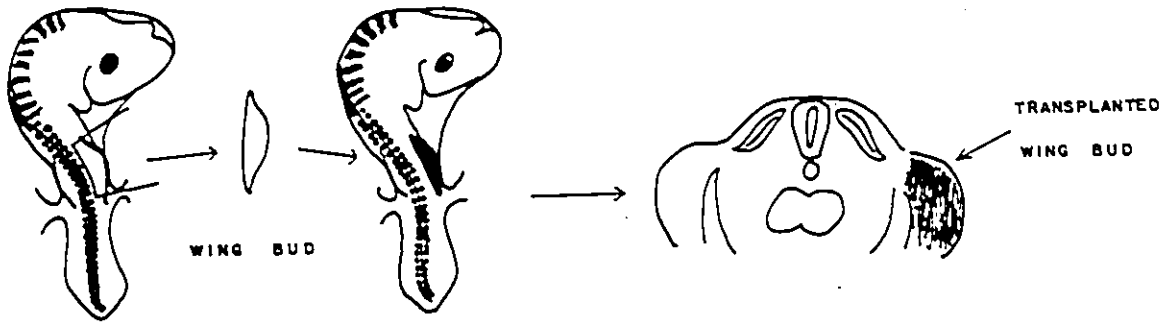
QUAIL DONOR

CHICK HOST

CHIMAERA

CROSS SECTION

4



QUAIL DONOR

CHICK HOST

CHIMAERA

CROSS SECTION

5

Preparation of Host

At stage 17-18 the right wing bud of the host embryo was removed by cutting along its base with a microscalpel. If necessary, remnants of the bud were removed by suction.

Preparation of Donor

The donor embryo was removed from the egg and flattened in a dish with the dorsal side upward. A tungsten needle was used to cut the wing bud away from the embryo and trim it to fit the host. It was transferred to the host with a pipet and manoevered into place with a needle. If necessary, it was held in place with a piece of shell.

Survival of chimaeras

Somite transplants are extremely traumatic for embryos. A total of 805 somite transplantation experiments were performed during the course of this investigation. Sixty-seven of these chimaeras have been used for the results of the present study. The remainder of the chimaeras died before they had developed to the desired stages, or contained morphological abnormalities due to improper healing of the grafted tissue. Most of the chimaeras died within two days after the operation.

In addition to somite transplants, 66 somite extirpation experiments were performed, of which two survived to 10 days in ovo. Ten somatopleure extirpation experiments were performed, of which one survived to 10 days in ovo.

In general, the survival rates for the other experimental procedures (grafts of somite dorsal walls or limb buds) were higher. Details are given in the appropriate places in Section 5.

Staining

At the desired stage of development, the embryo, now called a chimaera, was removed from the egg and rinsed in HBSS. The exact stage of development was determined according to the criteria of Hamburger and Hamilton (1951). Whole chimaeras were placed in Zenker's fixative for one to 24 hours, depending on the size of the embryo. In some embryos older than stage 34 (8 days of development) the pectoralis major muscles from both operated and unoperated sides were removed from the embryo and fixed and stained separately. Whole muscles were always removed using a dissecting microscope as a visual aid.

After fixation, the chimaeras or muscles were washed, dehydrated in a series of alcohols, cleared in xylene, and embedded in Paraplast (Sherwood Medical Industries, St. Louis, Mo.). Embedded tissues were sectioned in 5 or 6 micron sections on a Jung microtome and placed on glass slides. The staining procedure was that of Feulgen and Rossenbeck (1924) (see Table 2). The sections were counterstained in picro-indigo-carmine (2% indigo-carmine in saturated picric acid). Chick nuclei were stained pink; quail nuclei were pink with one or more large dark spots; the cytoplasm was stained light green (Figure 6).

Whole muscles were used in a quantitative determination of the quail contribution to the musculature.

Quantitative Determination of Quail Contribution to Musculature

Whole pectoralis major muscles from chimaeras of stage 35 or older were serially sectioned and stained as described above. Every twentieth section was designated to be counted. Each designated section was arbitrarily divided into 5 equal regions. In each region, one field containing a preponderance of muscle cells was chosen under high magnification. All the

TABLE 2

STAINING PROCEDURE TO DISTINGUISH CHICK FROM QUAIL TISSUE

(Feulgen - Rossenbeck Reaction)

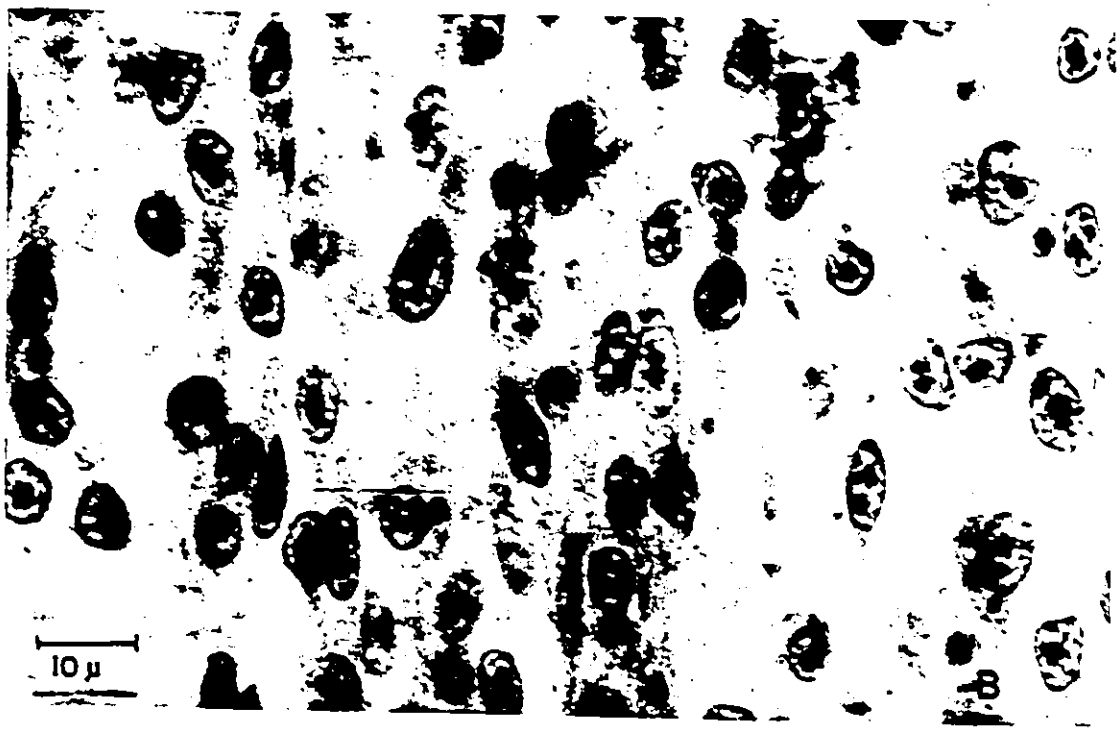
1.	Toluene	5 minutes
2.	Toluene	5 minutes
3.	Absolute Alcohol	5 minutes
4.	Collodion	3 minutes, then drain for a few seconds
5.	Formol-alcohol	5 minutes
6.	80% alcohol	5 minutes
7.	4% iodine in 70% alcohol	7 minutes
8.	5% sodium thiosulphate	3 minutes
9.	Running tap water	15 minutes
10.	1.0 Normal HCl at 60°C	5 minutes (8 minutes for formalin-fixed tissues)
11.	Running tap water	15 minutes
12.	Distilled water	5 minutes
13.	Schiff reagent	60-90 minutes
14.	1% $K_2S_2O_5$ in 1/10 N HCl	1 1/2 minutes
15.	1% $K_2S_2O_5$ in 1/10 N HCl	1 1/2 minutes
16.	1% $K_2S_2O_5$ in 1/10 N HCl	1 1/2 minutes
17.	Running tap water	15 minutes
18.	Distilled water	2 minutes
19.	Picro-indigo carmine	5 seconds
20.	Absolute alcohol	brief passage 5 minutes total if collodion was used
21.	Absolute alcohol	brief passage
22.	Toluene	5 minutes

All solutions were kept at room temperature except Schiff, which must be refrigerated, preferably in a dark brown bottle. All solutions were saved and re-used except sodium thiosulfate.

Figure 6

Nuclei of chick cells (A) and quail cells (B) in the pectoralis major muscles of stage 35 embryos that have been stained with the Feulgen-Rossenbeck procedure for DNA. The chromatin in the chick nuclei is more dispersed than that in quail nuclei, where the chromatin is condensed in one or more conspicuous masses.


10 µ
A


10 µ
B

muscle nuclei in this field were counted and the number recorded. Then all quail nuclei were counted and recorded. When this had been done in all five regions, the next designated section was counted in a similar manner. When all designated sections of one muscle had been counted, the numbers were added and the quail nuclei were represented as a proportion of all the muscle nuclei.

Four of the muscles had already been sectioned longitudinally rather than transversely at the time that the counting method was devised. These muscles were counted also, but each designated section was arbitrarily divided into nine equal regions, and one field in each region was counted. One additional field chosen at random in the section was also counted, making a total of ten counted fields in each longitudinal section.

Five of the muscles were counted a second time by another person to determine investigator error in counting. The final proportion of quail muscle to total muscle determined by two people varied by plus or minus 7%.

Another possible source of error in the quantitative determination is the reliability of the distinction between quail and chick nuclei. In cross-section or longitudinal sections of pectoralis muscles from 10-day unoperated quail embryos, approximately 5% of the nuclei did not show the characteristic condensations of chromatin seen in the other quail nuclei. In a chimaera, such nuclei could be mistaken for chick nuclei. Therefore, the percentage of quail nuclei in chimaera muscles given in section 2 of the Results may be 5% lower than the actual percentage.

RESULTS

SECTION 1.

SECTION 1

COMPARATIVE STUDY OF THE DEVELOPMENT OF SKELETAL MUSCLES IN CHICK AND QUAIL EMBRYOS

Introduction

This study was undertaken for two reasons: (1) to investigate the morphological changes that occur at wing and thorax levels during the early stages of muscle development, and (2) to determine whether quail and chick muscles develop in a similar way, an important consideration when quail and chick muscles develop together in a chimaera.

A total of 34 embryos (20 of chick and 14 of quail) between stages 13 and 35 were examined for this study.

Observations

CHICK

Stage 13 (2 days of incubation, 19 pairs of somites)

Somites form in a cranio-caudal sequence. At stage 13, the wing-level somites (roughly somites 15-21) are forming. In transverse section, these somites are seen to be spherical epithelial structures with a few cells in the center (Fig. 7A). The cells in the somite are undifferentiated, i.e., they all appear alike, with no evidence that muscle or cartilage has begun to form. Somites with this morphology are referred to as primary somites to distinguish them from the secondary somites into which they evolve (Christ, Jacob and Jacob, 1978b).

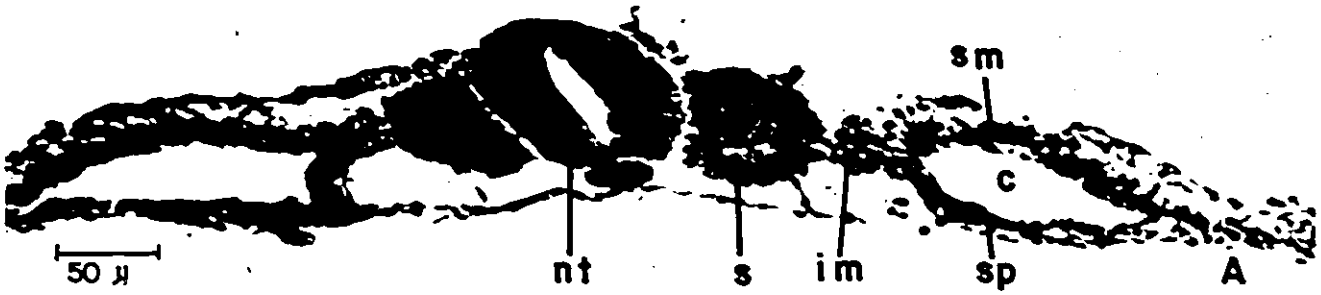
The lateral plate mesoderm consists of two layers: the somatopleuric mesoderm, which will form the body wall, and the splanchnopleuric mesoderm, which will form the visceral organs. These two layers are separated by a space, the coelom. The intermediate mesoderm, between the somite and lateral plate, is the primordium of the excretory system. The limbs have not yet appeared.

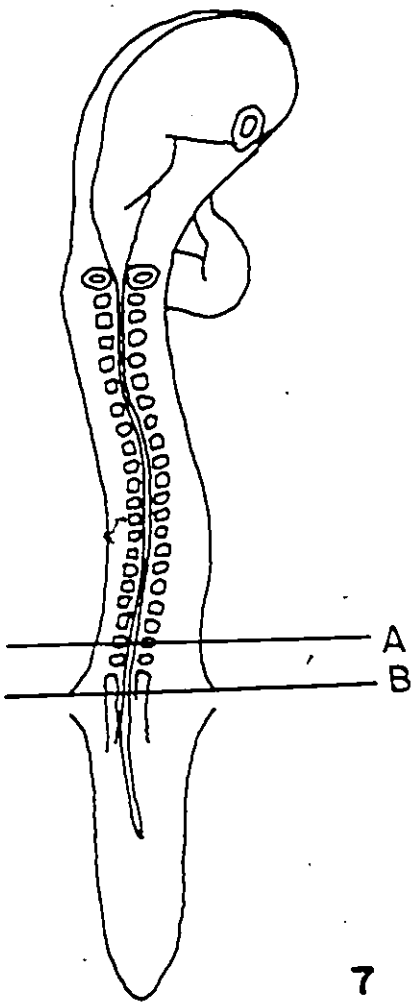
Figure 7 Stage 13 chick embryo (19 somites; 2 days)

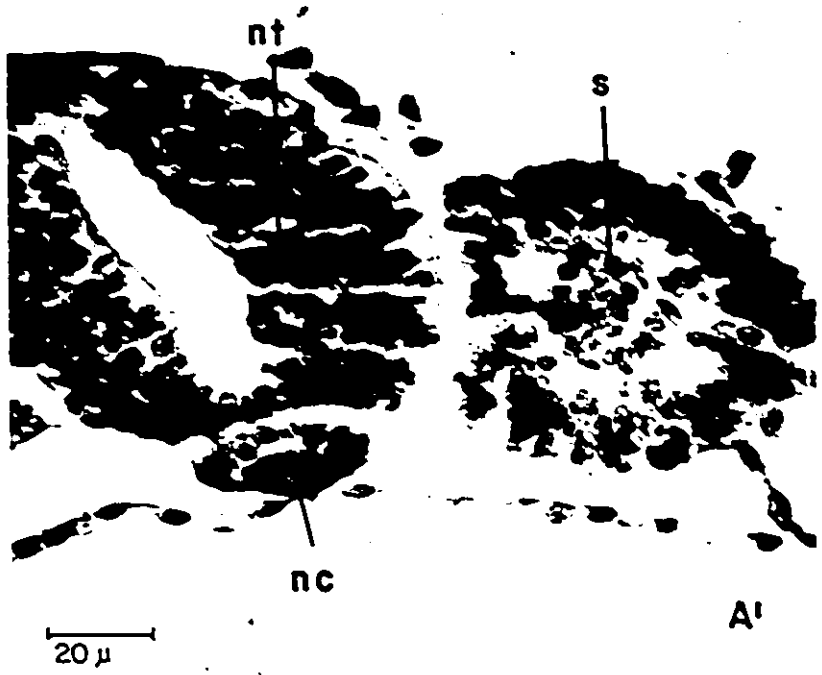
A. Cross-section at the brachial level. The primary somite is a spherical epithelial structure with a few cells in the center. The somatopleure and splanchnopleure are epithelial. nt: neural tube s: somite im: intermediate mesoderm sm: somatopleure sp: splanchnopleure c: coelom

A'. Detail of A. The apparent difference in staining intensity between the dorsal and ventral regions of the somite is an artefact caused by uneven thickness of the section. nt: neural tube nc: notochord s: somite

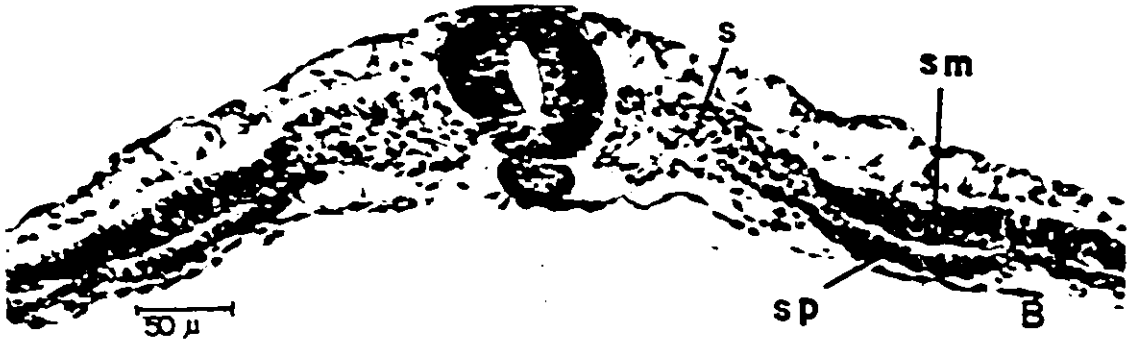
B. Cross-section through a more posterior level. The somitic mesoderm is in the form of mesenchyme. It is not yet organized into the epithelium of the primary somite. The somatopleure and splanchnopleure, however, are epithelial. s: somitic mesenchyme sm: somatopleure sp: splanchnopleure.







7



Caudal to somite 19, (Fig. 7B) the somitic mesoderm is unsegmented and is mesenchymal (loose and unorganized), while the two layers of the lateral plate are epithelial.

Stage 15 (2+ days; 25 somites) Figure 8

At the level of the wing somites, morphogenetic changes are occurring within the somites. The ventral wall is no longer epithelial but has become mesenchymal. The dorsal epithelium persists.

The somatopleuric mesoderm at this level has formed a fold, the first step in limb development.

Stage 17 (2.5 days; 29-32 pairs of somites) Figure 9

Wing and leg buds are visible as swellings in the somatopleure. The wing is located opposite somites 15-21. The leg is opposite somites 27-33.

In cross section at the level of the wing, it can be seen that the somites have acquired their secondary morphology. Each somite has differentiated such that it now consists of two dorsal epithelial layers, the dermatome and myotome, and a ventral region of mesenchyme called the sclerotome. The dermatome is destined to give rise to dermis, the myotome to muscle, and the sclerotome to cartilage. When stained with the Feulgen reaction, the myotome nuclei stain more lightly than other cells, and the cytoplasm of these cells is stained more intensely by the counterstain. This fact permits easy identification of the myotome layer in older stages. The two dorsal epithelial layers, collectively called the dermomyotome, extend to the medial edge of the wing.

The mesoderm of the wing bud is a mass of apparently undifferentiated mesenchyme. Although this mesenchyme will ultimately form both muscle and cartilage, there are, at this stage, no cells that are obviously myogenic (muscle-forming) or chondrogenic (cartilage-forming).

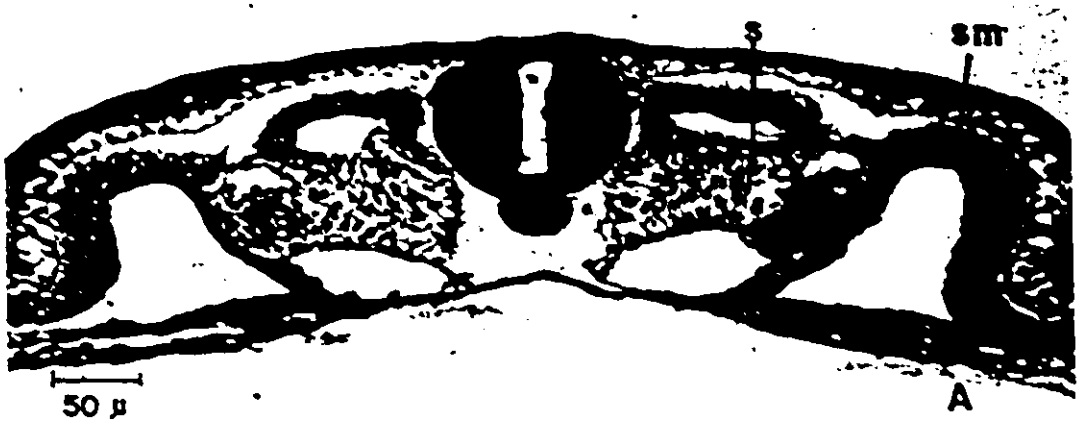
At thoracic levels the somites are similar to those at the wing level.

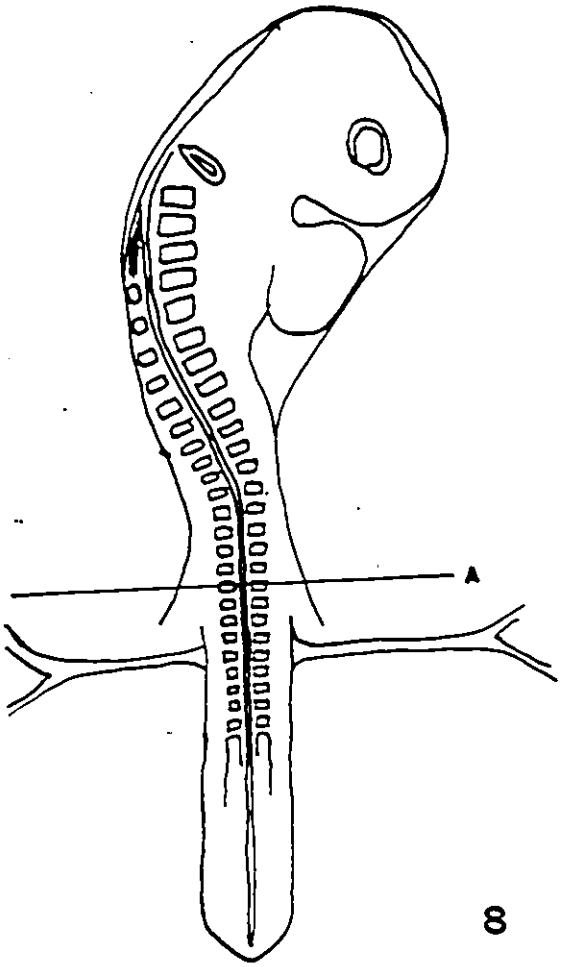
Figure 8 Stage 15 chick embryo (25 somites; 2 + days)

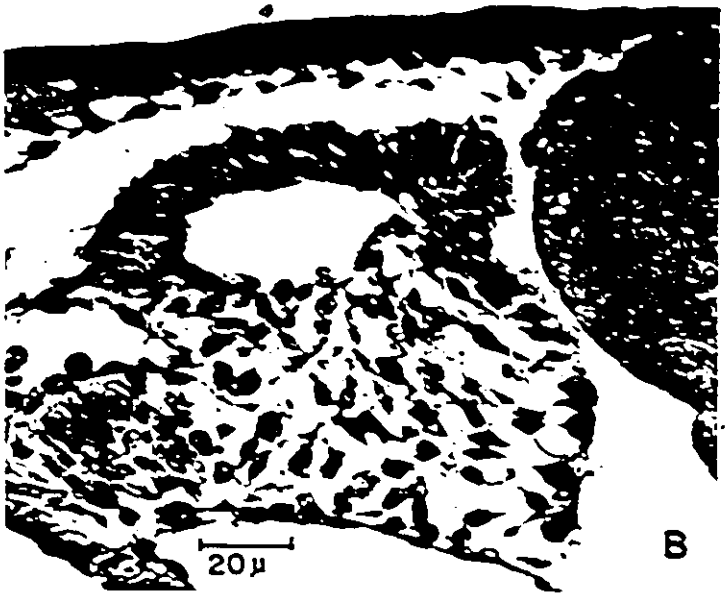
A. Cross-section at the brachial level. The ventral wall of the somite, which was epithelial at stage 13, has been replaced by mesenchyme. The somatopleure has become folded. s: somite; sm: somatopleure.

B. Detail of A. The dorsal epithelium persists.

s: somite k: developing kidney tubule.





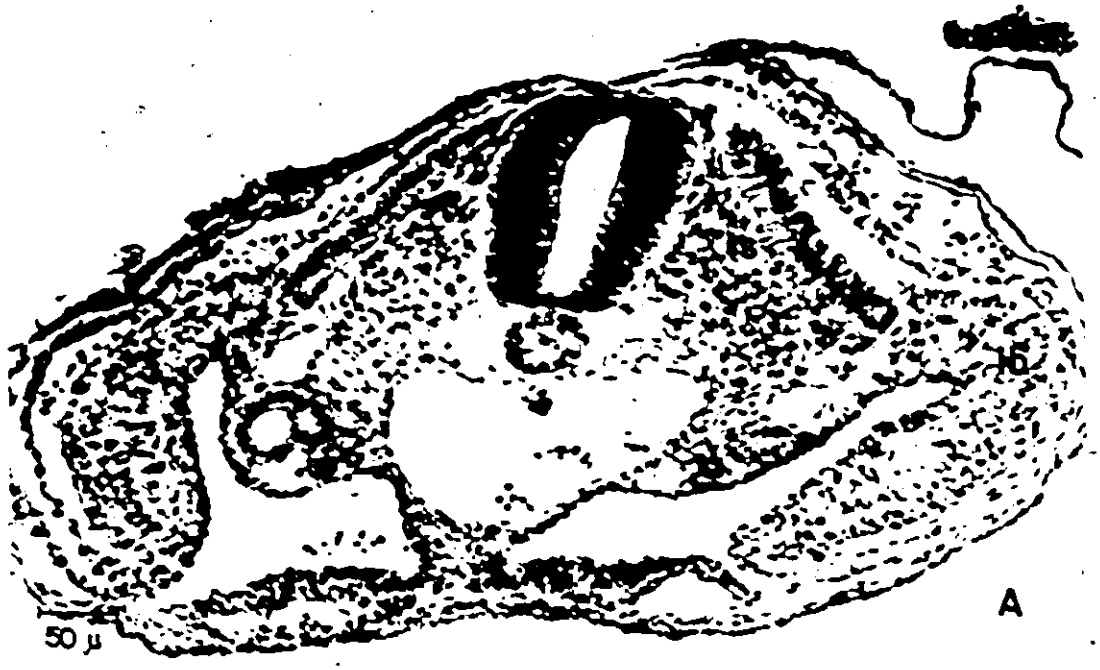


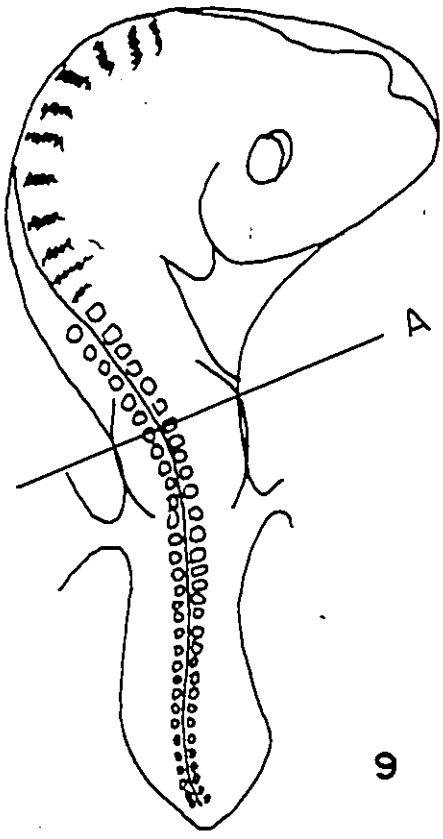
8

Figure 9 Stage 17 chick embryo (29 - 32 somites; 2.5 days)

A. Cross-section at the level of the wing bud. The somite is now in its secondary configuration and consists of three layers. s: somite; lb: limb bud.

B. Detail of A. D: dermatome; M: myotome; Sc: sclerotome The dermatome and myotome are epithelial; the sclerotome is mesenchymal.


50 µ
A


A


Sc
M
D
C
20 µ
B

Stage 19-20 (3 days; 37-43 somites) Figure 10

The dermomyotome has become more vertical than at stage 17, due to the expansion of the sclerotome dorsally between the dermomyotome and the neural tube. The limb buds are larger and are becoming vascularized.

Somites at thoracic levels are similar.

Stage 23 (3.5 - 4 days) Figure 11

At the level of the wing, the dermomyotome is epithelial only at its medial edge. More distally, the epithelium has disappeared. The myotome, recognizable due to its staining properties, does not extend into the limb.

In the limb, there is still no apparent differentiation of muscle or cartilage. However, the vasculature is confined to the peripheral areas of the limb, which are the future sites of muscle formation. The central core of the limb will form cartilage and does not appear to be vascularized.

At thoracic levels, the distal end of the myotome extends deeper into the body wall than it does at the wing level.

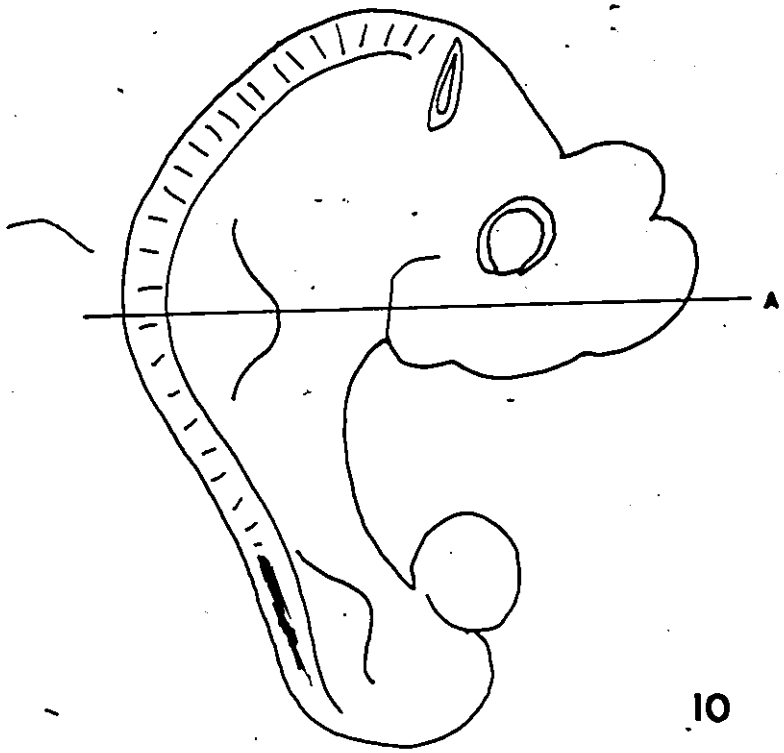
Stage 25 (4.5 days) Figure 12

In the limb, presumptive cartilage areas in the central core are represented by condensations of mesenchyme. Myogenic areas around the periphery are recognized by the fact that the cells are not as dense as in the chondrogenic areas. The myotomes of the somites are not continuous with the myogenic areas of the limbs. At this stage, there are no divisions of the limb myogenic areas into specific muscle primordia.

At thoracic levels, the myotomes of the somites extend into the body wall. Unlike the situation in the limb, thoracic myotomes are continuous with myogenic areas in the body wall.

Figure 10 Stage 20 chick embryo (3 days)

A. Cross-section at the level of the wing bud. The somite still has a dermatome, myotome and sclerotome, but the dermatome and myotome are more vertical due to the expansion of the sclerotome into the space between the dermomyotome and the neural tube. The limb buds are larger than at stage 17. Dm: dermomyotome; Sc: sclerotome; Bv: blood vessel



10

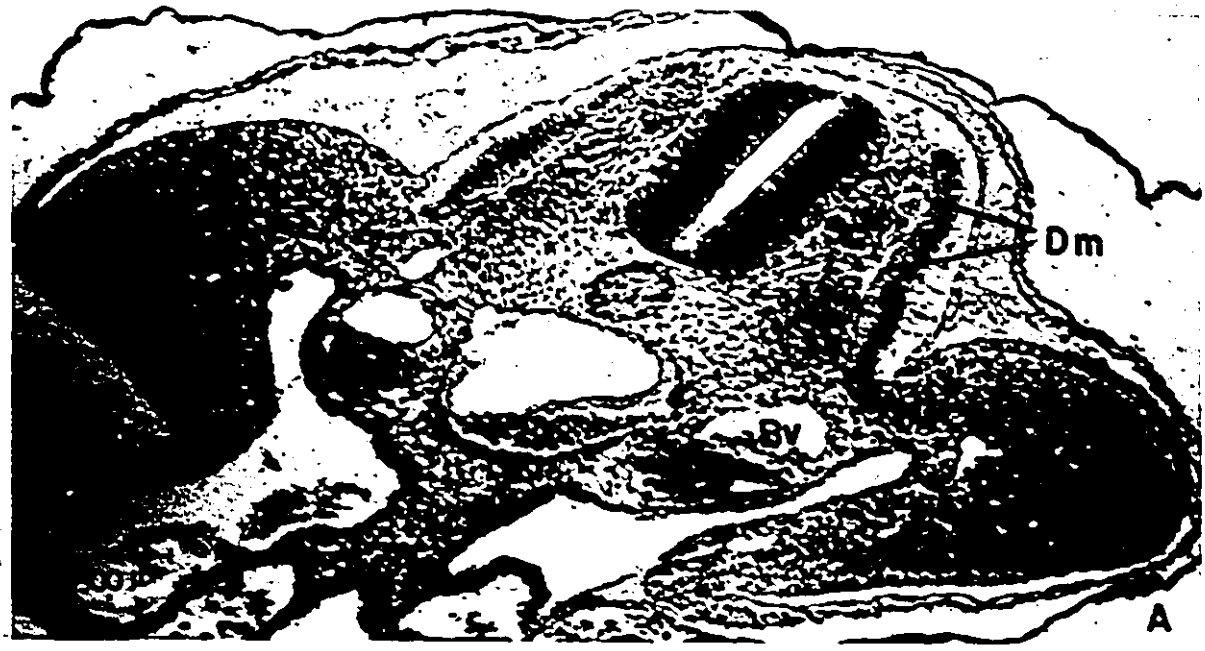
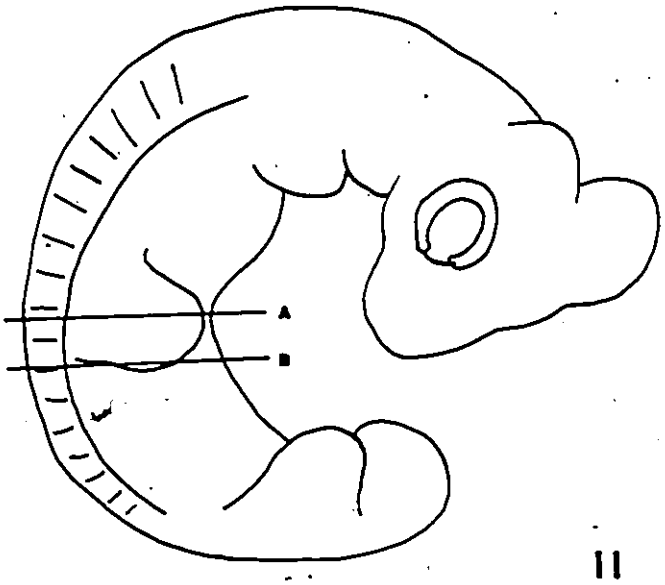


Figure 11 Stage 23 chick embryo (3.5 - 4 days)

A. Cross-section at the level of the wing. The myotomal layer of the somite is indicated by arrows. It is epithelial at the dorsal edge but becomes mesenchymal ventrally. It extends toward the wing but disperses before it reaches the limb mesoderm. N: a spinal nerve. S.G.: a spinal ganglion.

B. Cross-section through the thorax caudal to the wing. Here the myotome, indicated by arrows, extends from the somite into the body wall.



11

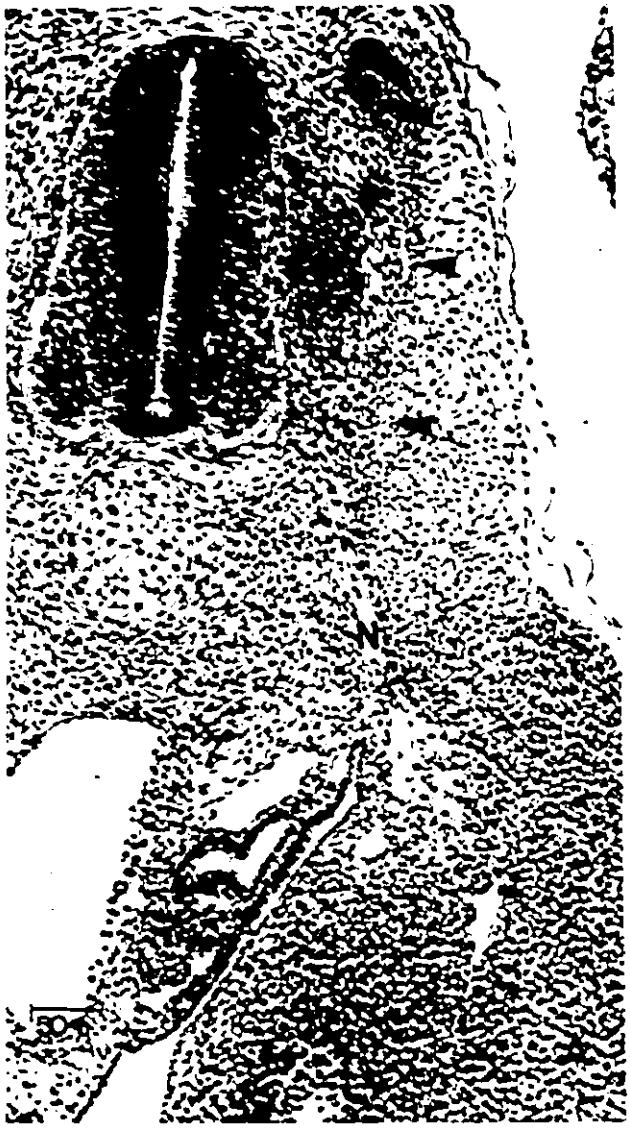
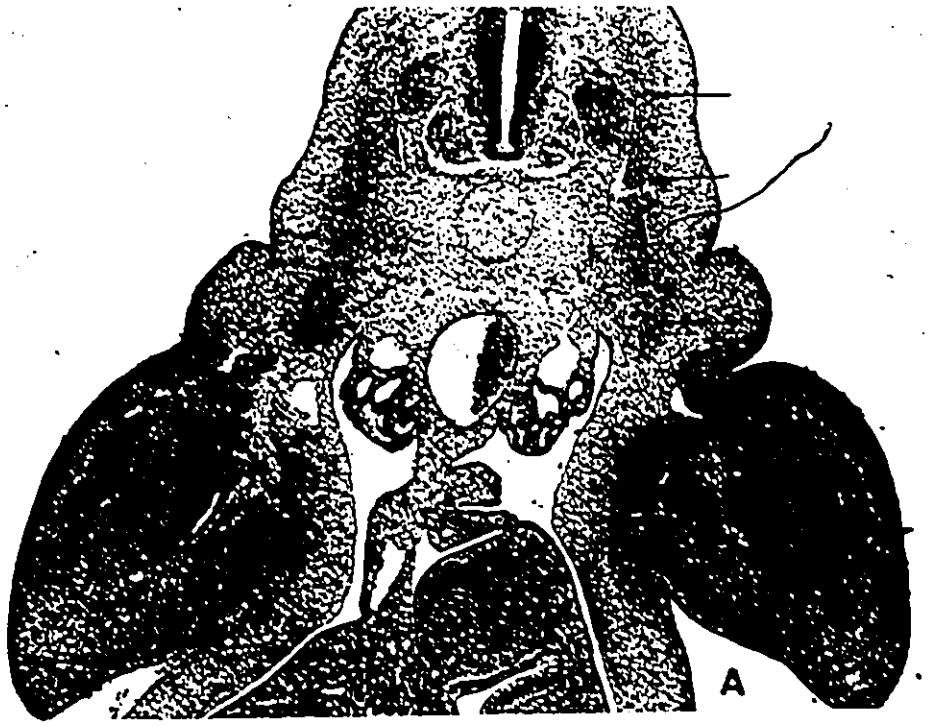
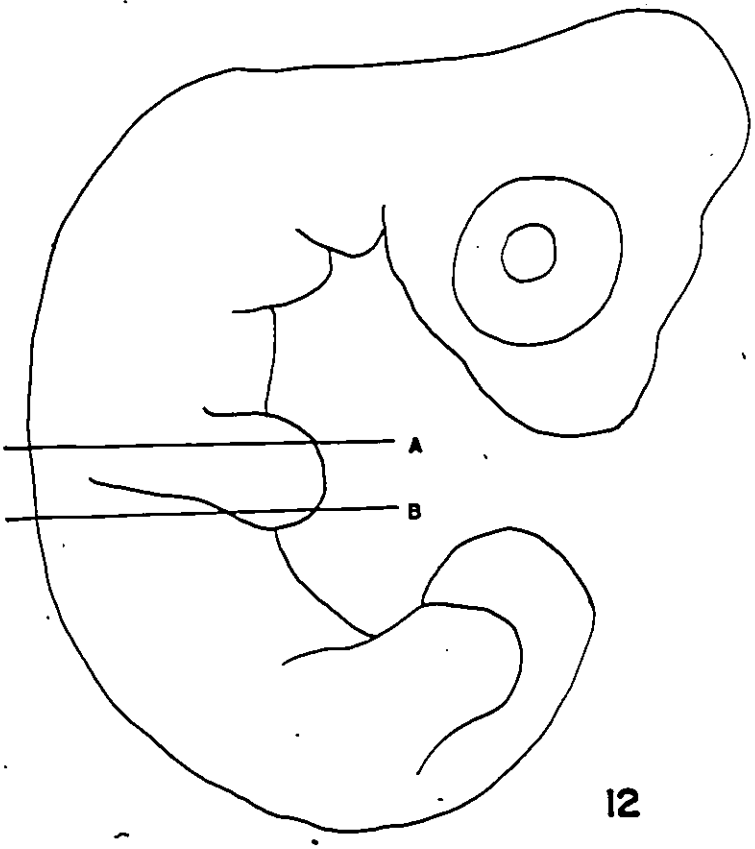


Figure 12 Stage 25 chick embryo (4.5 days)

A. Cross-section at the wing level. The myotome, indicated by arrows, disperses before it reaches the limb mesoderm. S.G.: spinal ganglion.

B. Cross-section through the thorax. The myotome, indicated by arrows, extends deeply into the body wall. S.G.: spinal ganglion.





12

Stage 27-28 (5 days) Figure 13

Development has advanced to the point where developing bones and muscles are distinguishable. The body wall bulges outward ventral to the wing, where the primordium of the pectoralis major muscle is recognizable. Myotubes are present in this primordium, which extends from the shoulder joint caudally to the level of the second true rib (formed by somite 22). Medial to the pectoralis in the body wall is the developing sternum. It arises as two cartilaginous primordia, one in the body wall on the left side of the embryo and one on the right side. At a later stage, the two halves will meet at the ventral midline and fuse.

Other wing-associated muscles are beginning to separate out of common primordia. The anterior latissimus dorsi (ALD) extends from a dorsal mass in the wing medially toward the spinal cord. Ultimately, it will attach to several thoracic vertebrae, but at this stage it has not yet reached as far as the scapula. The posterior latissimus dorsi (PLD) arises from the same mass and extends a short way caudally. The primordia of the biceps, triceps and deltoid muscles are becoming distinct but are not yet separate from one another.

Stage 29 (6 days) Figure 14

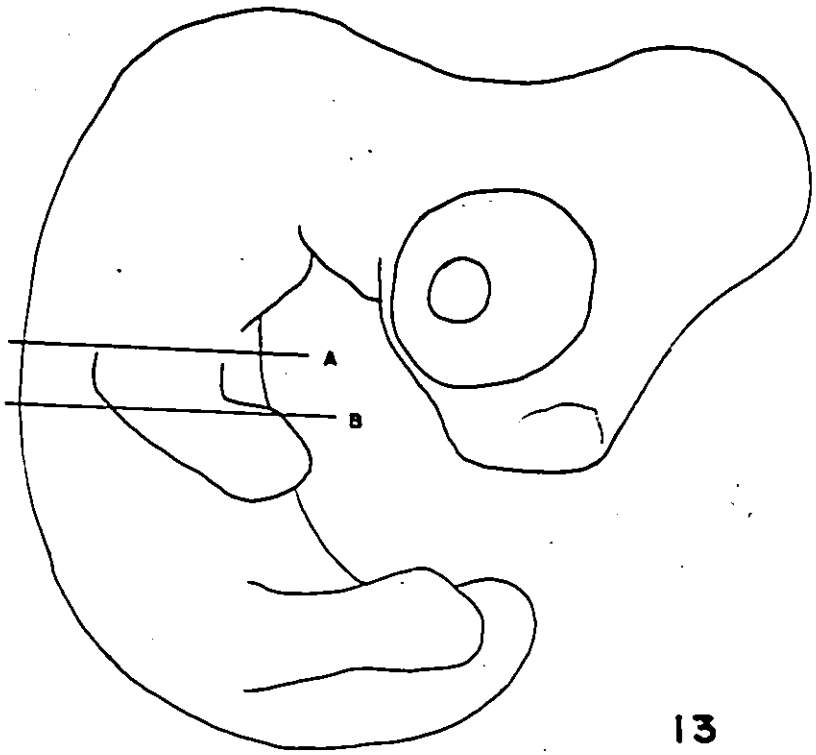
The primordia of the pectoralis major and minor are larger. There is more obvious cartilage in all skeletal structures. The ribs are evident and extend all the way to the sternum, which has moved ventrally. The

Figure 13 Stage 27 chick embryo (5 days)

A. Cross-section at the level of the shoulder. Cartilage and muscle are distinct. V: developing vertebra; H: humerus; S: scapula; C: coracoid; P: pectoralis major muscle; T: triceps muscle.

B. Cross-section through the thorax. The pectoralis extends from the wing (A) caudally into the thorax. P: pectoralis major; S: sternum; L: limb.

C. Higher magnification of pectoralis primordium. Myotubes are present in the primordium of the pectoralis major (arrows).



13

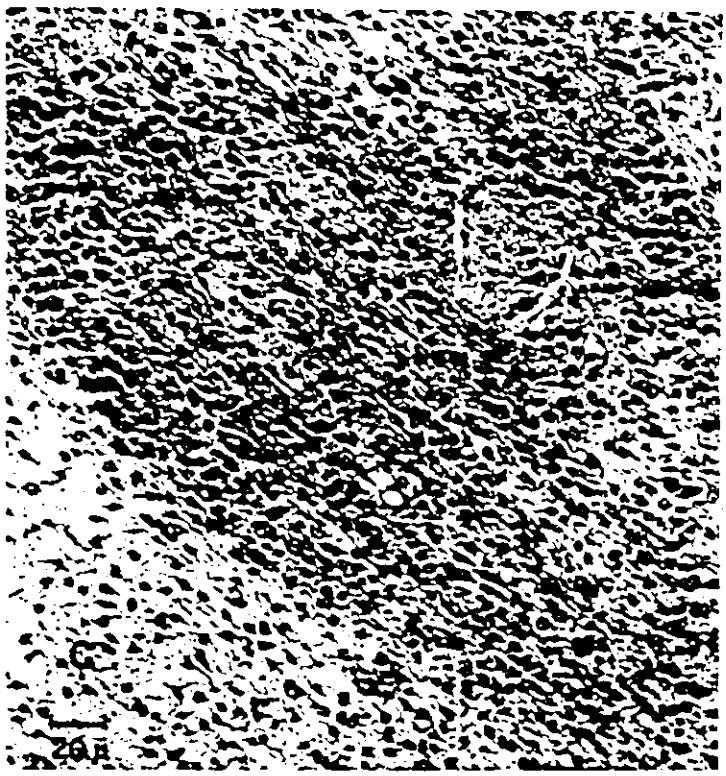
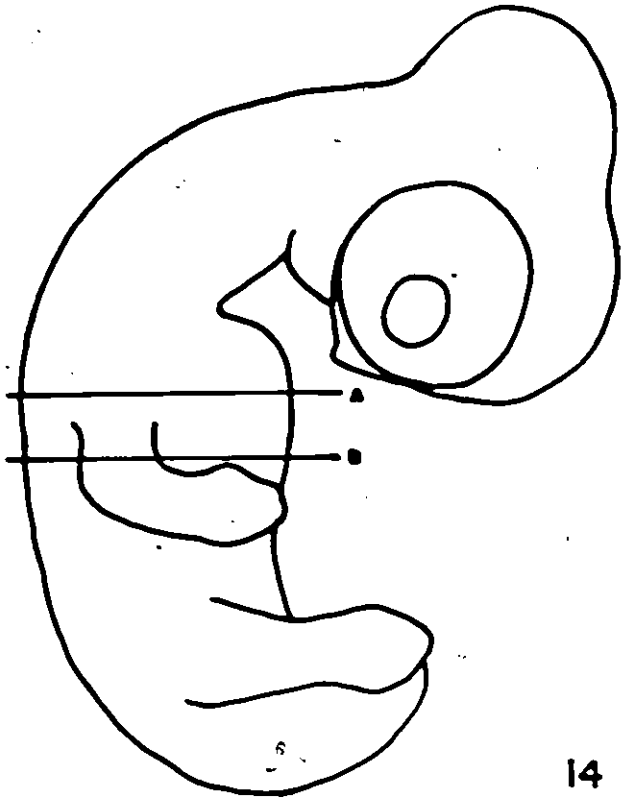


Figure 14 Stage 29 chick embryo (6 days)

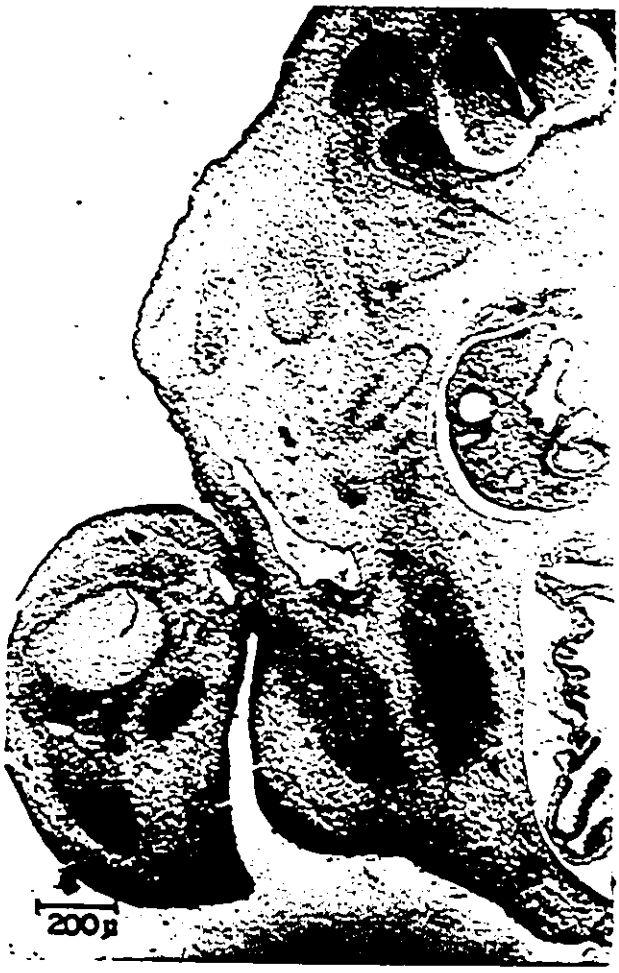
A. Cross-section at the level of the shoulder. P: pectoralis major; D: deltoid muscle; T: triceps muscles.

B. Cross-section through the thorax. The pectoralis is similar to that at stage 27 but it is larger. P: pectoralis S: sternum L: limb

C. Higher magnification of part of the pectoralis major. More myotubes are present than at stage 27.



14



pectoralis major extends caudally to the level of the third rib.

The ALD has extended further toward the spinal cord and ends at the level of the scapula. The PLD extends caudally to approximately the level of the first rib.

All muscles in the shoulder and upper wing are becoming more distinct and all of them clearly contain myotubes.

Stage 33 (7.5 - 8 days) Figure 15

In the pectoralis, there are many more myotubes present and more space between them. This muscle now extends caudally to the level of the abdomen. The ALD and PLD are both more elongated. The two heads of the biceps and the three heads of the triceps are distinct. The two halves of the sternum are much closer together in the anterior region but have not begun to fuse.

Stage 35 (9.5 days) Figure 16

The sternum has fused through most of its length. The abdominal walls have not closed entirely, but at wing and thoracic levels, all muscles and bones are in their final positions.

The pectoralis major muscle can be seen to consist of two parts separated by a connective tissue barrier. The pectoralis minor shows a similar division.

QUAIL

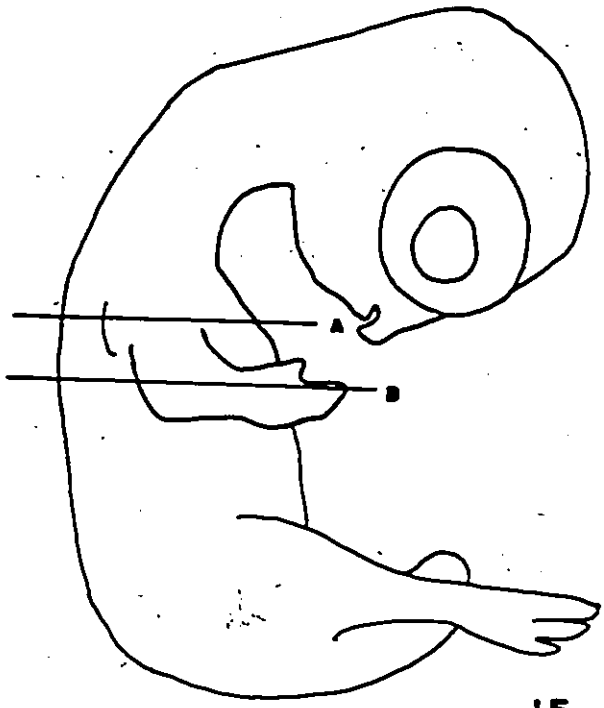
Development of muscle in the quail is similar to that in the chick. At stage 13 (Figure 17), the somites are spherical epithelial structures with a few cells in the center. The two layers of the lateral plate are epithelial. At stage 17 (Figure 18), the limbs are visible as accumulations of mesenchyme in the somatopleure. As in the chick, the wings arise at

Figure 15 Stage 33 chick embryo (7.5-8 days)

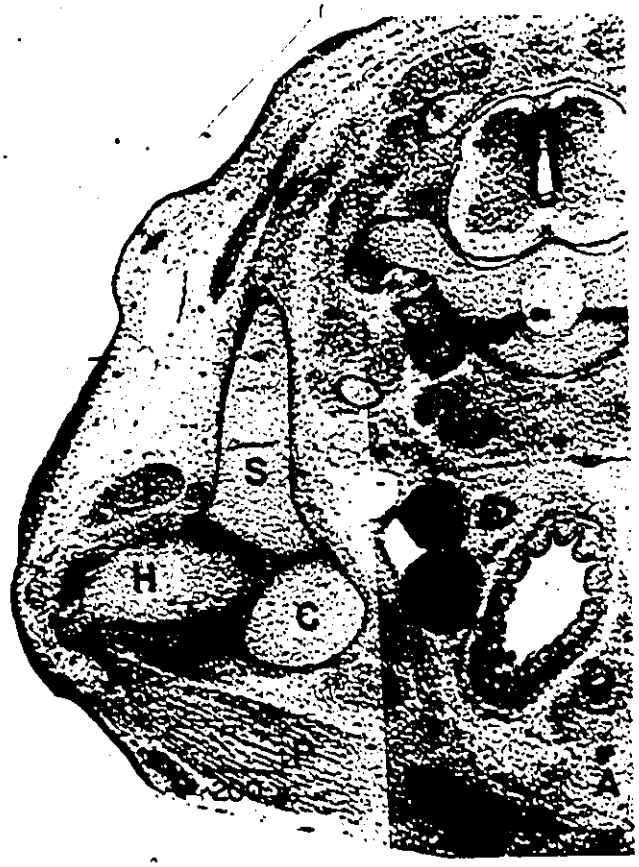
A. Cross-section at the level of the shoulder (composite). Although the sternum has not fused, the structures in this region are in their final positions. P: pectoralis T: triceps muscle S: scapula H: humerus C: coracoid

B. Cross-section through the thorax. The two halves of the sternum have not yet reached the ventral midline. The pectoralis extends caudally to the abdomen. P: pectoralis S: sternum R: ribs

C. Close-up of part of the pectoralis major muscle. There are larger spaces between the myotubes than at earlier stages.



15



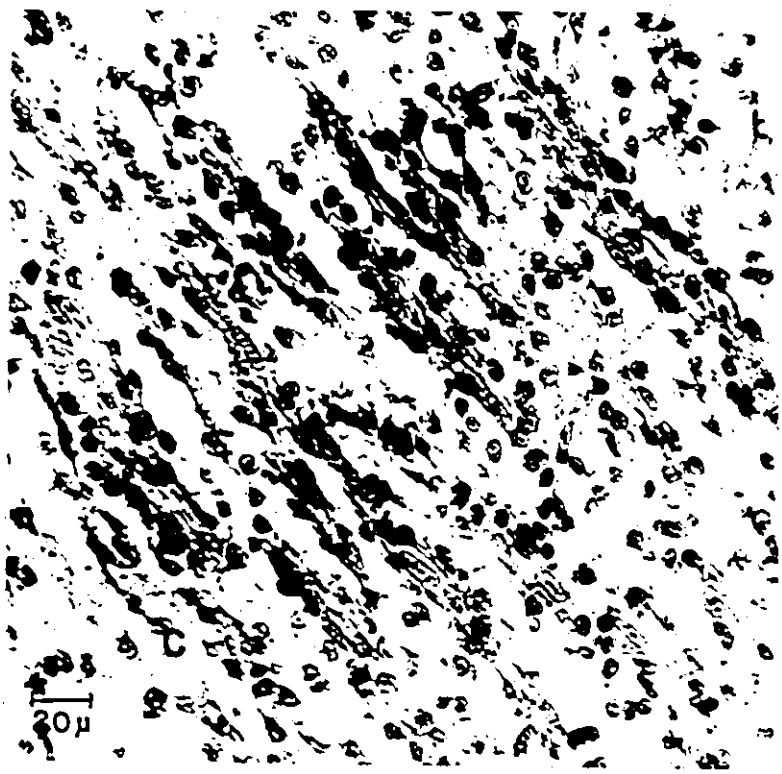
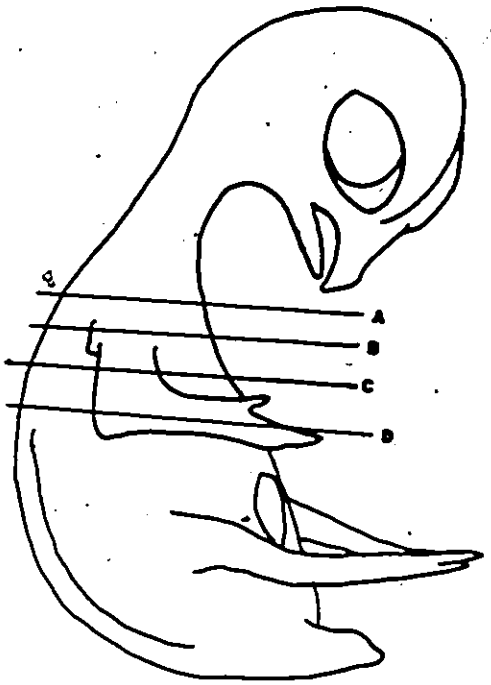


Figure 16 Stage 35 chick embryo.

All muscles and bones are in their final positions.

A. Cross-section at the level of the shoulder. (composite)

1. scapula
2. humerus
3. coracoid
4. clavicle
5. pectoralis major muscle (medial region)
6. deltoid muscle
7. coracobrachialis anterior
8. intervertebral muscles.



16

Figure 16

B. Cross-section at a level slightly caudal to A. (composite)

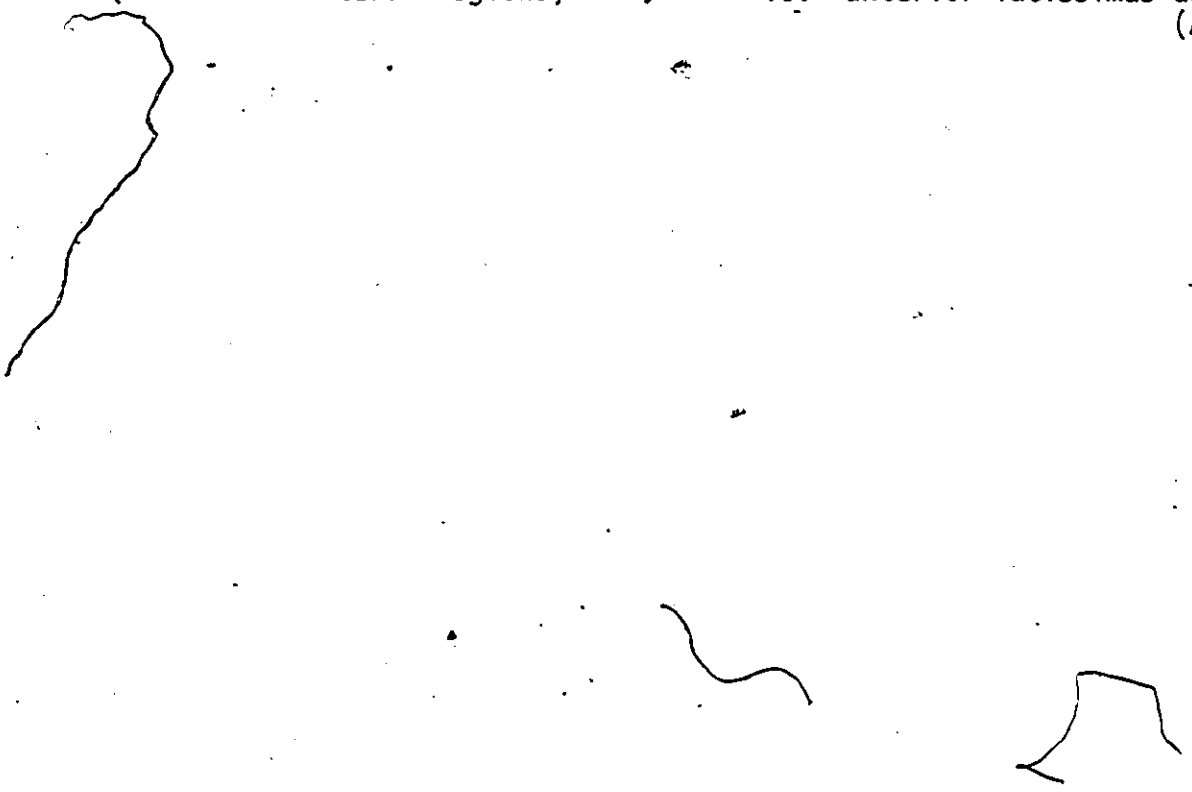
1. scapula
2. humerus
3. coracoid
4. pectoralis minor muscle
5. pectoralis major muscle (medial and lateral region)
6. coracobrachialis anterior muscle
7. deltoid muscle
8. triceps muscle

1
2
3
200μ

Figure 16

C. Cross-section through the upper thorax. (composite)

1. scapula
2. humerus
3. coracoid
4. sternum
5. pectoralis minor muscle (medial and lateral regions)
6. pectoralis major muscle (medial and lateral regions)
7. coracobrachialis posterior muscle
8. biceps muscle
9. triceps muscle
10. anterior latissimus dorsi muscle (ALD)



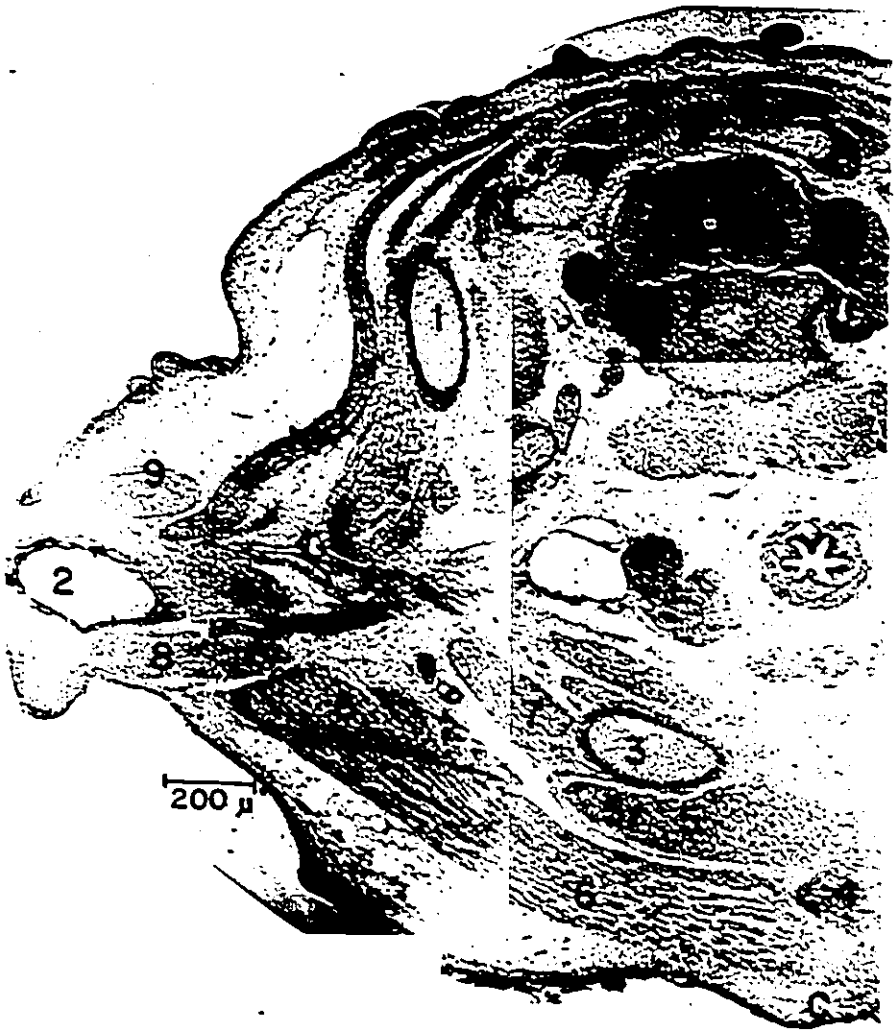
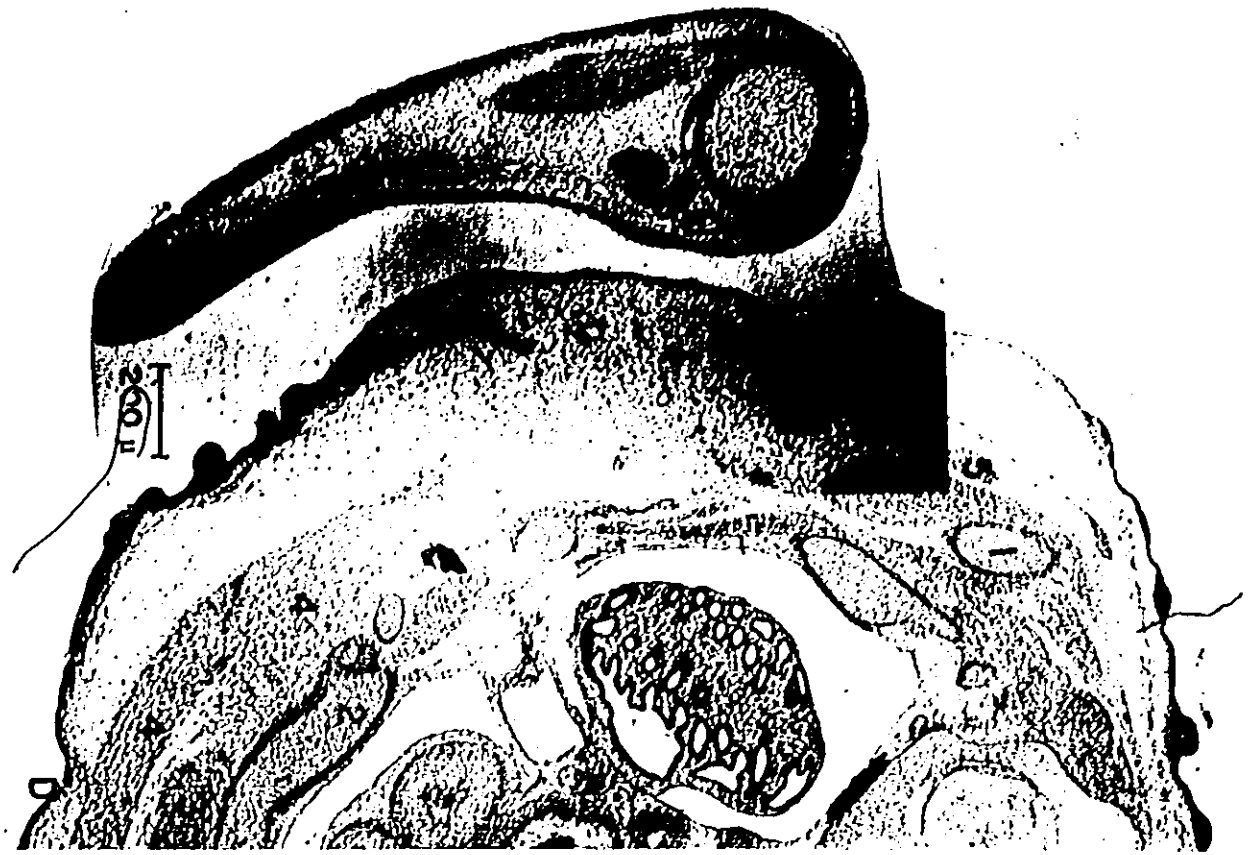

1
2
3
200 µ

Figure 16

D. Cross-section through the lower thorax. (composite)

1. scapula
2. sternum
3. pectoralis minor muscle
4. pectoralis major muscle (medial and lateral regions)
5. posterior latissimus dorsi muscle (PLD)



the level of somites 16-21 and the legs at somites 27 - 33. The dermomyotome of the stage 20 somite (Figure 19) is more vertical than at stage 17. No differentiation of muscle or cartilage is evident in stage 23 limbs (Figure 20). In the somites, the myotome does not extend into the limb; at thoracic levels, the myotome invades the body wall.

In later stages, the distinction between muscle and cartilage appears at the same stage as in the chick. Myotubes are not present in the primordium of the pectoralis at stage 26 (Figure 21), but they do appear by stage 28 (Figure 22). Morphogenetic movements resulting in growth and separation of distinct muscles are similar in chick and quail. By stage 35 in the quail, the two halves of the sternum have fused down the ventral midline, and the pectoralis clearly has two parts separated by connective tissue (Figure 23).

DISCUSSION

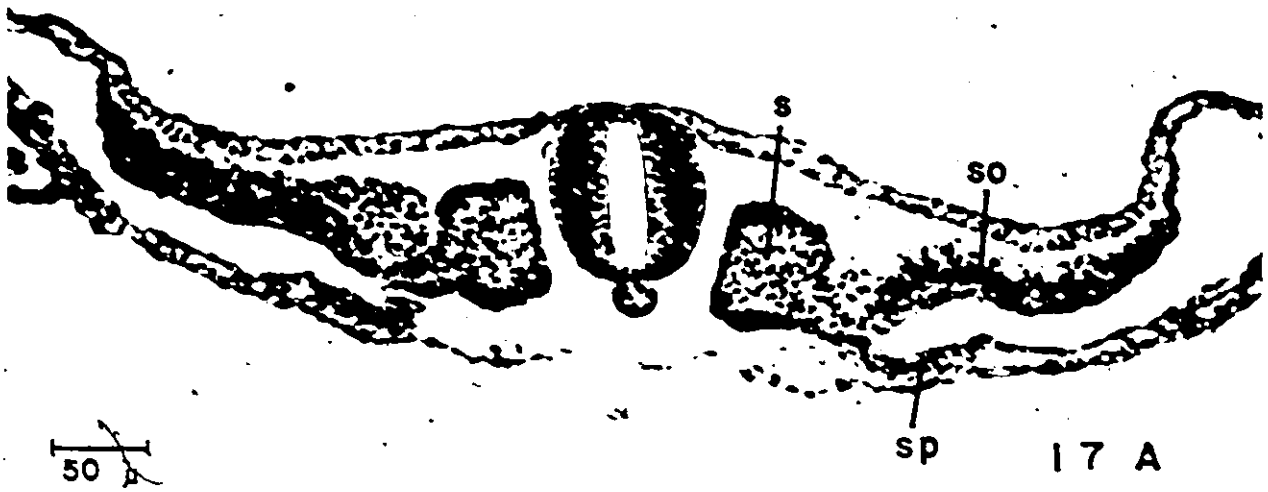
It is apparent from this study that somites are transient and dynamic structures. Within hours after a somite becomes structurally distinct, its morphology begins to change. The structured ventral epithelium is replaced by the loose mesenchyme of the sclerotome, which spreads around the neural tube to form the vertebrae. The dorsal epithelium, on the other hand, first acquires a more complex structure in that it becomes layered to form the dermomyotome. In later stages, this epithelium also disperses, but the cells of the myotome remain and differentiate *in situ* to form the intervertebral muscles.

These observations are consistent with those of previous investigators (Paterson, 1888; Williams, 1910) who observed that the myotome of the wing somites do not appear to invade the limb somatopleure and they are not continuous with

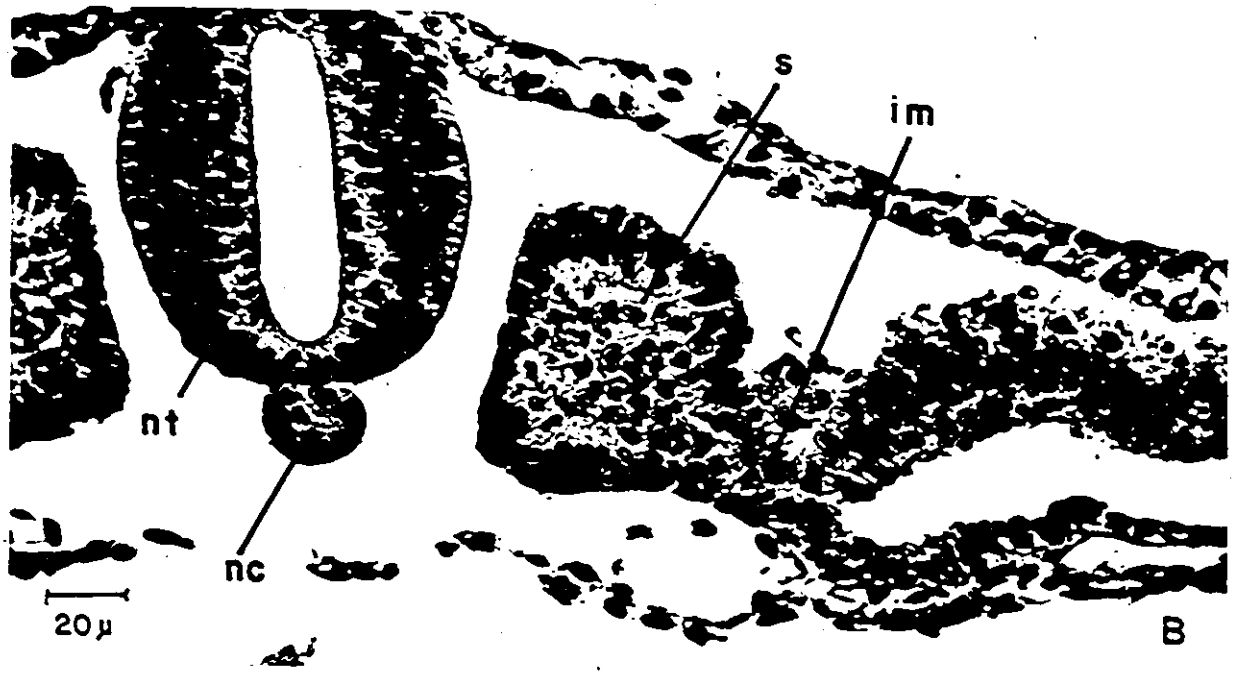
Figure 17

A. Cross-section through the brachial region of a stage 13 quail embryo. As in the stage 13 chick embryo, the primary somite is a spherical epithelial structure with a few cells in the center. The somatic and splanchnic mesoderm are also epithelial. s: somite so: somatopleure sp: splanchnopleure.

B. Detail of A. s: somite im: intermediate mesoderm nt: neural tube nc: notochord



17 A



B

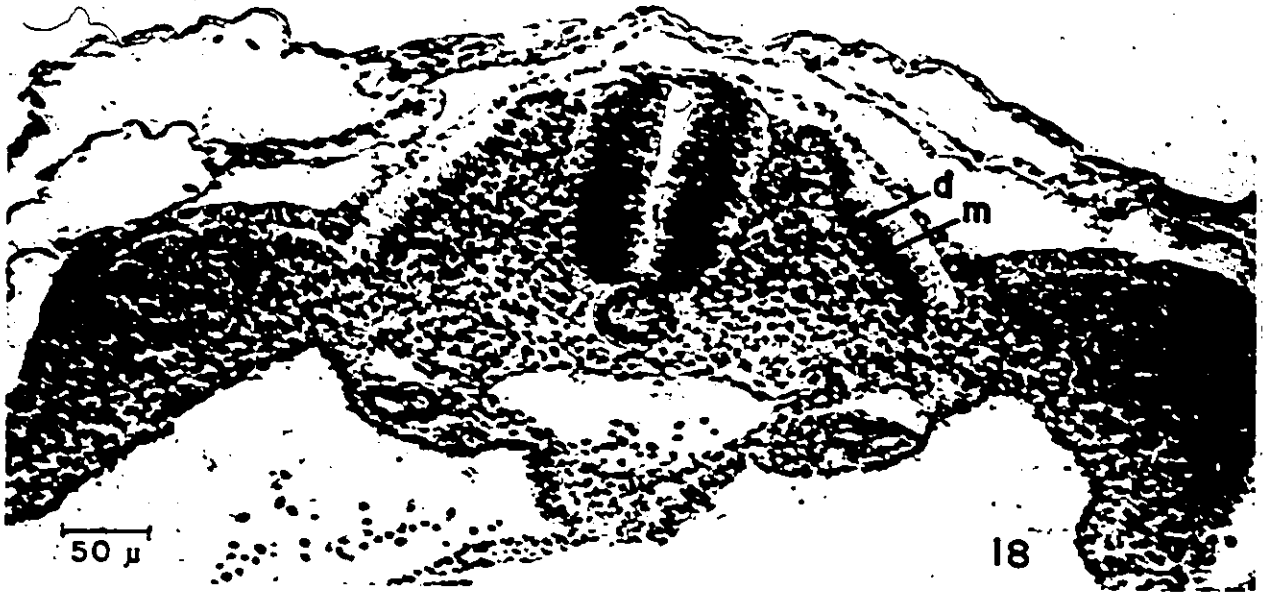
Figure 18

Cross-section through the level of the wing buds of a stage 17 quail embryo. The somites have taken on the secondary morphology, which consists of a dermatome, myotome and sclerotome.

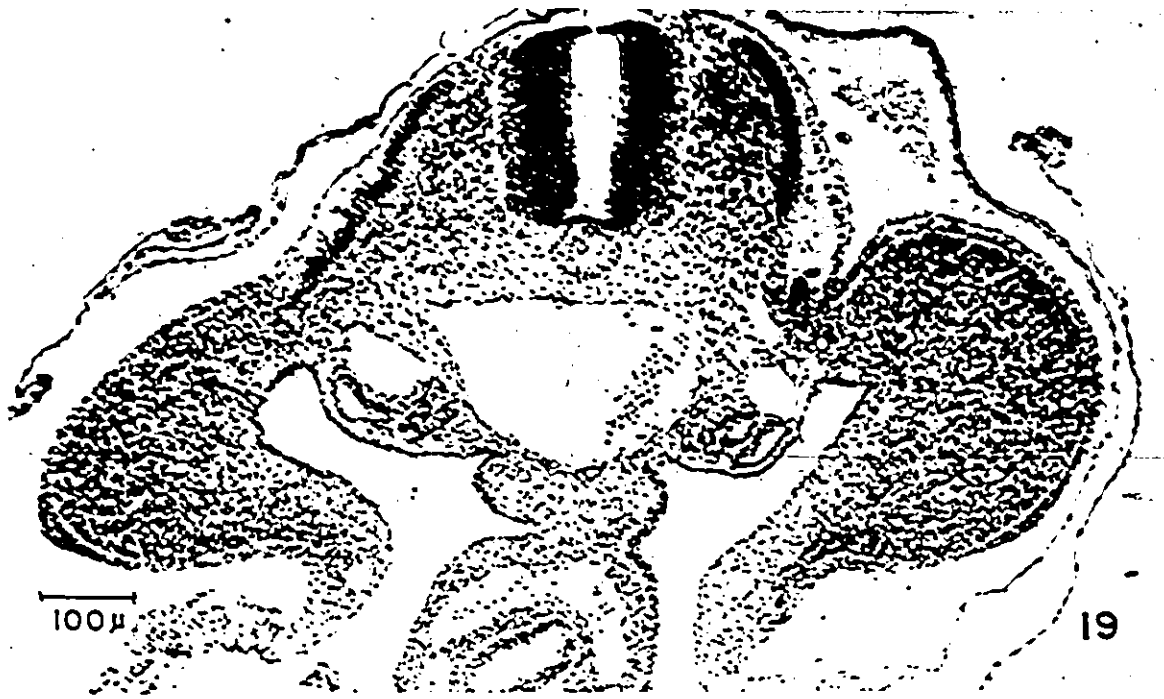
d: dermatome m: myotome s: sclerotome

Figure 19

Cross-section through the level of the wing buds of a stage 20 quail embryo. As in the stage 20 chick embryo, the dermomyotome has become more vertical. The wing buds are larger.



18



19

Figure 20

Cross-section through the wing level of a stage 23 quail embryo. As in the stage 23 chick embryo, the dermomyotome is epithelial at its medial edge but is mesenchymal in lateral regions. The myotome, indicated by the arrows, extends toward the wing but disperses before it reaches the limb mesoderm.


100 µ
20

Figure 21

A. Cross-section at the wing level of a stage 26 quail embryo. The central chondrogenic (cartilage-forming) area of the wing is evident due to the difference in cell density between it and the myogenic (muscle-forming) areas. C: chondrogenic area in limb; M: myogenic areas; P: the myogenic mass that will ultimately form the pectoralis muscle.

B. Detail of A showing the primordium of the pectoralis. There are no myotubes in evidence at this stage.


100μ

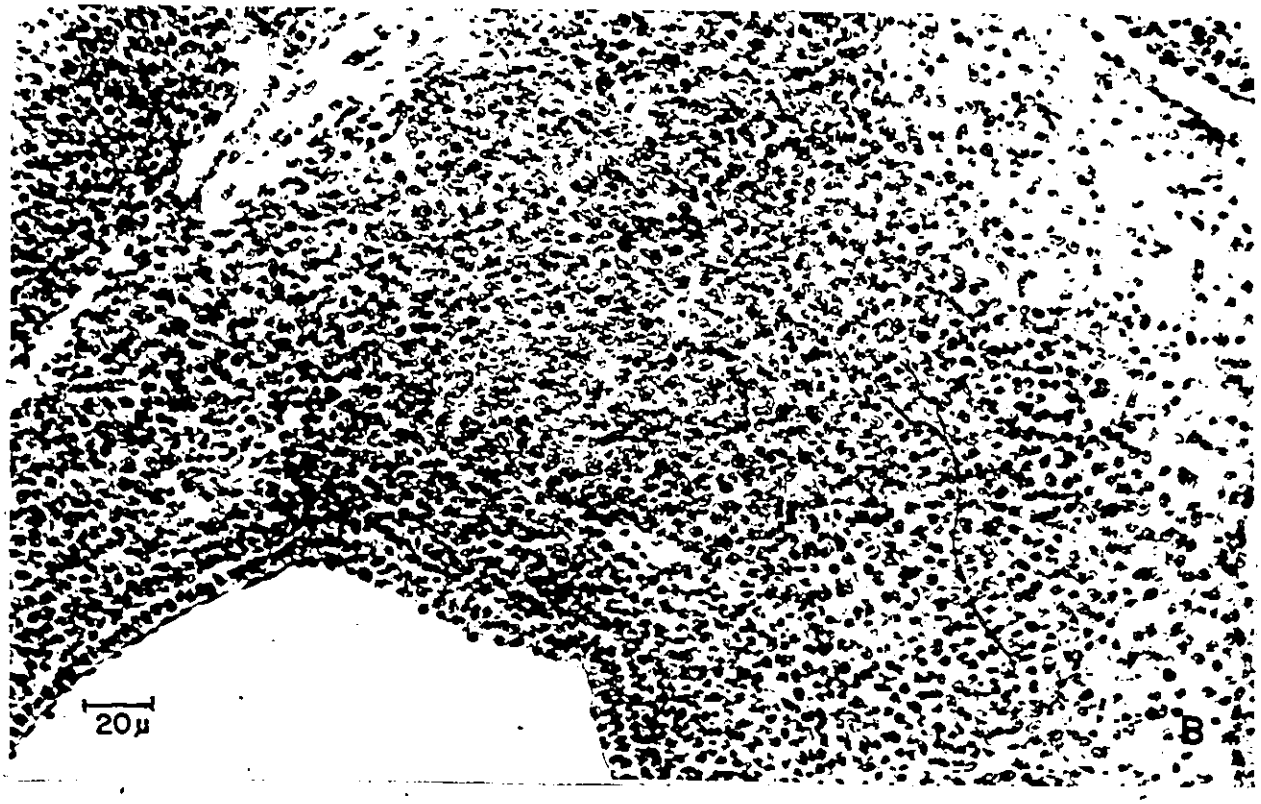
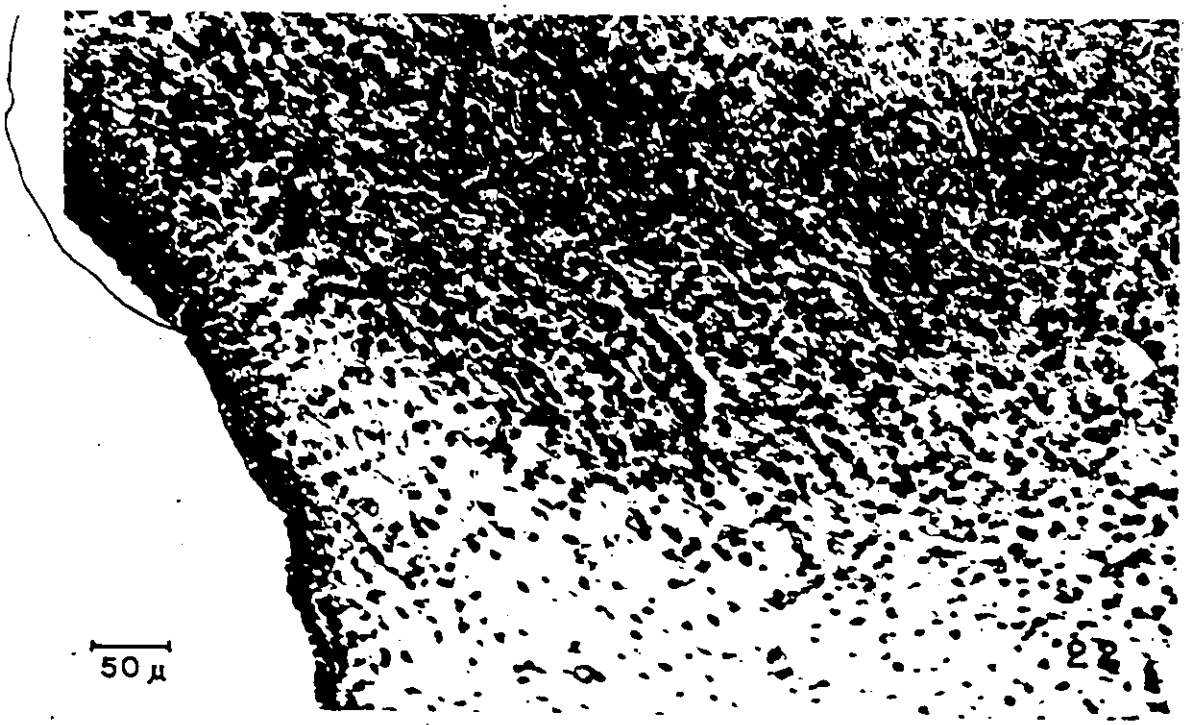

20μ
B

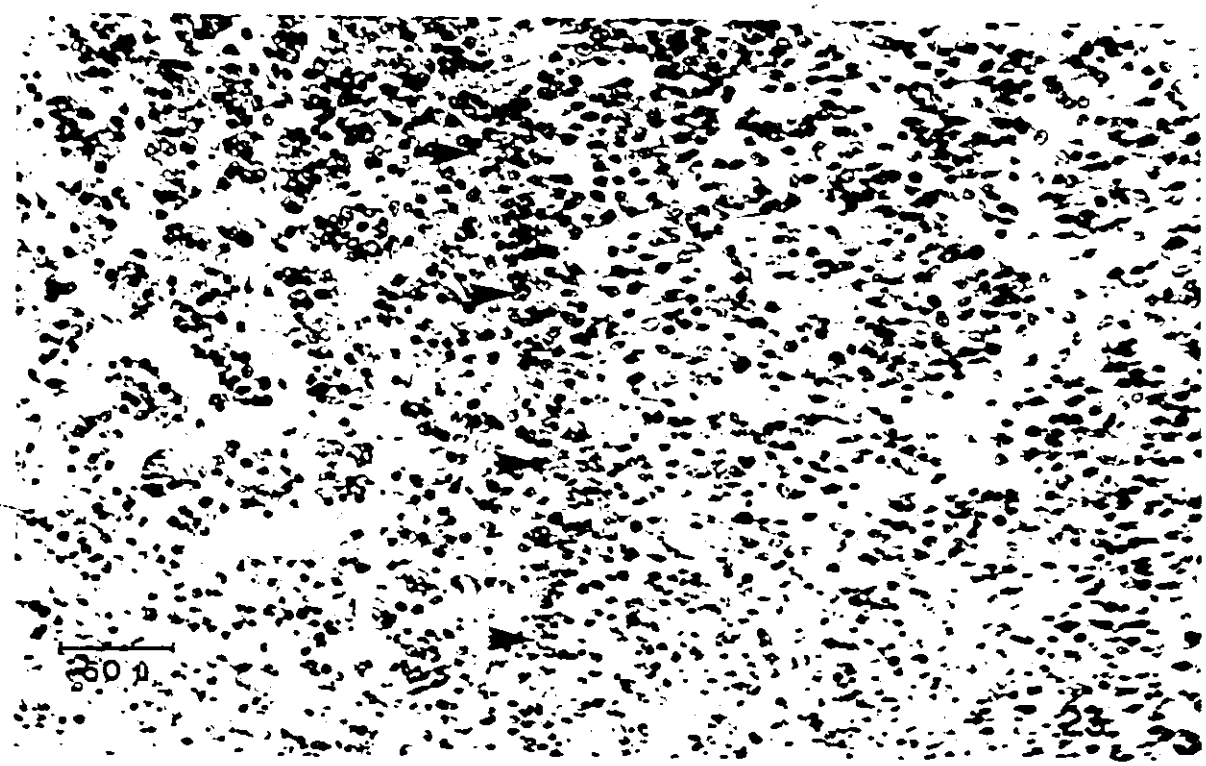
Figure 22

Pectoralis major muscle of a stage 28 quail embryo. Myotubes are present.

Figure 23

Pectoralis major muscle of a stage 35 quail embryo showing the medial and lateral regions of the muscle separated by a band of connective tissue (arrows).


50 µ


50 µ

the myogenic areas of the limb. However, the myotomes of the thoracic somites do invade the body wall and appear to give rise directly to thoracic muscles such as the intercostals. From experimental studies, we know that somites are the source of limb muscles as well as intercostal muscles (Christ et al., 1974 a, b). It must therefore be the case that the cells that form the limb muscles migrate out of the somite and make their way to the myogenic areas of the limb. From descriptive studies such as the present study, it is not possible to identify these cells and therefore to determine when they begin to leave the somite and when this migration has ended.

The present study shows that muscle development in quail and chick embryos are similar at all developmental stages up to stage 35. Although the quail develops faster than the chick, it goes through the same stages of development. A comparison of quail and chick embryos at the same stage of development reveal no major morphological differences, other than a difference in size.

This study also confirms the observations of Sullivan (1962) that the pectoralis, like all other wing-associated muscles of the shoulder and thoracic regions, develops from a myogenic primordium at the level of the wing. The pectoralis primordium cannot be identified prior to stage 27.

The observations of the present study show that the pectoralis major muscle and the pectoralis minor muscle each consist of two parts that are separated by a thin layer of connective tissue. This fact has been noted by previous investigators (Sullivan, 1962; Koch, 1973; Nickel et al., 1977; George and Berger, 1966). Since I will have occasion to discuss the pectoralis major in detail, I will refer to the two parts respectively as the medial and lateral regions of this muscle.

RESULTS

SECTION 2

SECTION 2

SOMITE TRANSPLANTS: THE LEVEL OF ORIGIN OF THE PECTORALIS MAJOR MUSCLE

Introduction

The vertebral column of the chick consists of five regions: cervical, thoracic, lumbar, sacral and caudal. The number of cervical and thoracic vertebrae total 21. However, the division of these 21 vertebrae into cervical and thoracic groups seems to be a matter of some dispute. The last seven vertebrae in this group all bear ribs. The last five pairs of ribs are true ribs, with a vertebral component that articulates with a sternal component attached to the sternum (see Figure 24). The two pairs of ribs in front of these five are called "false" or "floating" ribs because they lack a sternal component and do not attach to the sternum. According to some sources, the vertebrae to which the false ribs are attached are the last two cervical vertebrae, giving the chick 16 cervical and 5 thoracic vertebrae (e.g. Robinson, 1970). However, according to others, the vertebrae to which the false ribs are attached are the first two thoracic vertebrae, giving the chick 14 cervical and 7 thoracic vertebrae (Pinot, 1969; Kieny, Mauger and Sengel, 1972). In this thesis, the latter designation will be used because it seems to be most widely accepted.

All of the vertebrae and ribs arise from the sclerotome of the somites. The somites are sometimes named for the vertebrae to which they give rise. This is a convenient way to designate groups of somites and it is a practice I follow. Thus, somite 18 is the last cervical somite, and somite 19 is the first thoracic somite. It must be kept in mind, however, that each somite contributes to two vertebrae, not one. Somite 19 contributes to the caudal half of the last cervical vertebra and to the cephalic half of the first thoracic vertebra. It is designated as a thoracic somite because it forms the first false rib (Seno, 1961; Pinot, 1969; Kieny, Mauger and Sengel, 1972;

Figure 24

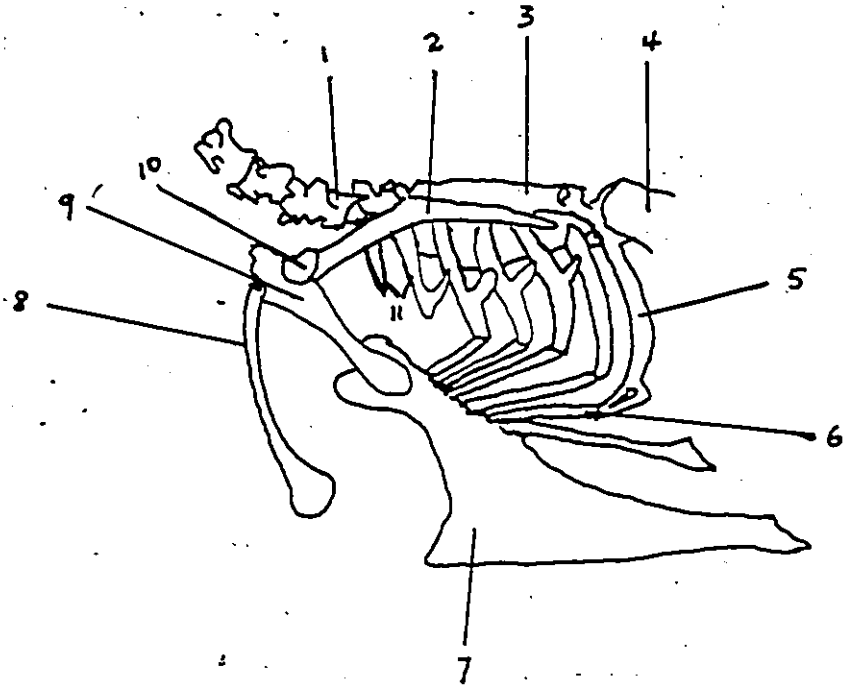
Thoracic skeleton of the chicken.

A. Left Lateral View

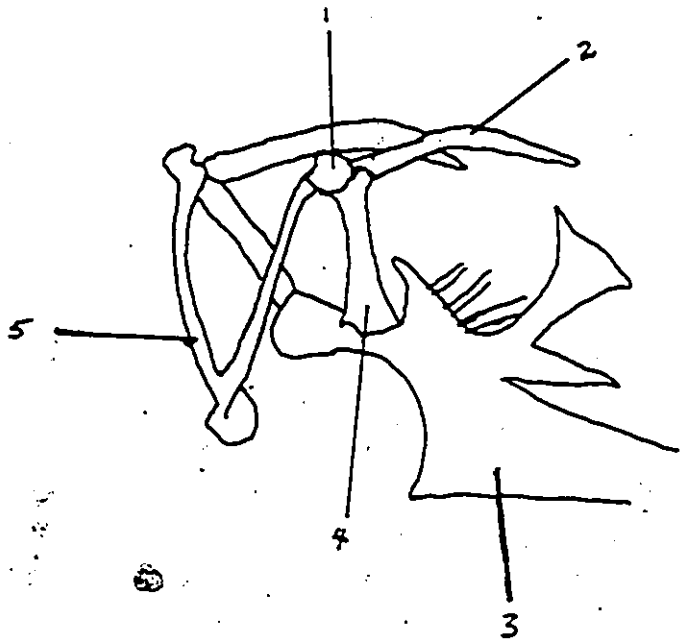
1. last cervical vertebra
2. scapula
3. fused thoracic vertebrae
4. ilium (a pelvic bone)
5. vertebral component of rib
6. sternal component of rib
7. sternum
8. clavicle
9. coracoid
10. joint cavity for humerus (humerus not shown)
11. false ribs

B. Antero-lateral View

1. joint cavity for humerus (humerus is not shown)
2. scapula
3. sternum
4. coracoid
5. clavicle



24 A



B

Chevallier, 1975). Somites 5 - 18 are therefore cervical somites. Somites 19 - 25 are thoracic somites.

Since the pectoralis major muscle in the chicken occupies a large area in the thorax, extending from the clavicle to the abdomen, it is often considered to be a thoracic muscle. The results of three previous studies on the development of thoracic structures in the chick embryo have been interpreted to suggest that the pectoralis arises at the thoracic level. Seno (1961) and Pinot (1969) concluded that this muscle is formed by the somatopleuric mesoderm between somites 19 and 26. Christ et al. (1974a) concluded that it is formed by thoracic somites.

However, a study by Sullivan in 1962 described the development of the pectoralis from a myogenic mass in the wing bud of the chick embryo. My own observations are similar (Section 1). The wing bud arises in the somatopleure opposite cervical somites 15 or 16 - 18 and thoracic somites 19 - 20 or 21. These somites (15-21) are sometimes referred to as brachial somites. Moreover, the pectoralis and other brachial muscles are innervated by the brachial plexus, which is derived from spinal nerves that leave the spinal cord at the level of the wing bud (Roncali, 1970). It therefore seems possible that the brachial somites are the source of the pectoralis muscle.

A total of 805 somite transplants were performed for the studies described in this section and the next two sections. For the experiments described in this section, quail somites from cervical (somites 13-15), brachial (16-20), and thoracic (21-25) levels were grafted orthotopically to chick embryos at stages 12-14 (16 - 22 pairs of somites) to determine the levels of origin of the pectoralis major muscle. The results are based on 15 chimaeras that received grafts of brachial somites, 3 chimaeras that received grafts of cervical somites, and 4 chimaeras that received grafts of thoracic somites. All chimaeras in this series were analyzed at stages between 9 and 17 days in ovo.

In 7 cases, the pectoralis on the operated side was removed from the chimaera and fixed and stained separately so that the quail contribution to the muscle could be quantitatively determined. In another 4 cases, the quantitative determination was done on the pectoralis in situ. The chimaeras were also examined to determine what other structures were derived from the grafted somites.

In a series of control embryos, brachial somites were removed from 2-day embryos. Sixty-six such extirpations were performed. Two embryos survived and were fixed at 9 - 10 days of incubation and examined for deficiencies caused by somite removal. In another 10 embryos, the somatopleure opposite somites 16 - 20 was removed. Only one embryo from this series survived to stage 35. It was examined for deficiencies caused by somatopleure removal.

Results

In the three chimaeras that received grafts of cervical somites 13 - 15, no quail nuclei were found in the pectoralis major muscle or in any other structure at the brachial or thoracic level. Quail cells were found in cervical vertebrae, in muscles in the neck and in dorsal dermis at the level of the graft.

In the 4 chimaeras that received grafts of thoracic somites 21 - 25, some quail cells were found in the most lateral region of the pectoralis. The majority of the muscle contained only chick cells. A few quail cells were found in some other muscles of the wing, but their contribution was extremely small. Quail cells in these chimaeras were also found in thoracic vertebrae, ribs, dorsal dermis, and intercostal and abdominal muscles.

In all 15 chimaeras that received grafts of quail brachial somites, quail cells were found in all muscles of the wing, shoulder and thorax on the operated side, including the pectoralis major. These chimaeras are listed in Table 3.

TABLE 3

QUANTITATIVE STUDY OF QUAIL CONTRIBUTION TO PECTORALIS MAJOR

CHIMAERA	AGE IN SOMITES AT TIME OF TRANSPLANT		SOMITE(S) TRANSPLANTED	AGE WHEN ANALYZED	% QUAIL IN PECTORALIS
	Donor	Host			
112	17	18	16 - 18	11 days	
124	21	20	17 - 20	9 days	
231	18	16	18 - 21	15 days	75*
321	16	20	16 - 21	14 days	36
403	15	18	15 - 19	16 days	
425	20	17	16 - 19	13 days	80
553	15	15	16 - 20	11 days	90
629	22	23	18 - 21	12 days	87*
633	19	19	18 - 21	14 days	88*
675	15	16	16 - 20	18 days	72
842	19	18	17 - 19	15 days	
934	17	16	16 - 20	Stage 37 (11 days)	84
1027	15	15	16 - 21	Stage 38 (12 days)	90
1077	14	16	14 - 21	Stage 37 (11 days)	88
1078	15	15	15 - 20	Stage 37 (11 days)	75

* Pectoralis not completely removed.

In the intervertebral muscles, quail cells were found in both muscle and connective tissue (Figure 25). However, in all muscles of the wing, shoulder, and thorax, quail nuclei were seen predominantly in myotubes. The connective tissue was of chick origin (Figure 26). The peripheral muscles derived from brachial somites are shown in Figure 27.

The results of the quantitative study is shown in Table 3. The average percentage of quail nuclei in the muscles is 78%, with a range of 36% to 90%. In every case but one, quail cells were in the majority.

Three chimaeras are marked with an asterisk. In these cases, the pectoralis on the operated side was removed from the chimaera for counting. When the rest of the chimaera was sectioned and stained, it was found that a small but significant portion of the pectoralis had been left behind. This portion contained mostly chick cells. The quantitative determinations for these muscles are therefore inaccurate. All three of these chimaeras received grafts of somites 18-21. The portion that was left behind and that was derived from chick probably came from somites 16 and 17. If the results of these three chimaeras are excluded, the average number of quail in the muscle is 76%. This is based on 8 chimaeras that received grafts of at least 5 somites between somites 15 and 21.

In addition to muscles, the quail somites formed cartilage structures, including the vertebrae and the scapula at the graft level. Quail cells in the vertebrae were restricted to the operated side, while the remainder of the vertebrae were formed by chick somites on the unoperated side. Figure 28 shows the midline of a chimaeric vertebra. The quail and chick nuclei form opposite halves of this structure and do not occupy each others' normal territory.

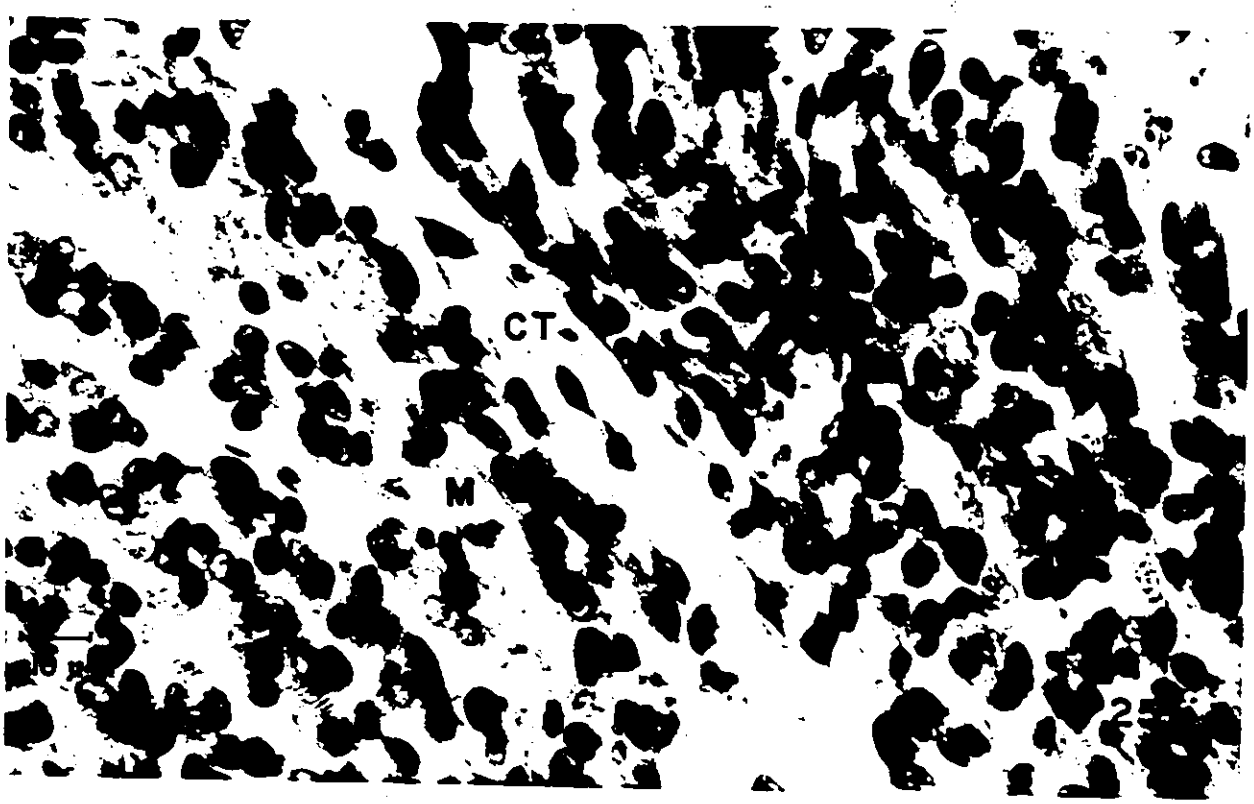
Quail somites contributed the dermal layer of the skin of the back distally to the level of the scapula (Figure 29). The dermis of the skin of the wing and body wall is derived from somatopleuric mesoderm.

Figure 25

Intervertebral muscle in a cross-section at the graft level of a stage 35 chimaera that received brachial somites from a quail donor at stage 13. In these muscles, both muscle and connective tissue are derived from the transplanted somites. M: muscle; CT: connective tissue.

Figure 26

Pectoralis major muscle in a cross-section of the same chimaera as in Figure 25. In this peripheral muscle, the nuclei that are associated with aggregations of the myotubes (muscle cells) are quail, while the connective tissue cells between the aggregations of myotubes are chick cells. M: aggregations of myotubes; CT: connective tissue; arrows: quail nuclei.


CT
M
Z

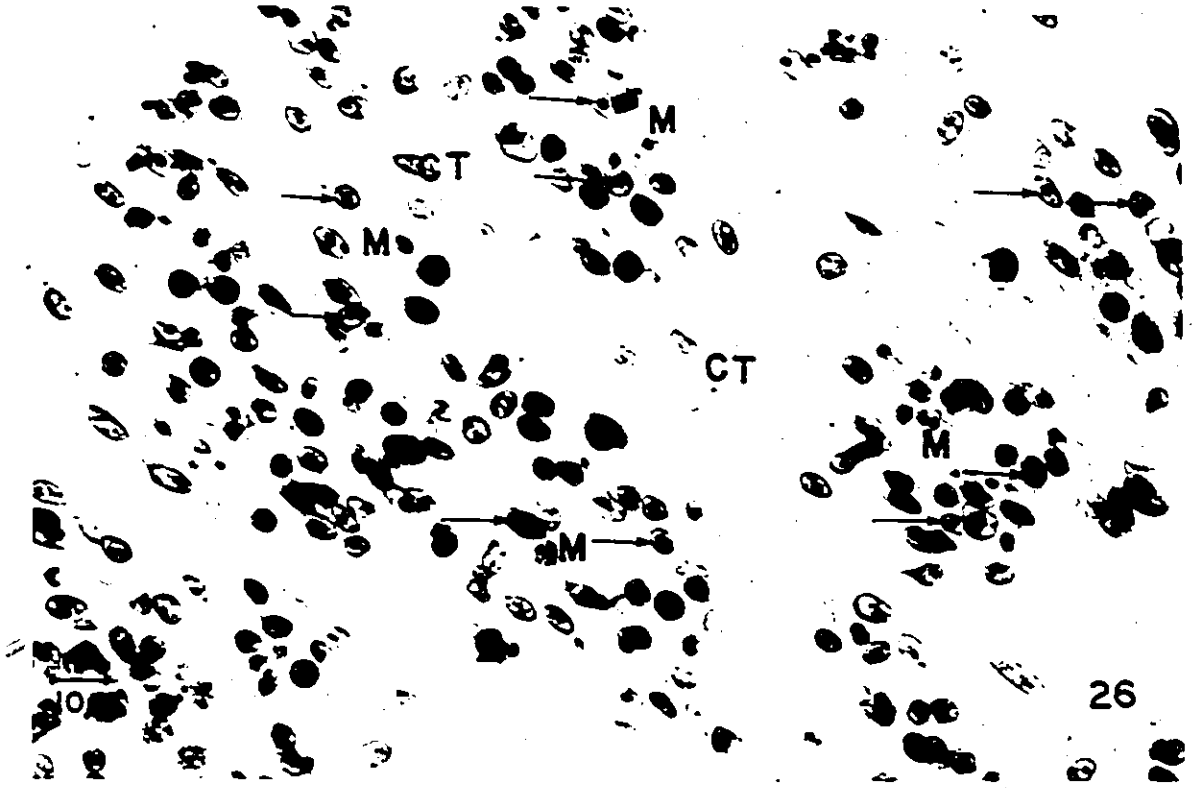
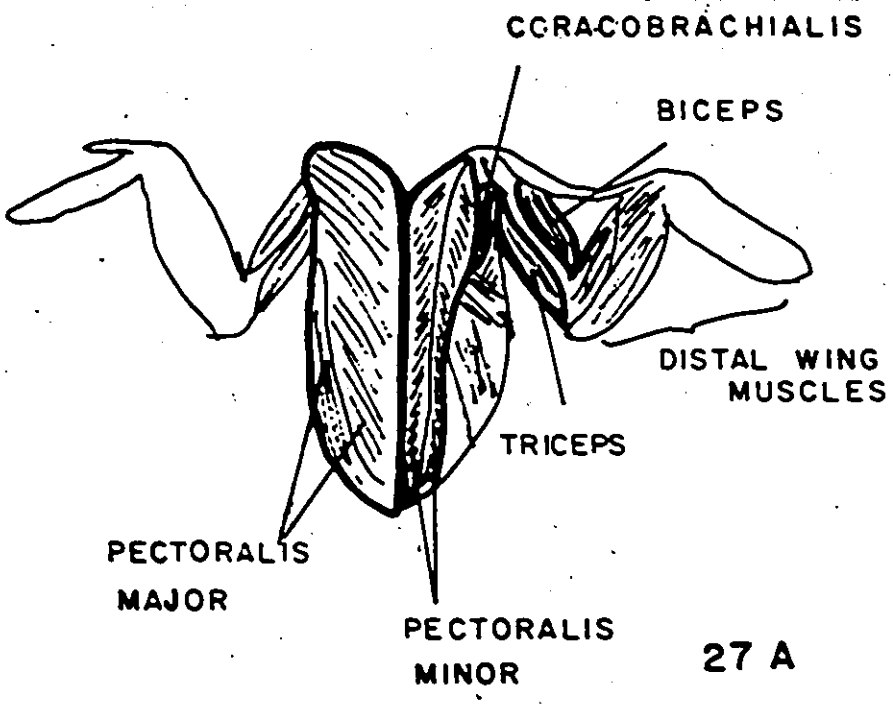

M
CT
M
CT
M
M
Z
26

Figure 27

The muscles considered in this study that are derived from brachial somites, as seen in an adult chicken.

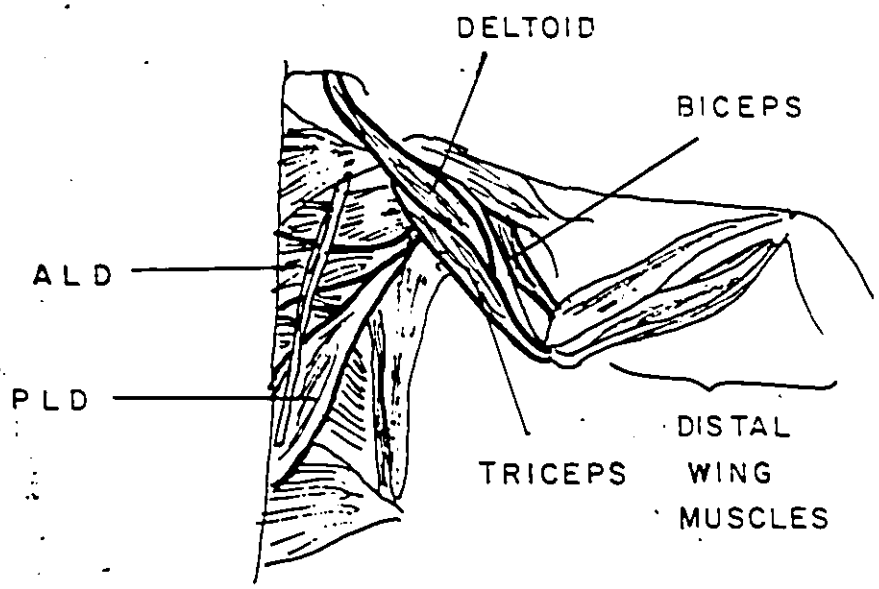
A. Ventral view. On the left side of the drawing are the muscles as seen after removal of the skin. The pectoralis major is the largest muscle and covers several other thoracic muscles. On the right side are the thoracic muscles as seen after removal of the pectoralis major. Both the pectoralis major and pectoralis minor muscles consist of two parts that are separated by connective tissue. The muscle fibers in the two regions in each muscle are aligned in different directions.

B. Dorsal view, right side only. ALD: anterior latissimus dorsi; PLD: posterior latissimus dorsi. All of the skeletal muscles of the wing and thorax are derived from the brachial somites. However, only the muscles that are labelled in these drawings were studied in detail.



27 A

VENTRAL VIEW



B

DORSAL VIEW

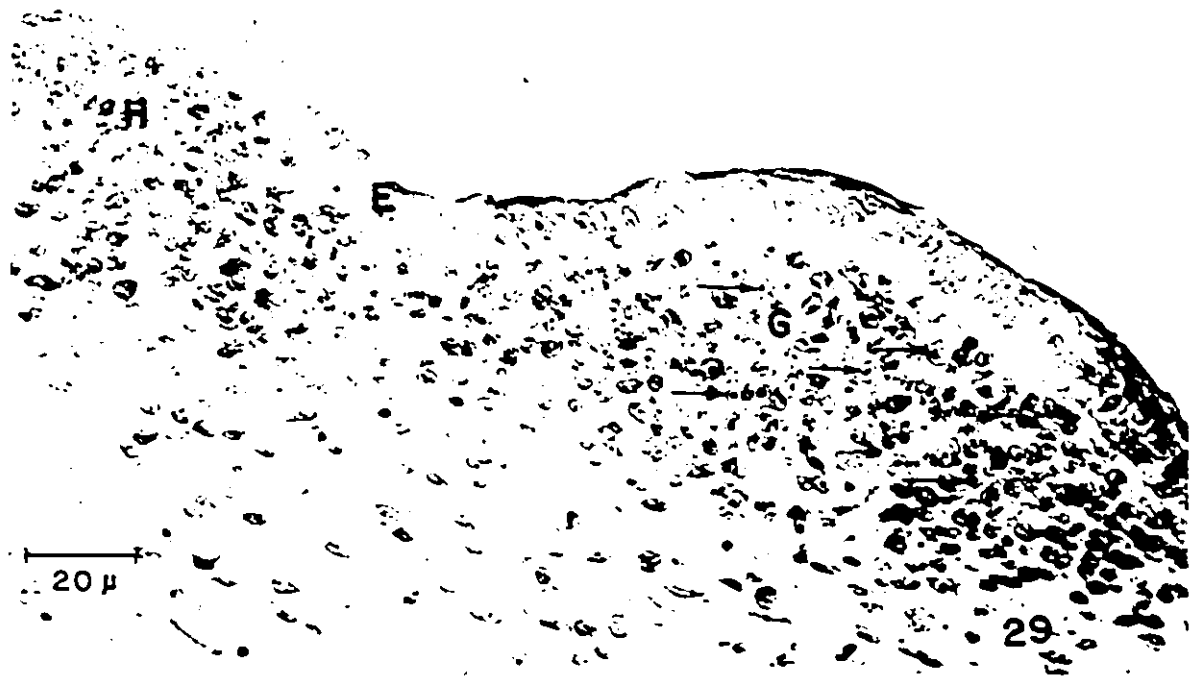
Figure 28

Mid-dorsal region of vertebra in a cross-section through the graft level of a stage 35 chimaera that received a graft of quail brachial somites at stage 13. On the operated side of the animal, the vertebra is composed of quail cells (indicated by arrows). On the unoperated side, the vertebra is composed of chick cells.

Figure 29

A region of dorsal skin in a cross-section at the graft level of the same chimaera as seen in Figure 28. The ectoderm is of host origin. The dermis in the most dorsal region of the back is derived from somites and is therefore quail (indicated by arrows). More peripherally, the dermis is of host origin. E: ectoderm; G: graft-derived dermis; H: host-derived dermis.


20 µ
28


A
E
G
20 µ
29

Quail cells were also found in the developing kidney tubules at the level of the graft. These structures were actually derived from the intermediate mesoderm that lies between the somites and somatopleure. This tissue was transplanted along with the somites.

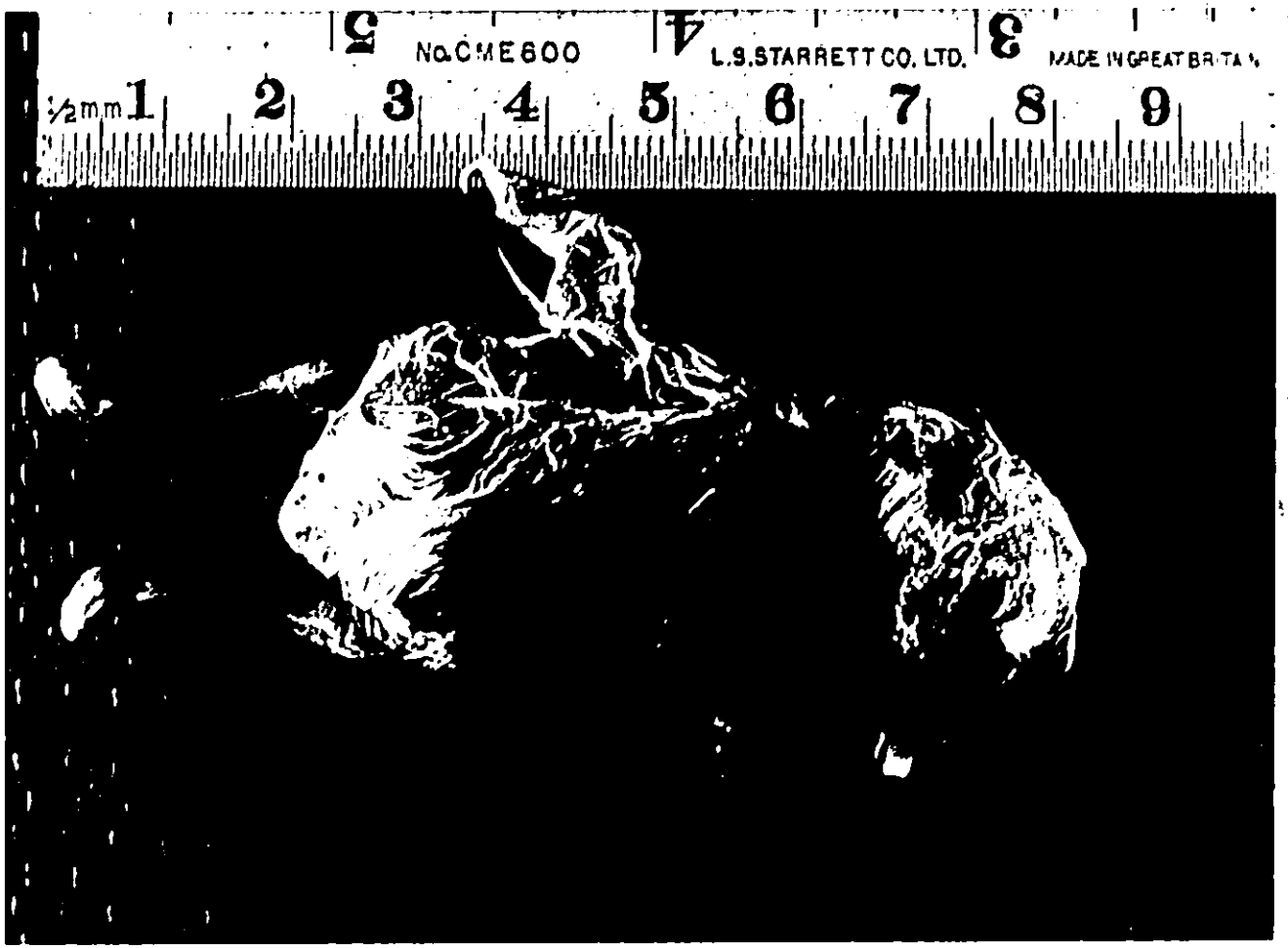
In addition to the structures listed above, quail cells were sometimes found in dorsal root ganglia and sympathetic ganglia at the graft level. This is due to the presence of neural crest cells that were migrating through or around the somites at the time of the transplant. Quail nuclei were sometimes found along the length of a spinal nerve that left the cord at the graft level. These were assumed to be Schwann cell nuclei, also neural crest derivatives (Weston, 1970). Neural crest cells also give rise to melanocytes, which conferred quail coloring to the plumage of some chimaeras (Figure 30). In general, neural crest derivatives were excluded from the graft by performing the transplant at stage 12 (15 - 16 somites), before the neural crest cells have migrated away from the neural tube.

Embryos whose brachial somites had been removed at 2 days in ovo were analyzed at 9 - 10 days in ovo. These embryos showed no deficiencies in the musculature on the operated side. All muscles were present and apparently normal. However, the scapula, which is derived from the somites, was absent in one embryo and abnormally small in the other. In both embryos, the dorsal root ganglia that formed in the operated region were reduced in number, and most of them were either larger or smaller than normal.

In the embryo whose somatopleure had been removed at 2 days in ovo, extensive regulation had also occurred. The limb on the operated side was present, but its site of attachment was slightly caudal to the normal position. In cross-section, it was apparent that all muscles were present and in normal or near-normal positions. Those muscles that normally attach to the humerus did so in this embryo, even though the position of the humerus was abnormal.

Figure 30

Chimaera after 17 days *in ovo*. This embryo received a graft of quail brachial somites at stage 14. The black feathers on the operated side indicate that prospective melanocytes were transplanted with the quail somites.


No.CME600
L.S.STARRETT CO. LTD.
MADE IN GREAT BRITAIN
½mm
1
2
3
4
5
6
7
8
9

All cartilage structures were present, but there was no joint between the scapula and the coracoid bone. The two bones in this case were united in a single structure.

Discussion

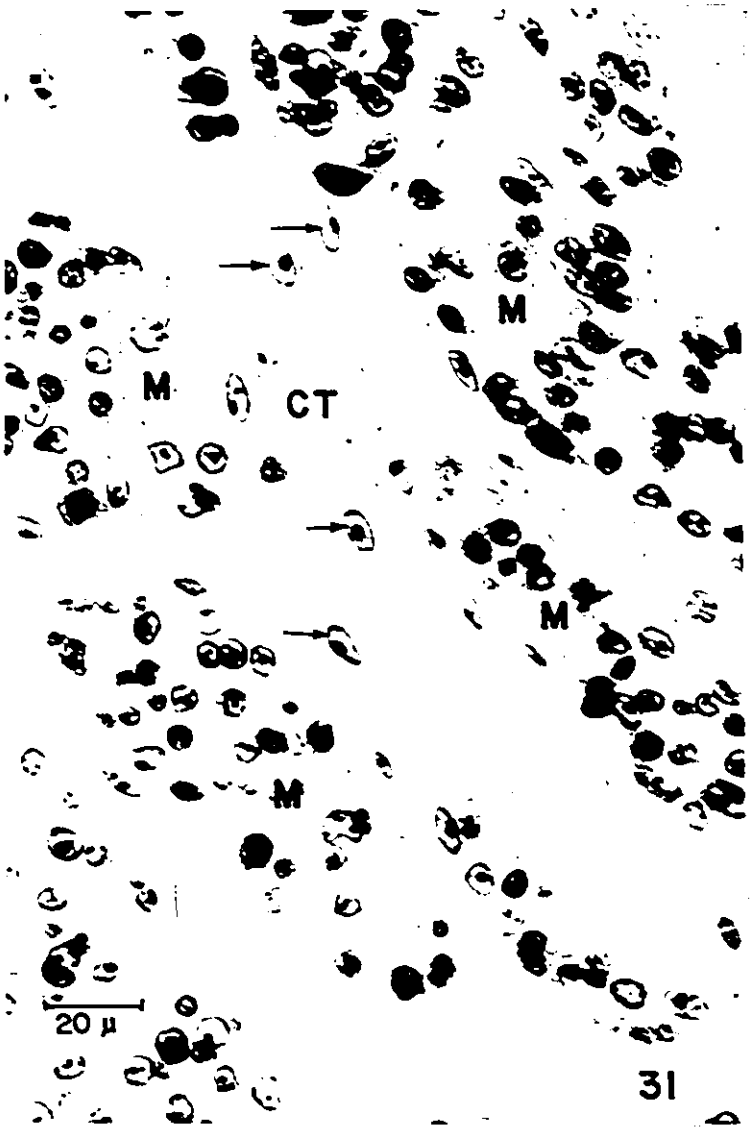
The results of the grafting experiments indicate that the brachial somites are the source of all of the brachial muscles, including the pectoralis major. Somites above and below this level contribute little or nothing to these muscles. The cells from the somites mingle with cells in the somatopleuric mesoderm to form a structure that is therefore heterogeneous with respect to the tissues of origin, the muscle cells deriving from somites and the connective tissue from somatopleuric mesoderm.

This conclusion is supported by a series of experiments performed by Christiane Le Lievre (Beresford, Le Lievre, and Rathbone, 1978). She transplanted quail somatopleure from the brachial level orthotopically into chick embryos at stage 13 - 14 and allowed the chimaeras to reach stage 35 (9.5 days) or older. In these chimaeras, the somatopleuric mesoderm formed the dermis of the wing and body wall, the cartilage of the wing, and the connective tissue of all brachial muscles, including the pectoralis major. The musculature, however, was formed from chick cells (Figure 31). These results are consistent with my conclusions that the somites are the source of muscle and the somatopleuric mesoderm is the source of connective tissue in the peripheral muscles at the brachial level.

These results are not at variance with the results of Seno (1961), Pinot (1969) and Christ et al. (1974a) (see Introduction), although they contradict the interpretations the above authors gave to their results. Seno marked the somatopleure opposite somites 19 - 28 with carbon particles. When the particles were subsequently found in the pectoralis, he concluded that he had marked the source of the musculature, when most likely he had marked the presumptive connective tissue.

Figure 31

Pectoralis major muscle in a cross-section of a stage 38 chimaera that received a graft of quail somatopleure at the wing level at stage 14. The nuclei associated with the aggregates of myotubes are mostly chick nuclei; the nuclei found in the connective tissue are quail nuclei (indicated by arrows). A few quail nuclei are also found among the myotubes. These nuclei are presumed to belong to connective tissue cells. Compare with Figure 26. M: aggregations of myotubes; CT: connective tissue. (This chimaera was kindly provided by Dr. Christiane Le Lievre).



31

Pinot destroyed somites 21 - 25 with X-irradiation. When the pectoralis formed, she concluded that it arose in the somatopleure at this level. It is likely, however, that the muscle formed from the intact brachial somites.

Christ et al. (1974) demonstrated that the pectoralis formed from somitic tissue. They concluded that it is derived from thoracic somites. However, their protocol clearly shows that they transplanted a substantial length of somitic tissue starting with cervical somite 16. Their graft therefore included all of the brachial somites.

It is a point of interest that, although the somite itself gives rise to dermis, muscle, connective tissue, and cartilage, those somitic cells that migrate into the somatopleuric mesoderm are found primarily in muscle. This fact raises the possibility that these cells are committed to a myogenic lineage once they enter the somatopleure. This question is discussed more fully in Section 4.

The results of the extirpation studies indicate that embryos at 2 days of incubation are capable of extensive regeneration. When the brachial somites were removed at 2 days in ovo, all muscles that are normally derived from these structures were present and normal at 10 days in ovo. Cartilage derivatives were generally normal, except for the scapula.

It is not possible to determine from the above results the tissue that gives rise to these somite derivatives. There are three possibilities:- 1) cells of brachial somites that were left behind after somite removal; 2) cells from somites above and below the brachial somites, and 3) the somatopleuric mesoderm. This issue is discussed further in Section 3, where some evidence is given as to the origin of these cells.

One abnormality that was found in the embryos discussed above was a disruption in the regular pattern of dorsal root ganglia at the brachial level. This is not surprising, since it has long been known that the somites are required for the normal formation of these structures. Detwiler (1936) showed

that in urodele embryos, somite removal resulted in abnormalities in both the size and number of dorsal root ganglia that develop at that level, but that peripheral muscles formed normally and were innervated. My results indicate that the situation is similar in the chick embryo.

The one surviving embryo from the somatopleure extirpation series was also virtually complete. All structures in the limb and thorax were present, although the wing appeared to be abnormally attached. In this case, some indication of the source of compensating cells is given by the fact that the scapula and coracoid were one structure, rather than two structures as is normally seen. In primary development, the scapula is derived from somites, while the coracoid is formed by the somatopleure. Possibly some of the structures normally derived from the somatopleure, such as the coracoid, were formed by somitic tissue in this embryo.

Taken together, the results of the extirpation studies reveal that at two days in ovo, the chick embryo is capable of remarkable and extensive regulation of subsequent development. These results are consistent with similar studies done by other investigators, who showed that somite removal or somite destruction by X-irradiation in chick embryos did not prevent the formation of the wing muscles (Chevallier, Kieny and Mauger, 1978) or the rib cage (Kieny, Mauger and Sengel, 1972).

All of the chimaeras discussed in this section were morphologically normal, indicating that the graft had healed properly. Other chimaeras survived the operation but showed various abnormalities due to improper grafting. In most cases, such abnormalities were accompanied by a reduction of quail participation in muscle formation. The reduction ranged from slight to a virtually complete absence of quail cells in peripheral muscles. The graft could always be found close to the vertebrae at the level of the operation, but the size of the structural abnormalities that formed were extremely varied and bore no

relationship to the number of graft cells that formed peripheral structures. Sometimes large structural aberrations were accompanied by an apparently normal participation of graft cells in the peripheral muscles. In other cases, the graft formed small, insignificant structures near the vertebrae, and quail cells were not found in any other structure in the embryo.

The variable contribution of quail cells in peripheral muscles in the presence of structural abnormalities emphasizes the major hazard in experiments that involve the transplantation of somites between two species that cannot be histologically distinguished from one another: the participation of donor cells to host structures cannot be verified. For example, if somites are transplanted between two chick embryos, one of which is normal and one of which carries the gene for muscular dystrophy, there is no independent means of identifying the structures derived from the donor tissue. Such chimaeras must be thoroughly examined for even the slightest abnormalities that might indicate a reduced involvement of the graft in peripheral muscles.

RESULTS

SECTION 3

SECTION 3

SINGLE SOMITE TRANSPLANTS: THE LIMITS OF THE PECTORALIS PRIMORDIUM AND THE FATE OF SPECIFIC SOMITES

Introduction

This study was undertaken to define precisely the somites that contribute to the pectoralis major muscle. The results of the previous section indicate that the limits of the primordium of this muscle are somite 16 anteriorly and somite 20 or 21 posteriorly. This could be verified by using a more precise approach. In addition, the origin of other muscles that derived from these somites could be similarly evaluated.

Each chimaera received one somite (or in some cases, two), transplanted orthotopically (same level), from a quail donor at stage 13 - 14. Transplants were made between somites 15 and 23 inclusive. Most of the resulting chimaeras were analyzed at developmental stage 35 - 36 (9.5 - 10 days).

Results

The chimaeras that were analyzed for this study are listed in Table 4. Although the results are based on relatively few chimaeras, the observations are consistent. Quail cells derived from grafted somites 15, 22, or 23 were generally not found in the pectoralis major muscle or in any of the other muscles in the wing and thorax, whereas somites 16 to 21 always contributed cells to these muscles. The results are shown in Table 5. All somites transplanted between somites 16 and 21 inclusive contributed myoblasts to the pectoralis major muscle, whereas various subgroups of only 3 to 4 somites contributed to the remaining wing and thorax muscles.

In Table 5, the muscles have been placed in two groups, designated Dorsal and Ventral. Sullivan (1962) described the development of the wing and wing-associated muscles in the chick embryo between 4.5 and 12 days *in ovo*. In the youngest embryos that he examined (stage 25-26), he observed

TABLE 4

CHIMAERAS ANALYZED IN SINGLE-SOMITE STUDY

CHIMAERA	AGE IN SOMITES AT TIME OF TRANSPLANT		SOMITE(S) TRANSPLANTED	AGE WHEN ANALYZED
	Donor	Host		
952	20-21	20	18-19	17 days
1009	17	18	17-18	Stage 38 - 39 (12 days)
1015	17-18	17-18	16-17	36 (10 days)
1049	21	20	19-20	35 (9 days)
1050	21	20	19-20	35 (9 days)
1085	16	17	15-16	35 (9 days)
1126	24	20	19	35 (9 days)
1127	24	20-21	20	36 (10 days)
1167	16	16	15	35 (9 days)
1170	15-16	18	15	32 (7.5 days)
1176	22	18	20	35 (9 days)
1202	17-18	17-18	17	35 (9 days)
1207	21	21	21	35 (9 days)
1223	23	22	22	35 (9 days)
1224	23	22	22	35 (9 days)
1225	23	20	21	35 (9 days)
1226	20-21	20	21	35 (9 days)
1228	22-23	22	23	35 (9 days)
1229	22-23	26	22	36 (10 days)

TABLE 5

SOMITIC ORIGINS OF BRACHIAL MUSCLES

	MUSCLE \ SOMITE	15	16	17	18	19	20	21	22
Dorsal	Deltoid		X	X	X				
	Coraco-brachialis posterior		X	X	X	X			
	Anterior latissimus dorsi				X	X	X		
	Posterior latissimus dorsi					X	X	X	
	Triceps					X	X	X	
Ventral	Coraco-brachialis anterior			X	X				
	Biceps		X	X	X				
	Pectoralis Major · Medial		X	X	X	X			
	Pectoralis Major · Lateral					X	X	X	
	Pectoralis Minor		X	X	X				

two pre-muscle masses in the wing bud, one of which was located dorsal to the developing humerus and one ventral. According to his observations, all proximal wing and thorax muscles form by cleavage from one of these masses: the pectoralis major, pectoralis minor, coracobrachialis anterior, and biceps muscles form from the ventral mass; the triceps, coracobrachialis posterior, deltoid, ALD, and PLD are derived from the dorsal mass (the distal wing muscles do not derive from these masses). My results show no correlation between a myogenic mass and a particular group of somites.

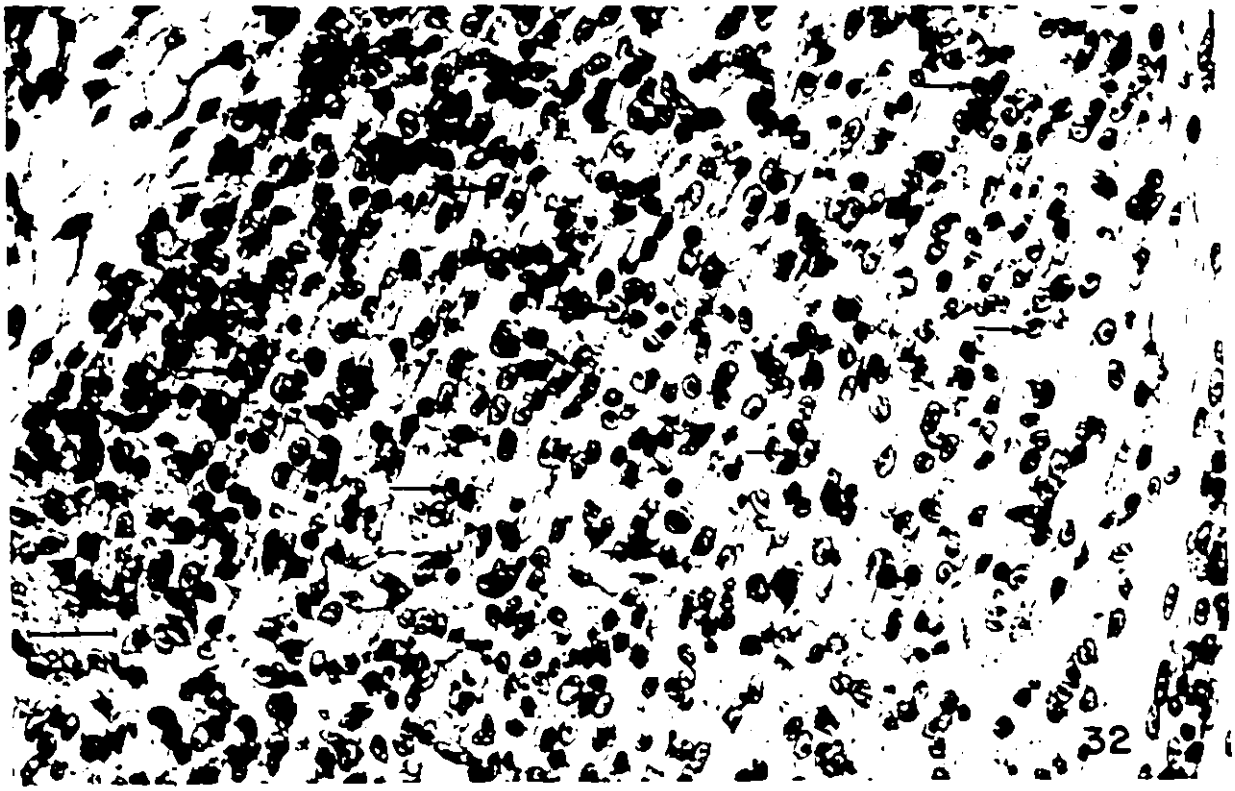
Each somite in the 16 - 21 group contributes to at least three muscles. Moreover, the particular group of muscles that receive myoblasts from each somite is specific for the somite. For example, somites 17 and 18 contribute myoblasts to the same seven muscles, but in addition, somite 18 contributes to the ALD. Similarly, somites 19 and 20 contribute to the same four muscles; however, somite 19 contributes to both regions of the pectoralis major, whereas somite 20 contributes only to the lateral region.

Although all six somites in the group 16 - 21 contribute to the pectoralis major muscle, the medial and lateral regions appear to be derived from a different group of somites: the medial region from 16 - 19 and the lateral region from 19 - 21. Both regions have somite 19 in common, but this somite contributes only a few cells to the medial region. Most of the myoblasts from somite 19 are found in the lateral region.

A particularly interesting feature of the chimaeras in this series is the distribution of quail cells within certain muscles. Since each chimaera received only one somite, or in some cases two somites, many individual muscles were therefore derived partly from the donor somite/somites and partly from the host somites. The triceps muscle, for example, is derived from somites 19, 20, and 21. Figure 32 shows the triceps muscle of a chimaera that received a graft of quail somite 19. All chimaeras that received grafts of somites 19,

Figure 32

Triceps muscle in a cross-section of a stage 35 chimaera that received a graft of quail somite 19 at stage 12. Quail cells (indicated by arrows) are found in all regions of the muscle, as are chick cells.


32

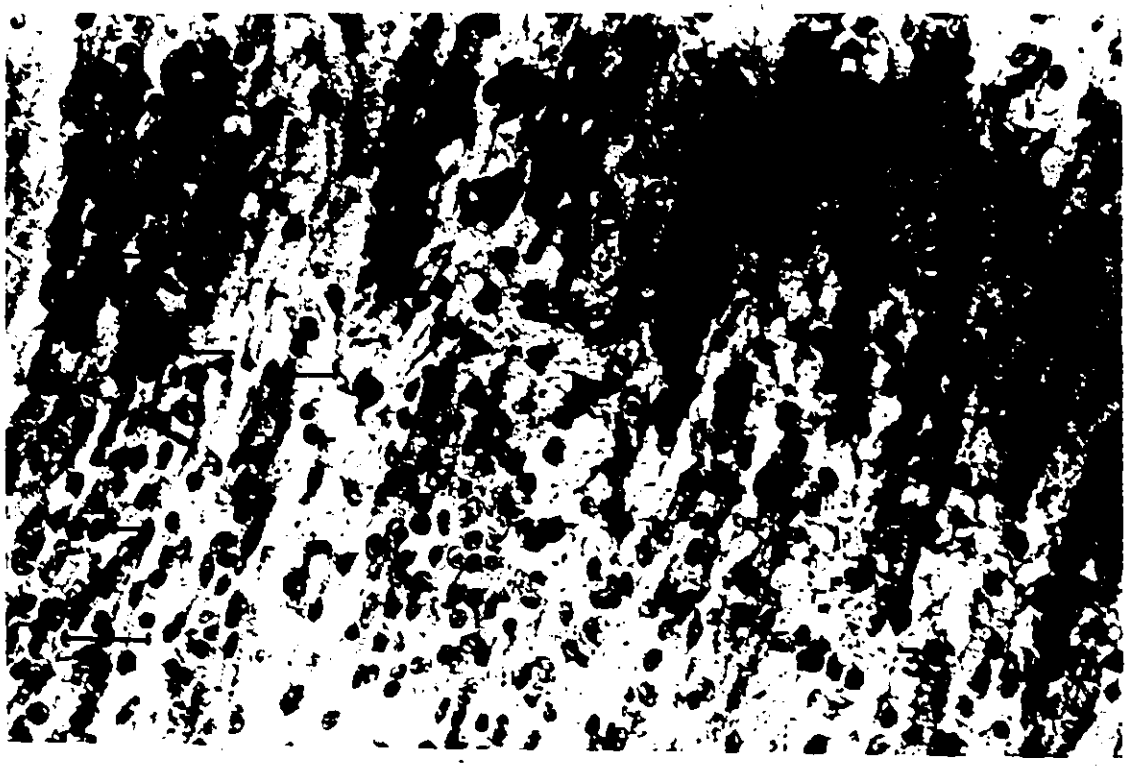
20 or 21 showed a random distribution of graft and host cells in the triceps muscles. Similar results were found in most of the muscles of the wing and thorax; whenever a muscle was derived from both donor and host somites, both quail and chick myoblasts from those somites were found in all regions of the muscle. However, this was not the case for all muscles. The biceps, the deltoid, and the pectoralis major muscles consistently showed a non-random distribution of quail and chick cells in this series of experiments. The pectoralis provides the clearest example of this phenomenon. Figure 33A shows a cross-section of the pectoralis in a chimaera that received a graft of quail somite 16. The quail cells are concentrated in the most dorsal region of the muscle. The region next to it is composed predominantly of chick cells. Figure 33B shows a similar section from the pectoralis of a chimaera that received a graft of quail somite 17. The region of the muscle that was derived from somite 16 in the first chimaera is composed predominantly of chick cells in this chimaera, while the region next to it is composed predominantly of quail cells. Similarly, in every chimaera that received a graft of one or two somites in the 16 - 21 range, the myoblasts from the grafted somite(s) were largely restricted to one region of the pectoralis major muscle. A few quail cells were sometimes seen in other regions, and usually a quail region and a chick region were separated by a small mixed region. Nevertheless, the general tendency for quail cells to form primarily one region of the pectoralis was striking. This regionalization was evident within both the medial and lateral regions of the muscle.

In addition to skeletal muscle, each somite forms cartilage and dermis. In the single-somite chimaeras, quail cells were found in the intervertebral muscles, in the scapula, in the vertebrae, and in the dermis of the skin of the back at the level of the graft. In some cases, quail cells were also found in the dorsal root ganglion and along the length of the spinal nerve

Figure 33

A. Pectoralis major muscle in a cross-section of a stage 35 chimaera that received a graft of quail somite 16 at stage 13. Quail nuclei are found predominantly in the most dorsal region of this muscle (arrows).

B. Similar region as in A of the pectoralis major muscle in a cross-section of a stage 35 chimaera that received a graft of quail somite 17 at stage 13. Quail nuclei are found predominantly in a more ventral region of the muscle (arrows).




B

that left the spinal cord at the level of the graft. These latter cells are presumably Schwann cells, which are neural crest derivatives (see Weston, 1970).

Somites 15, 22 and 23 generally did not contribute to any of the muscles in the brachial region. There was one exception. In two of three similar chimaeras that received grafts of quail somite 22, the operation was technically successful, i.e. only somite 22 in the host was removed, and an undamaged somite 22 from the donor replaced it. No quail cells from somite 22 were found in the pectoralis in either of these chimaeras. However, in a third chimaera, somite 21 of the host was inadvertently damaged during removal of somite 22. As in the other two, somite 22 in this case was replaced by an undamaged donor somite 22. No extra tissue was transplanted from the donor to compensate for the damage done to somite 21 of the host. Nevertheless, in this chimaera, quail cells from somite 22 were found in small numbers in the extreme lateral region of the lateral half of the pectoralis. Possibly the damaged somite 21 was not able to occupy its entire territory, a situation that permitted myoblasts from somite 22 to occupy atypical sites.

Discussion

The results of the single-somite transplants confirm that the pectoralis major muscle originates from somites 16 - 21 and that somites above and below this level do not normally participate in the formation of this muscle. These results further demonstrate that somites 16 - 21 give rise to all other wing and shoulder muscles and suggest that each muscle originates from specific somites within this group.

As discussed in Section 1, the pectoralis major muscle consists of a medial region and a lateral region separated by a band of connective tissue. The results of the single-somite transplants show that the two regions have

different embryonic origins. Since the two regions can be distinguished both anatomically and embryologically, it is possible that the pectoralis major muscle in the chick is phylogenetically derived from two muscles.

The origins of the anterior latissimus dorsi (ALD) from somites 18 - 20 and the posterior latissimus dorsi (PLD) from somites 19 - 21 are of interest in view of a controversy among avian myologists regarding the phylogenetic derivation of these two muscles. Some investigators believe that the two muscles are derived phylogenetically from one muscle, while others maintain that they are and always have been two distinct muscles. Grim (1971) reviewed the literature on this issue and described the development of these two muscles in the chick embryo. He concluded that the early separation of the two muscles from a common primordium, as well as the persistence of separate tendons, designated them as two separate muscles. My observation (Table 5) that they have different embryonic origins lends further support to the dual origin hypothesis.

The results of the single-somite transplants demonstrate that each somite plays a unique role in the development of the brachial muscles. Although two adjacent somites may contribute to many of the same muscles, the full array of myoblast recipients is specific to each somite.

The factors that determine the role played by each somite are possibly related to the position of the somite along the rostro-caudal axis. Somites form in an anterior-posterior sequence. Therefore, each somite is, developmentally, slightly delayed with respect to the somite ahead of it and slightly advanced with respect to the somite behind it. It is thus in a position to contribute myoblasts to available sites that the somite ahead of it has not filled and that the somite behind it is not yet capable of filling. Myoblasts would thus migrate into the periphery and form muscle in locations determined by environmental conditions. If this hypothesis is correct,

then any somite grafted to a particular position along the rostro-caudal axis should contribute to muscles appropriate to that position. There is evidence that this is so. Heterotopic transplants of quail somitic mesoderm from flank and leg levels grafted to the wing level of chick hosts can participate in the formation of wing muscles (Chevallier et al., 1977; Chevallier, 1979). Heterotopic transplants of single somites, however, have not been done.

The single chimaera that showed quail cells derived from somite 22 in the pectoralis deserves further comment. It is possible that in some embryos somite 22 does contribute myoblasts to the pectoralis. However, the fact that somite 21 was damaged during the operative procedure in only this chimaera and not in the other two that received similar grafts indicates that it is this unusual circumstance that permitted the participation of somite 22. If intact somites are thus capable of substituting for adjacent damaged somites, it suggests that in control embryos, in which somites were removed and not replaced with other tissues (Section 2), normal muscles developed by participation from intact somites above and below the level of extirpation.

One of the most interesting and unexpected observations in this series of experiments was the distribution of quail cells within the biceps, deltoid and particularly the pectoralis major. The restriction of graft-derived cells to one region of the muscles means that the myoblasts from each somite did not mix randomly with myoblasts from other somites, but that they remained together, segregated from other myoblasts. It is as if the pectoralis major develops as six muscles rather than one. It is an unusually large muscle in the chicken and derives from a correspondingly large number of somites. It is conceivable that this regionalization phenomenon is a reflection of the evolutionary process of adaptation to flight. As a larger pectoralis became

increasingly advantageous, an alteration in development that permitted myoblasts from atypical segments to contribute to the formation of this muscle and thus increase its growth potential would confer a selective advantage to birds in which this alteration had taken place. As discussed above, the participation of atypical somites in the formation of the pectoralis can occur in the presence of damaged somites. Possibly it can occur in any situation in which the normal participants are unable to provide enough myoblasts to fill the available sites in the periphery.

RESULTS

SECTION 4

SECTION 4

GRAFTS OF BRACHIAL SOMITES: DEVELOPMENT OF THE PECTORALIS UP TO STAGE 35

Introduction

Once it was established that brachial somites are the precursors of the pectoralis and other wing muscles, it became of interest to study the manner in which the graft gave rise to these structures, in particular how early the cells began to leave the graft and the route that they took to reach the sites of muscles formation.

Results

The results in this section are based on 33 chimaeras that received grafts of quail brachial somites at stage 13 and were fixed between stages 15 (50 - 55 hours) and 34 (9 days). The chimaeras are listed in Table 6. Table 7 shows the distribution of the chimaeras with respect to developmental stages.

Stage 15 (Figure 34)

The entire somite consists of quail cells and is differentiating into dermatome, myotome and sclerotome. The limb fold is present, and quail cells are present in the somatopleuric mesoderm.

Stage 17 (Figure 35)

The dermatome, myotome and sclerotome of the somites are populated entirely by quail cells. The limb is mostly chick, but a few quail cells are found dispersed in the mesenchyme.

Stage 20 (Figure 36)

Quail cells are found in all layers of the somite and in the developing kidney tubules. There are many quail cells in all regions of the limb bud, most of them near blood vessels. The distal edge of the myotome has dispersed into the mesenchyme of the shoulder region.

TABLE 6

CHIMAERAS ANALYZED IN DEVELOPMENTAL STUDY

CHIMAERA	AGE IN SOMITES AT TIME OF TRANSPLANT		SOMITES TRANSPLANTED	AGE WHEN ANALYZED
	Donor	Host		
474	14	16	16 - 21	5 days
480	15	16	16 - 19	5 days
484	14	16	15 - 21	4 days
557	17	17	16 - 20	5 days
558	17	18	16 - 21	6 days
561	17	18	16 - 20	3 days
562	19	19	16 - 20	3 days
584	14	15	15 - 20	Stage 17 (2.5 days)
587	20	19	15 - 20	25 (4.5 days)
589	19	19	16 - 20	17 (2.5 days)
592	16	16	16 - 19	27 (5 days)
602	15	16	16 - 18	16 - 17 (2.5 days)
604	18	19-20	18 - 20	16 - 17 (2.5 days)
613	16-17	17	18 - 20	19 - 20 (3 days)
619	20-21	19	18 - 21	28 (5.5 days)
643	21-22	19-20	19 - 22	16 (2.5 days)
645	17-18	17	17 - 20	16 (2.5 days)
685	20	20-21	16 - 20	21 - 22 (3.5 days)
686	17	18	16 - 20	19 - 20 (3 days)
689	16	16-17	16 - 20	32 - 33 (7.5 days)
709	17	17	16 - 20	16 (2.5 days)
722	19	21	16 - 20	33 - 34 (8 days)
833	19	21	17 - 19	17 (2.5 days)
855	22	22	18 - 19	19 - 20 (3 days)
907	21	20	15 - 20	14 (2 days)
1070	16	16	16 - 20	20 (3 days)
1137	19	19	16 - 19	24 - 25 (4 days)
1168	16	19	17 - 20	26 - 27 (5 days)
1184	18-19	18	16 - 19	24 (4 days)
1185	16	17	16 - 19	23 (4 days)
1189	18-19	19	16 - 19	20 (3 days)
1218	17-18	17	16 - 20	18 (2.5 days)
1219	16	17	16 - 20	28 (5.5 days)

TABLE 7

DISTRIBUTION OF CHIMAERAS IN TABLE 6 WITH RESPECT TO DEVELOPMENTAL STAGES

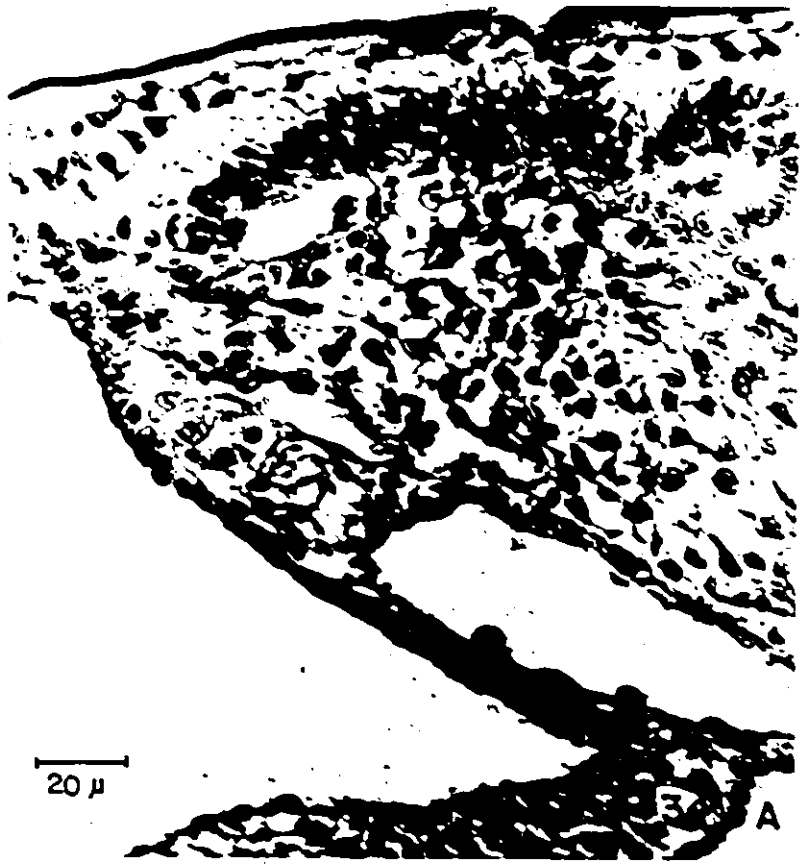
Stages of Development	Numbers of Chimaeras Analyzed
15 - 17	9
18 - 20	8
21 - 25	6
26 - 30	8
31 - 34	2

Figure 34

Cross-section through the brachial level of a stage 15 chimaera that received a graft of quail brachial somites at stage 13.

A. Somite. The grafted somite is undergoing the appropriate morphological change in a normal manner. D: dorsal wall S: developing sclerotome.

B. Limb bud. A few quail nuclei (arrows) can be seen in the somatopleure. S: somatopleure Ec: ectoderm


20 µ
A

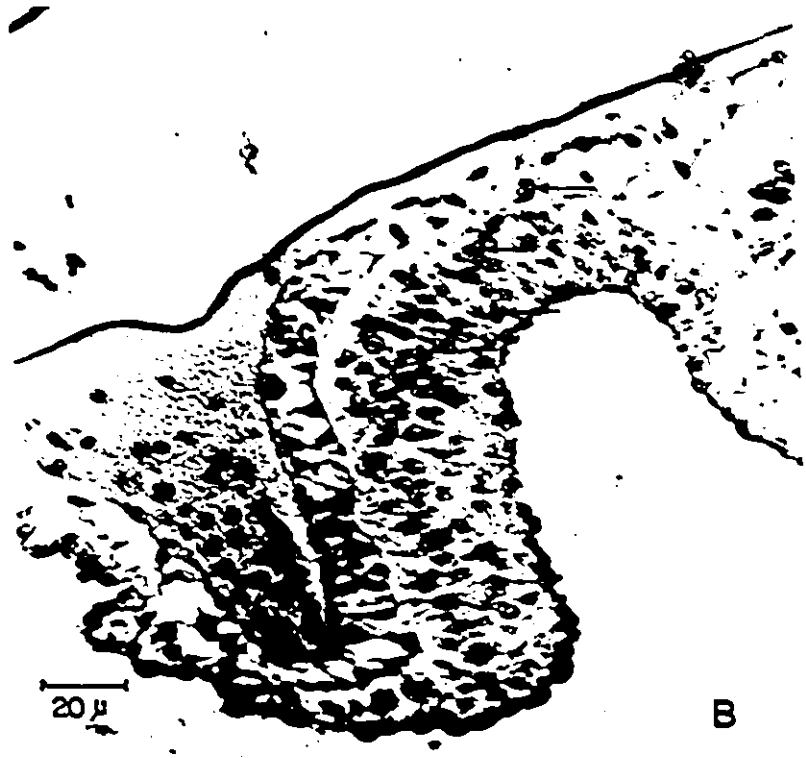

20 µ
B

Figure 35

Cross-section at the level of the wing bud of an early stage 17 chimaera that received a graft of quail brachial somites at stage 13.

A. Somite. The myotome layer is not yet fully formed. The entire somitic mesoderm is made up of quail cells. S: somite N.T.: neural tube.

B. Limb bud. Quail cells (indicated by the arrows) are present in the limb bud in small numbers.


S
20 µ
35 A

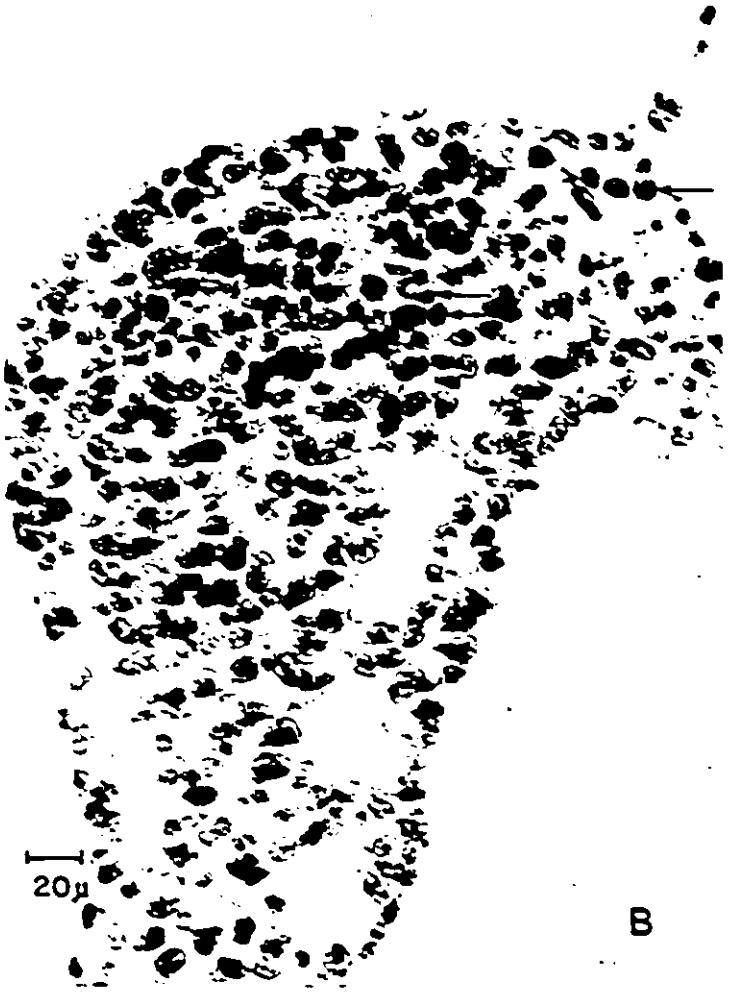
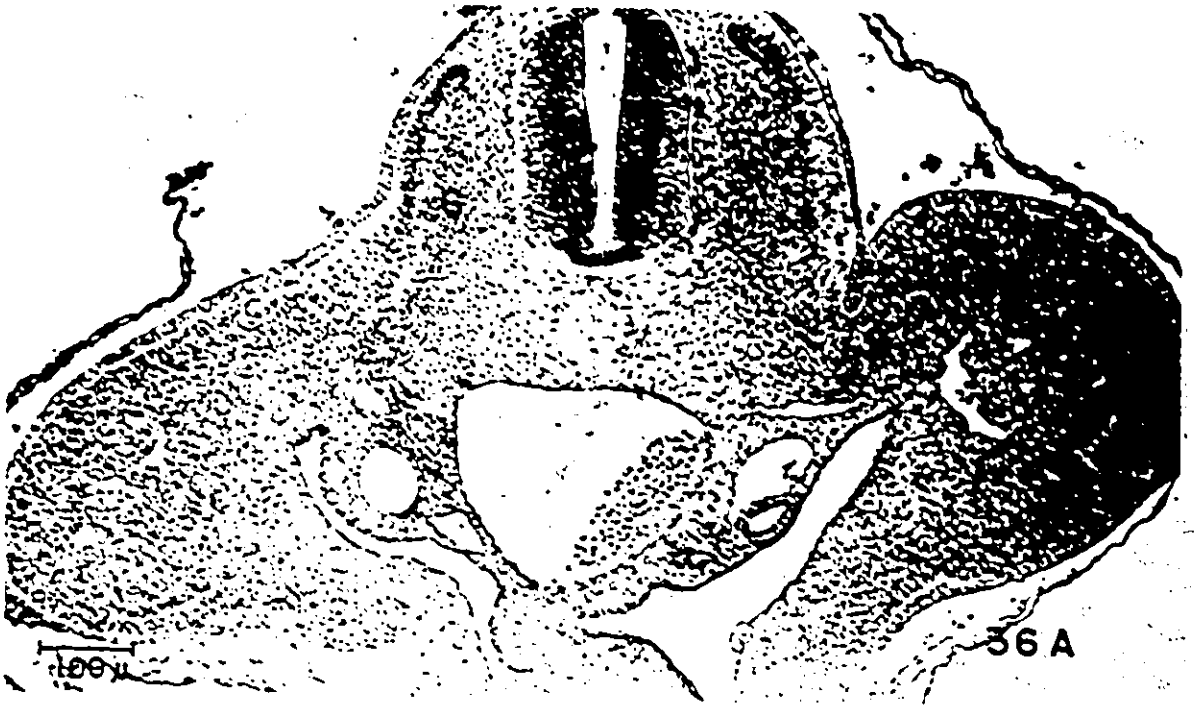

20µ
B

Figure 36

A. Cross-section at the level of the wing bud of a stage 20 chimaera that received a graft of quail brachial somites at stage 13. G: grafted region.

B. Limb bud of A. Quail cells (arrows) are seen in the limb bud in much larger numbers. They are found in all regions of the limb.


100μ
36 A


B

Stage 23 (Figure 37)

Quail cells in the limb bud appear to be migrating away from the central area of the limb, where cartilage will form. This central core is now composed largely of chick cells and is not vascularized. Blood vessels, like the quail cells, are found in more peripheral areas of the limb bud.

A number of quail cells are also found in the body wall medial to the limb. No quail cells are found in posterior levels.

Stage 24

Pre-chondrogenic areas in the somite and wing are obvious, although chondrogenesis has not begun. No quail cells are found in the pre-chondrogenic areas of the limb. They are restricted to the myogenic regions.

For the first time, quail cells can be seen in the body wall caudal to the limb.

Stage 25 (Figure 38)

The dorsal and ventral myogenic masses are evident in the limb bud. All quail cells in the limb are restricted to these two masses. More quail cells are evident in the body wall ventral and caudal to the wing.

Stage 26 (Figure 39)

The pectoralis primordium is clearly seen extending from the limb near the humerus into the body wall, which bulges outward below the wing. No myotubes can be seen yet.

The pectoralis minor muscle is represented by a very few quail cells in the medial body wall. Although other individual muscle primordia cannot be recognized in the limb on the unoperated side, the distribution of quail cells in the limb on the operated side shows that several muscle primordia have

Figure 37

Limb bud from a cross-section of a stage 23 chimaera that received a graft of quail brachial somites at stage 13. The quail cells are found predominantly in peripheral regions of the limb (encircled). The central region is nearly devoid of quail cells. C: central region; P: peripheral regions; BV: blood vessel.

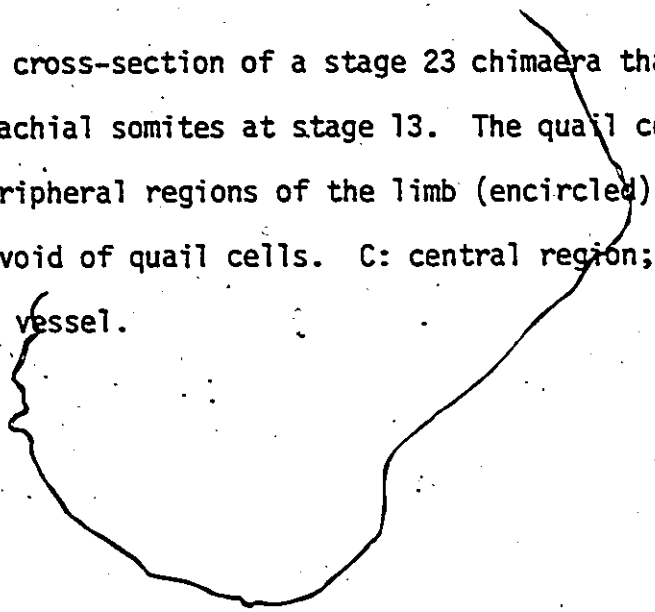
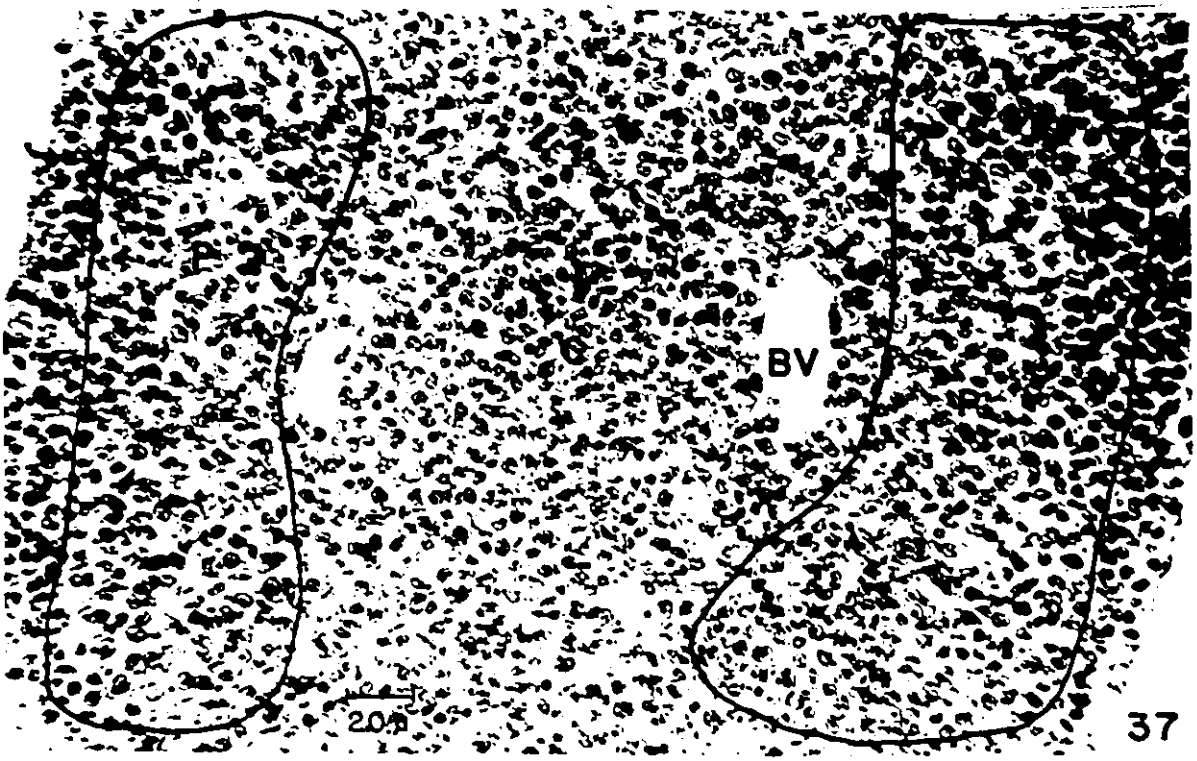
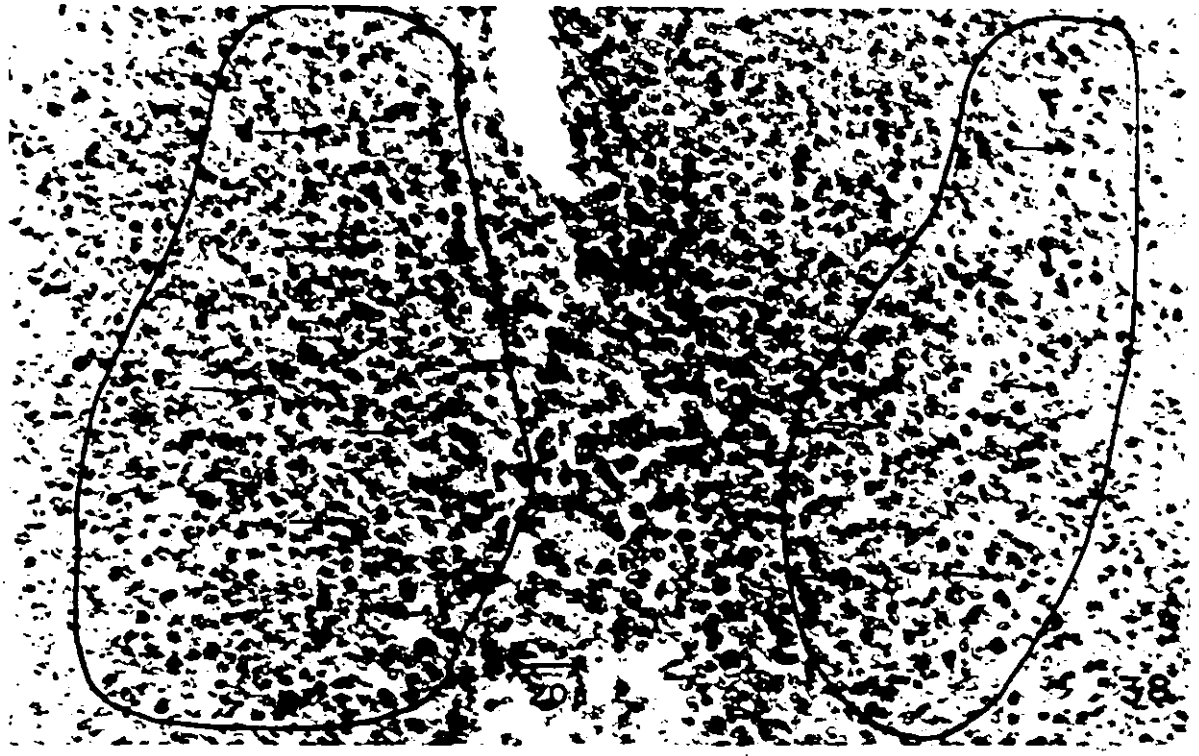


Figure 38

Limb bud from a cross-section of a stage 25 chimaera that received a graft of quail brachial somites at stage 13. As in stage 23, the quail cells are most prevalent in peripheral myogenic areas (encircled), and the central chondrogenic core is of host origin.



37



38

split away from the dorsal and ventral myogenic masses. The two masses have now become five, representing the pectoralis, biceps, triceps, deltoid, and latissimus dorsi.

Stage 28

All muscles are larger and more distinct than before. Quail cells are present in all the muscle primordia, the vertebrae, the scapula, and the dermis of the skin of the back.

Stage 32 - 34 (Figure 40)

In all muscles, spaces are present between myotubes, allowing the observation that quail cells are restricted to the myotubes, while the connective tissue is composed of chick cells. The two parts of the pectoralis major muscle are separated by connective tissue of chick origin.

Discussion

The results of this study show that cells begin migrating from the graft within a few hours after the operation and before the somatopleure thickens to form the limb bud. When the limb bud appears, all graft cells that have migrated peripherally are contained within it. Graft cells in the stage 17 limb are very few in number but are found in all regions of the mesoderm. Many more are present at stage 20. (It is not possible to determine from these results whether the quail cells increase in number by proliferation or by migration from the somites). Between stages 23 and 25, the quail cells become restricted to peripheral regions of the limb where muscle will form. At the same time the prospective muscle-forming areas become more vascularized than the prospective cartilage forming areas.

The pectoralis primordium is recognizable as a distinct entity by stage 26, although quail cells that probably contribute to it are seen in the body

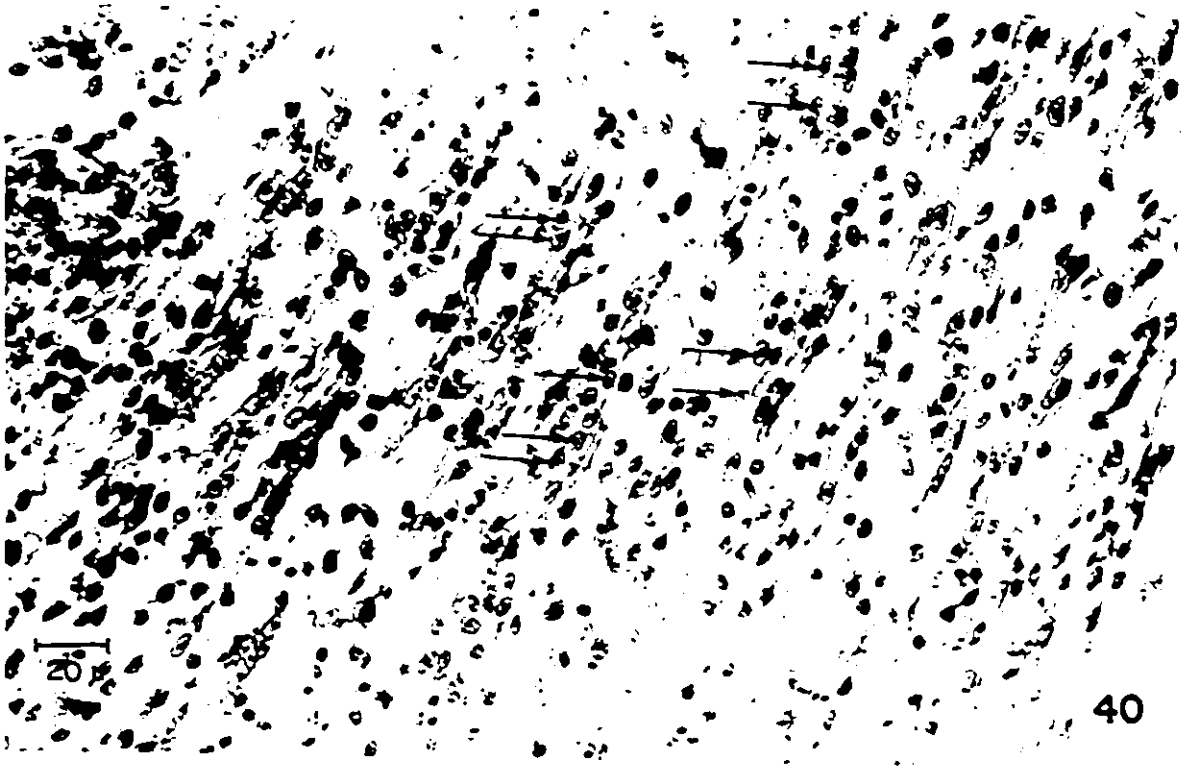
Figure 39

Pectoralis major muscle (encircled) from a cross-section of a stage 26 chimaera that received a graft of quail brachial somites at stage 13. This is the earliest stage in which the primordium of this muscle can be recognized. It consists of a mesenchymal condensation of quail (arrows) and chick cells. There are no myotubes present.

Figure 40

Pectoralis major muscle from a stage 34 chimaera that received a graft of quail brachial somites at stage 13. Quail cells (arrows) are seen in association with myotubes. Connective tissue cells are primarily of host origin.


20
39


20
40

wall medial to the limb by stage 23. By stage 24, quail cells are seen caudal to the limb. This means that cells are migrating to levels more posterior than the graft level. Myotubes are present by stage 28. As the muscle primordium grows larger, it continues to extend both ventrally and caudally. By stage 34, quail cells from the grafted somites have migrated a considerable distance away from the graft level. This is also true, though to a lesser extent, of cells in the pectoralis minor and posterior latissimus dorsi muscles.

The correlation between blood vessels, graft cells, and muscle differentiation seen in the limb from stages 20 - 25 may have relevance to studies of cell lineage. It has been suggested, and widely accepted, that limb bud mesoderm is a homogenous population of undifferentiated cells that become committed to a myogenic or chondrogenic lineage during a short but specific time period (Medoff, 1967; Zwilling, 1968; Searls and Janners, 1969; Finch and Zwilling, 1971). According to this hypothesis, prior to stage 22, each cell in the limb is capable of differentiating as muscle or as cartilage. Between stage 22 and 25, local environmental factors influence cell expression such that the cells differentiate as muscle in peripheral areas and as cartilage in the center of the limb (Caplan, 1970). The environmental cues responsible for phenotypic expression are thought to be metabolic gradients established by differential vascularization between myogenic and chondrogenic regions of the limb (Caplan, 1972). The uniform vascular network of early limb buds gives way to a vascularized periphery and an avascular core between stages 20 and 24, just prior to overt cell differentiation (Caplan and Koutroupas, 1973). This differential vascularization is believed to result in a high concentration of metabolites in peripheral areas, a condition that favours muscle differentiation, and a low concentration of metabolites in central areas, which favours cartilage development.

Such studies have been criticized on the grounds that they do not rule out the possibility that limb mesoderm is a heterogeneous population of committed myogenic and chondrogenic cells. Local environmental conditions would then favour selective migration or proliferation of one cell type over the other (Dienstmann, Biehl, Holtzer and Holtzer, 1974; Newman, 1977). My results lend support to this possibility. The limb bud mesoderm of the chimaeras is heterogeneous from the beginning, containing chick cells that form cartilage and quail cells that form muscle. My observations confirm those of Caplan and Koutroupas (1973), that myogenic cells are found in well vascularized areas. However, in my chimaeras, the cell population that contains the myogenic cell line can be recognized at earlier stages because they are also quail cells. These cells accumulate in specific areas of the limb before overt muscle differentiation occurs. It is therefore possible that cells of somitic origin are committed to a myogenic lineage from the time they enter the limb bud and are attracted to appropriate regions by environmental cues - conceivably the vascular system - before the final stages of differentiation.

An alternative explanation might be that quail cells are attracted to peripheral regions, not because they are myogenic cells, but because they are quail cells. It is conceivable that quail cells could be more strongly attracted to vascularized areas than chick cells, and that this would account for their predominance in muscle. If this were the case, in somatopleure transplants of quail to chick at stage 13, chick cells from the somites entering the quail limb somatopleure would likewise be excluded from muscle. The limb muscle would be formed from quail cells, whether the chimaera consisted of quail cells migrating into chick somatopleure or chick cells migrating into quail somatopleure. In somatopleure transplants, however, chick cells that migrate into the graft during limb formation are found exclusively

in muscle, while the cartilage is formed from quail cells (Section 2; Beresford, Le Lievre, and Rathbone, 1978). Somatopleure transplants, therefore, confirm the results of somite transplants that somitic cells form limb muscles, and these results strengthen the hypothesis that somitic cells are committed to a myogenic lineage from the time that they enter the limb region.

RESULTS

SECTION 5

SECTION 5

THE ORIGIN OF THE MYOTOME AND ITS RELATION TO MUSCLE DEVELOPMENT

Introduction

As described in the Introduction and in Section 1, the somite is originally an epithelial sphere that subsequently becomes divided into: 1) a ventral mesenchymal sclerotome that forms cartilage; 2) a 2-layered dorsal lamella, with (a) an outer layer or dermatome, which forms dermis, and (b) an inner layer or myotome, which forms skeletal muscle. It has generally been accepted that the myotome layer arises by proliferation and migration of cells from the edges of the dorsal wall of the primary somite (Lillie, 1908; Williams, 1910). However, a descriptive study by Mestres and Hinrichsen (1976) suggested that the myotome was formed by an aggregation of sclerotome cells, implying that the myotome arises from the ventral wall of the primary somite.

As interesting as the question of the origin of the myotome is the question of its fate. Previous investigators who supported a somitic origin for skeletal muscles (see Introduction) assumed that the myotome layer was the source of the myogenic cells that migrated into the lateral mesoderm. An alternative hypothesis suggests that these migrating cells may originate from the sclerotome (Elizabeth Hay, personal communication). In the secondary configuration of the somite, the myotome consists of a layer of epithelium in which the cells are held firmly to one another with little space or extracellular material between them. The sclerotome, on the other hand, consists of mesenchyme, a spongy tissue in which the cells are held together loosely and which might therefore be more likely to give rise to migrating cells.

Therefore experiments were performed in order to determine:-

1) which part of the primary somite forms the myotome of the secondary somite;
2) whether or not the myotome gives rise to peripheral muscles, and thereby

determine which part of the primary somite gives rise to peripheral muscles.

In order to carry out this study, transplants of parts of somites at stages 14 and 18 were carried out. At stage 14, when the somite is an epithelial sphere, the upper halves of somites 16 - 21 were transplanted from quail to chick. Some of these chimaeras were analyzed at stage 17 - 18, to determine which layers of the secondary somite are derived from the dorsal walls of the primary somite. Further chimaeras of this type were allowed to develop to stage 35 (9.5 days), in order to determine the contribution of the dorsal halves of the somites to peripheral muscles.

At stage 17 - 18, the upper halves of somites 16 - 21 were again transplanted; at this stage the graft would include the dermatome and myotome. These chimaeras were allowed to develop to stage 35, to determine the contribution of the dermomyotome to peripheral muscles.

The results of these studies led to the decision to perform limb-bud transplants at stage 18. These chimaeras were allowed to develop to stage 35 in order to determine the contribution of the limb bud to the pectoralis muscle.

In addition, extirpations of somite dorsal walls at stage 14, dermomyotomes at stage 18, and limb buds at stages 17 - 18 were carried out.

Results and Discussion

1. Dorsal Wall Grafts

Operations

Twenty-three chimaeras were made in which the dorsal walls of somites 16 - 21 were transplanted from quail to chick at stage 14. Two of these chimaeras were analyzed between stages 17 and 19 to determine the contribution of the dorsal wall to the myotome. Of the remaining 21 chimaeras, only two survived to stage 35 (9.5 days). These were analyzed to determine the contribution of the dorsal wall to the peripheral mustles.

Eleven chick embryo controls were subjected to removal of the dorsal walls of somites 16 - 21. No quail tissue was transplanted in their place. Two were analyzed at stage 17 - 18 to determine whether a deficiency in the somites was evident. Of the others that were left to develop to a later stage, only one survived to stage 35.

Observations

Two chimaeras that received grafts of the upper half of somites 16 - 21 at stage 14 were fixed between stages 17 and 19. In both cases, the dermomyotome of the somites were of quail origin, while the sclerotome was chick (Figure 41). These results support the prevailing view that the dorsal wall of the primary somite forms the myotome. In these chimaeras, a few quail cells were also found in the limb bud. These were assumed to be myogenic cells that had migrated from the graft, as is seen in whole somite transplants.

The results of dorsal wall removal are consistent with the results of dorsal wall transplants. At stage 20, one day after the removal of the upper walls of the somites, no dermomyotome is present (Figure 42). The ventral wall has given way to the mesenchymal sclerotome. These results support the view that the dorsal wall of the primary somite forms the dermomyotome, and the ventral wall forms the sclerotome.

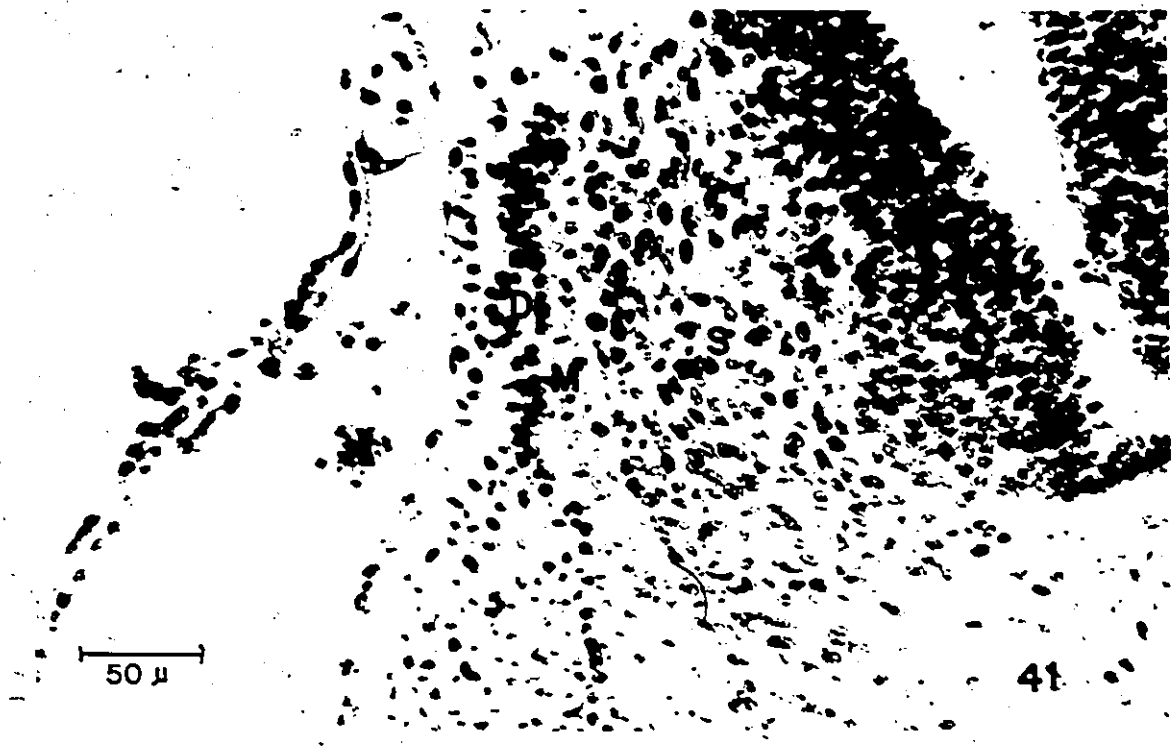
Two chimaeras that received grafts of the upper walls of somites 16 - 21 at stage 14 were fixed at stage 35 (9.5 - 10 days). The analysis of these chimaeras showed an abundance of quail cells in all of the muscles of the wing and shoulder girdle. Although the quail cells in the muscles were not counted, it is apparent that the majority of the nuclei within the myotubes are of quail origin (Figure 43).

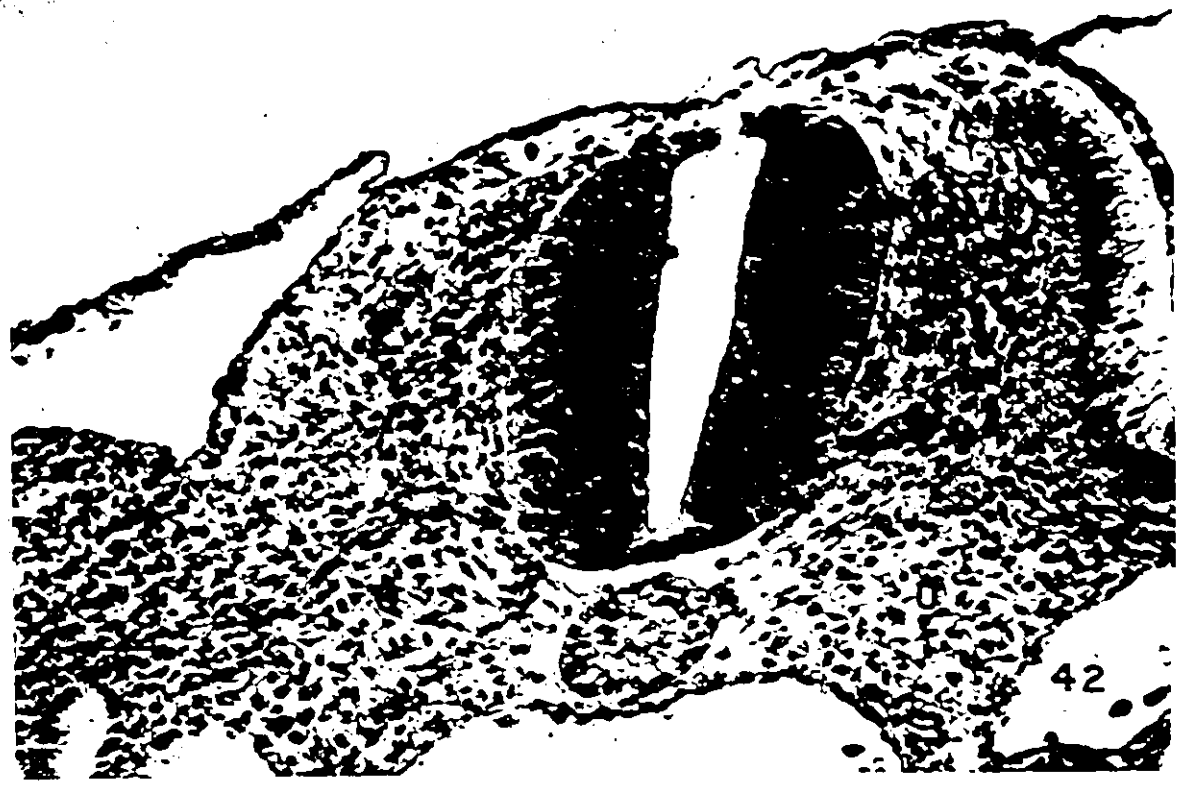
Figure 41

Cross-section of a stage 19 chimaera that received a graft of the dorsal halves of quail somites 16 - 20 at stage 13 - 14. The dermatome (D) and myotome (M) contain quail cells. The sclerotome (S) contains chick cells.

Figure 42

Cross-section of a stage 20 chick embryo from which the dorsal halves of the brachial somites were unilaterally removed at stage 13 - 14. The somite is normal on the unoperated side (U). On the operated side (O), there is no dermomyotome. All other structures appear to be normal.


D
S
50 µ
41


42

These results suggest that the dorsal wall of the primary somite is the source of the skeletal musculature that derives from the somite. Even though there are no apparent differences between cells in the dorsal wall of the somite and those in the ventral wall, it is the cells in the dorsal wall that are destined to give rise to dermis and muscle, while the ventral wall is destined to form cartilage.

One embryo from which the dorsal walls of somites 16 - 21 had been removed at stage 14 survived to stage 35. The analysis of this embryo showed no deficiencies as a result of the operation. Like the control embryos from which whole somites had been removed (Section 2), all muscles in the wing and shoulder were present and apparently normal.

2. Dermomyotome Grafts

Operations

Twenty chimaeras were made that consisted of a graft of the dermomyotomes of somites 16 - 21 from quail to chick at stage 18 (2.5 days; 32 - 36 somites). Only two survived to stage 35. They were analyzed to determine the contribution of the dermomyotome to the peripheral muscles.

Ten chick embryo controls were subjected to removal of the dermomyotomes of somites 16 - 21 at stage 17. Only one survived to stage 35.

Observations

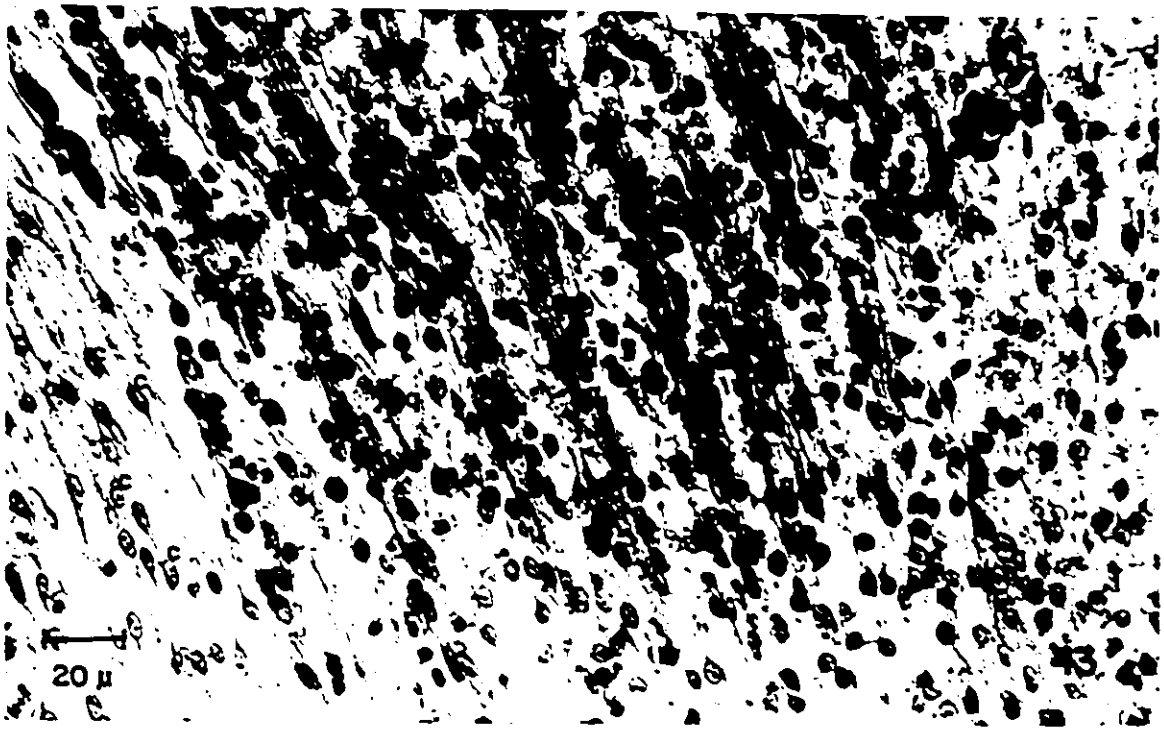
Two chimaeras that received a graft of the dermomyotomes of somites 16-21 survived to stage 35. In these chimaeras, the intervertebral muscles were derived from the graft. However, quail cells were not observed in any of the peripheral muscles of the wing, body wall, or shoulder girdle. All of the wing and wing-associated muscles were composed of chick cells (Figure 44). This result suggests that the myotome does not contain the primordium of peripheral skeletal muscles.

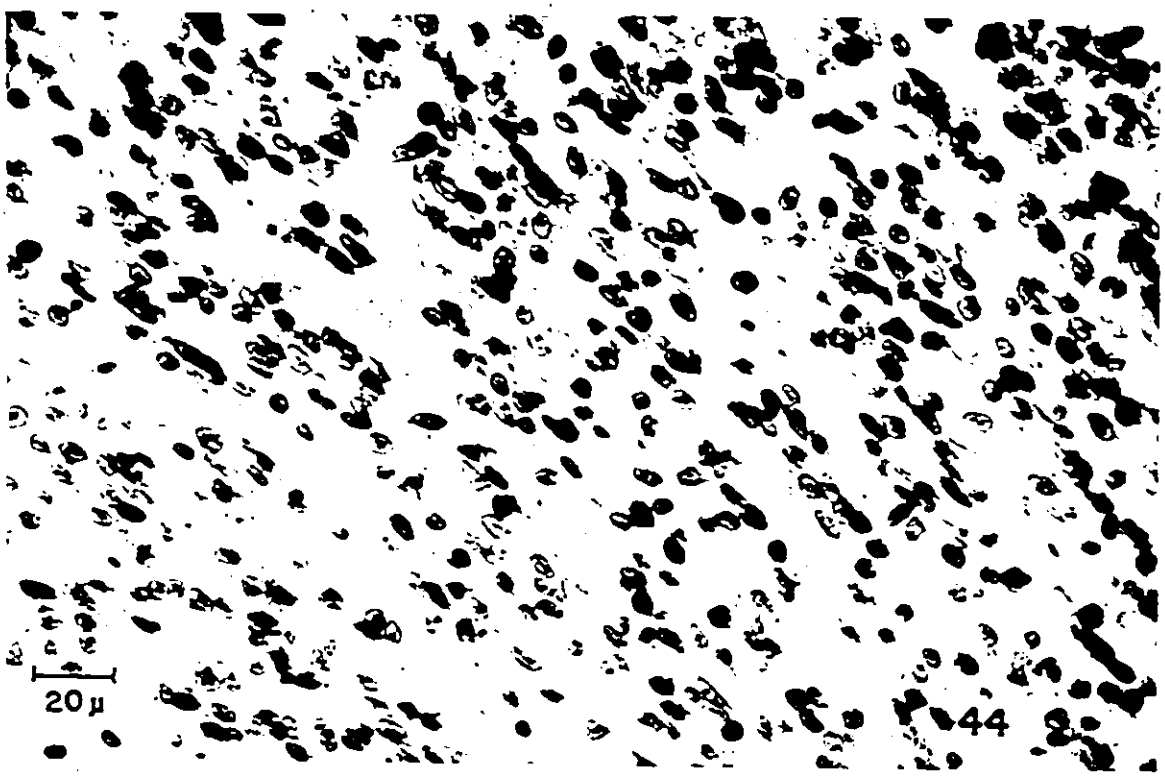
Figure 43

Pectoralis major muscle in a cross-section of a stage 35 chimaera that received a graft of the dorsal walls of quail brachial somites at stage 14. Most if not all of the nuclei associated with myotubes are quail nuclei. The connective tissue cells are chick cells. Compare with Figure 40. With respect to this muscle, transplanting the dorsal walls of the brachial somites is equivalent to transplanting entire, intact brachial somites. q: quail cells c: chick cells.

Figure 44

Pectoralis major muscle in a cross-section of a stage 35 chimaera that received a graft of the dermomyotomes of quail brachial somites at stage 17. Both muscle and connective tissue are derived from the chick host.


20 µ
43


20 µ
44

The primordia of all skeletal muscles at the brachial level are clearly in the somites at stage 14 (Sections 2, 3 and 4). More specifically, the primorida are in the dorsal walls of the somites. The dorsal walls of the stage 14 somites give rise to the myotomes of the stage 18 somites. They also give rise to all of the muscles of the wing and thorax in the stage 35 embryo. Yet the myotomes of the stage 18 somites do not give rise to these peripheral muscles.

Assuming that the results of the dorsal wall grafts and of the dermomyotome grafts are representative, it is possible that the cells destined to form the limb and thorax muscles migrate from the somite into the limb somatopleuric mesoderm between stage 14 and stage 17, and that no further migration from somite to limb occurs after stage 18.

3. Limb Bud Grafts

Operations

To test the above hypothesis, limb buds were transplanted from quail to chick at stage 18. Twenty-nine stage 18 limb transplants were performed, of which 8 survived to stage 35. Thirty control embryos were subjected to wing bud removal at stage 18, of which 8 survived to stage 35.

Observations

The chimaeras were analyzed at stage 35. All structures of the limb, both muscle and cartilage, were derived from the graft. In addition, all wing-associated muscles, including the pectoralis major, pectoralis minor, anterior and posterior latissimus dorsi muscles, were of quail origin. Both muscle and connective tissue were derived from the graft (Figure 45). These results indicate that after stage 18, there is little or no migration of myogenic cells from the somites to the limb.

Although all muscles and cartilaginous elements were present in these chimaeras, in every case the thorax was open. The two halves of the sternum had not successfully met and fused down the ventral midline. This observation indicates that the graft may not have healed properly, and therefore may have interfered with the normal migration of cells from the host somites into the donor limb. Young embryos are known to be capable of regulative activity to make up for deficiencies. It is possible that if the graft did not heal properly, the cells within the graft were effectively isolated. The myogenic cells present in the limb would be required to form all of the muscles, even though normally they would be joined by others migrating into the limb from the somites. This latter possibility would require that the limb bud at stage 18 is capable of complete differentiation, although normally it is not required to do so.

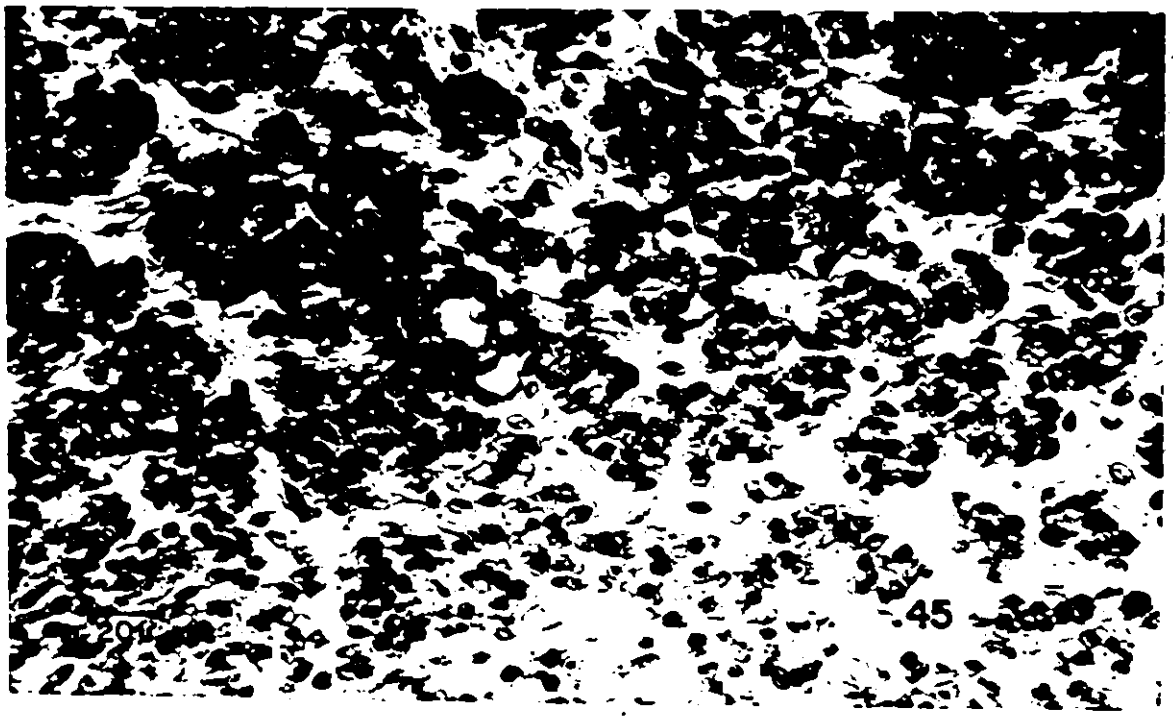
The above interpretation of the limb bud results appears unlikely in view of the fact that there is evidence that the grafted limb per se did heal properly. In particular, on the dorsal side of the graft, host and donor tissue attach to one another normally (Figure 46). Moreover, graft-derived muscles that attach the grafted limb to the host, such as the ALD and PLD, were normal in all chimaeras. There were, in short, no abnormalities in the embryos on the dorsal surface of the graft. On the ventral surface where the graft attaches to the host body wall, there is further evidence that successful healing did occur. The pectoralis major and minor muscles, of quail origin, were attached to the sternum, which is chick (Figure 47). It therefore appears most likely that the graft did heal as expected but that the chimaera developed abnormally after healing took place. Possibly the quail donor tissue was not capable of growing at an acceptable rate to permit the sternum of the operated side to meet its counterpart in the ventral midline.

Figure 45

Pectoralis major muscle in a cross-section of a stage 35 chimaera that received a graft of a quail wing bud at stage 17. The entire muscle, including connective tissue, is of quail origin. Compare with Figure 26.

Figure 46

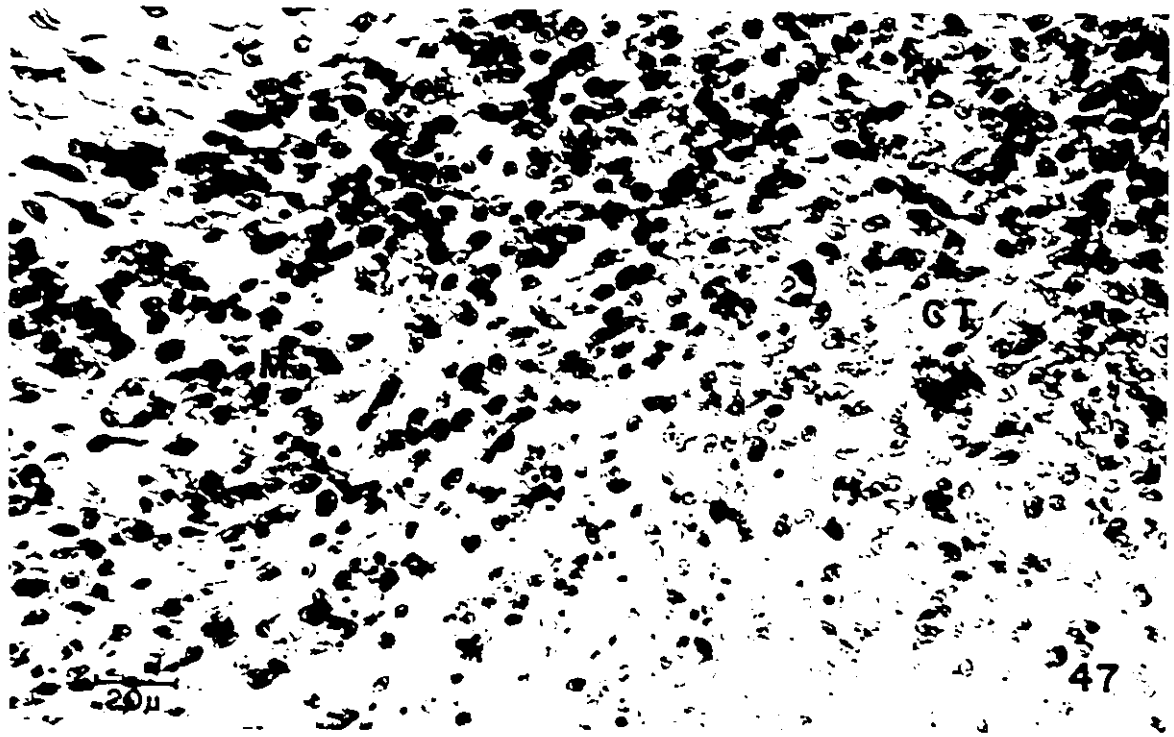
Shoulder region in a cross-section of a stage 35 chimaera that received a graft of a quail wing bud at stage 17. The ALD muscle is derived from the grafted tissue and attaches in a normal manner to a host vertebra. The scapula(S) is derived partly from grafted tissue and partly from host tissue. Two muscles (M) that are derived from the host attach the scapula to the host vertebrae in a normal manner.


45


ALD
50μ
46

Figure 47

Pectoralis major muscle from a cross-section of a stage 35 chimaera that received a graft of a quail wing-bud at stage 17. The pectoralis major muscle is derived from the grafted tissue. It attaches ventrally to connective tissue of host origin that is associated with the host sternum. M: muscle C.T.: connective tissue.


GL
47
20μ

Eight embryos that had been subjected to limb bud removal at stage 18 survived to stage 35. In every case, there was no limb and the muscles normally associated with the pectoral girdle were absent.

The results of the limb transplant and limb removal studies support the hypothesis that after stage 18, there is no further migration of myogenic cells from the somites to the limb bud. The entire migration occurs between stage 14 and stage 18.

In summary, the results of the dorsal wall, dermomyotome, and limb bud studies demonstrate the following:

The cells that are destined to give rise to the muscles of the wing and shoulder girdle are located in the dorsal walls of somites 16 - 21 at stage 14, when these somites are in their primary configuration. Between stage 14 and stage 18, as the ventral walls of the primary somites form the mesenchyme of the sclerotome, the dorsal walls give rise to myogenic cells, some of which migrate into the adjacent somatopleuric mesoderm, and some of which remain in the somite to form the myotomal layer of the secondary somite. By stage 18, when the change from the primary to the secondary configuration is complete, the migration of myogenic cells into the limb mesoderm has ended. The myotomal layer of the somite will ultimately give rise to the intervertebral muscles. The myogenic cells in the limb mesoderm will give rise to all of the muscles of the limb and shoulder girdle.

These conclusions provide an explanation for the controversy in the literature regarding the origin of skeletal muscles in the chick. When Saunders (1948) marked the brachial somites of 2.5 - 4 day embryos, he subsequently found no carbon particles in peripheral tissues. He concluded that somites do not contribute to limb structures. My results suggest that the somitic contribution to the limb is complete by 2.5 days. Saunders, therefore, marked the somites after the migration had occurred.

This argument can similarly be applied to the study by Straus and Rawles (1953). These investigators were concerned with the origin of trunk muscles and did not study the limb, but like Saunders, they performed their experiments at 2.5 - 3.0 days of development. Their studies showed that somites did not contribute to the peripheral muscles and that the somatopleure contained myogenic cells at the time of the marking. If abdominal muscles are derived from somites in a manner similar to limb muscles, these experiments, like Saunders, were performed after the migration of myogenic cells from somite to somatopleure had been completed.

On the other hand, Seno (1961) performed similar experiments on 2.0 - 2.5 days in ovo embryos. His results showed a somitic contribution to abdominal muscles. He attributed the difference between his results and those of Straus and Rawles to a difference in the technique of applying carbon particles to the tissue in question. However, his results would be expected if the migration of myogenic cells from the somites into the somatopleure was occurring at this time.

Perhaps the most important point that these results emphasize is that the timing of such experiments is crucial to the outcome. If an experimentor wishes to study the contribution of somites to limb muscles in the chick embryo, the appropriate time to perform his experiments is not when the limb bud forms, but when the limb somites form. This point should be kept in mind when interpreting the results of similar experiments on all classes of vertebrate embryos. My results and those of others working on the chick embryo (Christ et al., 1974a, b; 1977, 1978; Chevallier et al., 1977, 1978) show that skeletal muscles in birds are derived from somites. This may be the case in amphibians, reptiles, and mammals also. All of the experimental results mentioned in the Introduction that have been interpreted to show a somatopleural origin for skeletal muscles are consistent with

the hypothesis that myogenic cells migrate into the periphery over a short, specific time period that begins as soon as the somites are formed. If the experiments are performed after this migration has occurred, the results will show that the somatopleure can give rise to muscle. However, if the experiments are performed before this migration takes place, the results will support a somitic origin for skeletal muscle.

Several other investigators have attempted to define the period of migration using descriptive studies. There is general agreement among them that cells leave the somite and enter the somatopleure up to stage 18 (Grim, 1970; Gumpel-Pinot, 1974; Christ et al., 1976; Jacob et al., 1978; see page 180 for these references). My results are consistent with this view and are, at present, the only experimental results that have established an end point.

FINAL DISCUSSION

DISCUSSION

The embryonic origin and formation of the pectoralis major muscle have been investigated in interspecific chimaeras between quail and chick. My observations are as follows:

1) The in ovo transplantation of quail brachial somites orthotopically into chick embryos at stage 13 leads to the appearance, at stage 35, of quail nuclei in the pectoralis major muscle, as well as in all the other wing and shoulder muscles on the operated side. Quail nuclei are found in and associated with myotubes. The connective tissue of the muscle is made up of chick cells (Section 2).

2) The in ovo transplantation of any one of somites 16 - 21 orthotopically from quail to chick at stage 13 leads to the appearance, at stage 35, of quail nuclei within some of the myotubes of the pectoralis major muscle. The majority of these quail nuclei are restricted to one region of the muscle. The region occupied by the quail nuclei is dependent upon the somite that is transplanted. The transplantation of somites anterior to somite 16 or posterior to somite 21 results in a pectoralis major muscle consisting entirely of chick cells (Section 3).

3) The in ovo transplantation of quail brachial somites orthotopically into chick embryos at stage 13 leads to the appearance of quail cells in limb somatopleure as early as stage 15. The number of quail cells in the limb mesoderm increases at each stage of development. From stage 23 onward, it is apparent that quail cells are restricted to the myogenic regions of the limb and body wall. Overt muscle differentiation begins at approximately stage 27 - 28, when mononucleated cells of quail origin fuse to form multinucleated myotubes. By stage 35, the two halves of the sternum have met

and fused in the ventral midline of the embryo, so that the pectoralis major muscle, and the other wing and shoulder muscles, have reached their definitive positions (Section 4).

4) The in ovo transplantation of the upper (dorsal) halves of somites 16 - 21 orthotopically from quail to chick embryos at stage 13 leads to the appearance of quail cells in the dermatome and myotome of the somite at stage 17. A few quail cells are also seen in the limb bud mesoderm. When similar chimaeras are allowed to develop to stage 35, quail cells are found primarily associated with the myotubes of the pectoralis major muscle, as well as all the muscles of the wing and shoulder (Section 5).

5) The in ovo transplantation of the dermomyotome of somites 16 - 21 orthotopically from quail to chick embryos at stage 18 results in a pectoralis major muscle consisting entirely of chick cells at stage 35. All other wing and shoulder muscles are also composed entirely of chick cells. The only muscles that contain quail cells are the intervertebral muscles at the graft level (Section 5).

6) The in ovo transplantation of the wing bud orthotopically from quail to chick embryos at stage 18 leads to the appearance, at stage 35, of quail cells in both myotubes and connective tissue of the pectoralis major muscle. This muscle and all other wing and shoulder muscles, as well as all cartilaginous elements of the wing, are composed exclusively of quail cells (Section 5).

These observations lead to the following conclusions: Cells that are destined to form the skeletal muscles of the brachial level, including the pectoralis major, are located in the dorsal walls of the somites 16 - 21 at stage 14. Between stages 14 and 18 (2 - 2.5 days in ovo), myogenic cells migrate from the dorsal wall in two directions: 1) ventrally, where they will form the myotomal layer of the somites and ultimately the intervertebral

muscles, and 2) laterally into the limb mesoderm, where they will form the wing and wing-associated peripheral musculature. Each somite plays a unique role in the formation of the brachial muscles. The myotomal layer of the somites does not contribute to peripheral muscles.

Validity of Technique

The above conclusions are based on the premise that quail tissue develops in the chick host in precisely the same manner as the chick tissue that it replaces, provided that (1) the donor and host are at equivalent stages of development; (2) the grafted tissue is placed in the same region of the host as the region of the donor from which it was removed, i.e. orthotopically, and (3) the original dorsoventral, anterior-posterior, and medio-lateral orientations of the tissue are retained during the grafting procedure.

There is one publication in the literature that calls into question the validity of quail tissue as a marker for developing tissue in the chick. In this study (Chevallier, Kieny and Mauger, 1977), the origin of limb muscles was investigated by grafting quail somites into chick hosts and vice versa. According to the authors, when quail somites were grafted to chick hosts, all the nuclei in the myotubes of the wing muscles were quail nuclei; but when chick somites were grafted to quail hosts, the muscles contained a mixture of chick and quail nuclei, and some muscles were formed entirely by quail cells. The authors speculate that, since quail embryos take 16 days to reach hatching and chick embryos take 21 days, quail tissue may have an intrinsically faster rate of development than chick tissue. Quail cells in both chick and quail hosts would begin to form muscle earlier and "prematurely occupy available spaces" (Chevallier et al., 1977). This phenomenon has been encountered in grafting experiments in insect

embryos (Morata and Ripoll, 1975) and in some mouse aggregation chimaeras (Peterson, 1978). In both cases, cells from one source become dominant in one or more tissues. Chevallier et al. (1977) suggest that this may be occurring in their chick-quail chimaeras. The evidence they provide in support of this hypothesis consists of three photographs of skeletal muscles showing a mixture of chick and quail nuclei in the myotubes. These muscles were found in one chimaera that consisted of a heterotopic graft of chick leg somites to the wing level of a quail host.

The suitability of this chimaera as evidence for the hypothesis discussed above is doubtful for two reasons:

1) According to the figure legend accompanying the photograph (Chevallier et al., 1977, Figs. 10 - 12), prospective wing somites 16 - 20 were removed from the quail host, which had 15 pairs of somites at the time of the operation, and replaced with prospective leg somites 26 - 32 from the chick donor, which had 19 pairs of somites. It is questionable whether the donor tissue was developmentally equivalent to the tissue that it replaced. Somitic mesoderm that is not scheduled to develop somites for many hours may have a reduced capacity to give rise to migrating myogenic cells when it is placed at a developmentally more advanced level.

2) Previous studies by members of this same group of investigators (Kieny, Mauger and Sengel, 1972) have shown that the cartilage derivatives of somites are determined in the somitic mesoderm before the somites form and cannot be altered by grafting the somitic mesoderm to a different level. Therefore, heterotopic grafts of leg somites to the wing level must have resulted in cartilage derivatives that were inappropriate for the wing level, although this is not mentioned by Chevallier et al. (1977). My own studies have shown that structural abnormalities at the level of the graft often

result in a reduced participation of graft-derived cells in the muscles derived from that level (Section 2). The reduced participation of leg somites in the formation of wing muscles observed by Chevallier et al. (1977) could be explained by the presence of atypical cartilaginous structures.

It is worth repeating in this context that in numerous studies performed by Le Douarin and her colleagues (see Introduction), involving chick embryos that received grafts of quail tissue and quail embryos that received grafts of chick tissue, the results of both types of chimaeras were the same. There was no indication that quail cells had a developmental advantage over chick cells.

In the absence of well-documented evidence to the contrary, and in view of the careful studies carried out by the originator of the technique, the validity of quail somites as a "biological" marker in chick hosts has been assumed throughout the course of the present investigation.

Relationship between Somites and Skeletal Muscles: Recent Investigations

During the course of the study presented in this thesis, a number of publications appeared that confirmed and extended the studies by Christ et al., (1974 a, b) showing a somitic origin for wing muscles. Christ, Jacob and Jacob (1977) reported their earlier studies in a more complete form and added the results of somatopleure transplants that, like those done by Christiane Le Lievre (discussed in Section 2), show that wing somatopleure forms the cartilage and connective tissue, but not the muscles of the wing. The study by Christ et al.(1977) was carried out using both somite and somatopleure grafts of quail to chick; the inability of the somatopleure to develop muscle was further tested by grafting quail wing somatopleure into the intra-coelomic cavity or onto the chorio-allantoic membrane of a chick host. The results led the authors to conclude that the wing somato-

pleure at stage 12 - 13 *in ovo* is incapable of forming skeletal muscle.

Another group has published the results of similar studies that were designed to test the role of the somite in the formation of wing muscles (Chevallier, Kieny and Mauger, 1977). They too grafted quail brachial somites orthotopically into chick hosts at stage 13 - 14. Their results, like my own observations and those of Christ *et al.* (1974 a; 1977), show that graft cells contribute to the musculature of the wing but that the cartilage and connective tissue is formed by host cells. Additional experiments by these authors (Chevallier *et al.*, 1978) included the replacement of brachial somites with quail non-somitic tissue, the destruction of somitic tissue by X-irradiation, or somite removal. Somite removal did not prevent the formation of wing muscles. Replacing somites with a piece of quail digestive tube also did not prevent the wing muscles from developing, and these muscles were entirely of host origin. Somite destruction by X-irradiation, however, led to severe deficiencies in the wing musculature. The authors concluded that somites are the source of the wing musculature and that the regulative ability of the embryo enabled it to develop wing muscles in the absence of wing somites or when these somites had been replaced with non-somitic tissue.

My results are in agreement with the somite transplant and somite removal investigations discussed above. When quail brachial somites are grafted orthotopically into a chick host at stage 13 - 14, graft cells are found in the musculature of wing and shoulder muscles. The removal of brachial somites from a chick embryo at stage 13 - 14 does not prevent the formation of wing and shoulder muscles.

Christ *et al.* (1978b) performed transplants of the dorsal walls of primary somites from quail to chick. After two more days of incubation, quail cells were found in the dermatome and myotome, but not the sclerotome. These

results, like mine, substantiate the view that the dorsal wall of the primary somite gives rise to the dermomyotome of the secondary somite, and the ventral walls form the sclerotome.

Chevallier (1978) investigated the migration of cells from the somite into the periphery. He labelled chick donor somites with tritiated thymidine and grafted them orthotopically into chick hosts at stage 13. He found labelled cells in the somatopleure within a few hours after the transplantation was performed. His examination of chimaeras at later stages led him to conclude that cells no longer migrated from the somites into the somatopleure after stage 17. His conclusion is based on the fact that the dermomyotome was fully differentiated by stage 18 and that from stage 18 onward there were no labelled cells in the immediate vicinity of the lateral edge of the dermomyotome.

The combined results of the dermomyotome and limb bud transplants that I performed on stage 18 embryos suggest that myogenic cells migrate from the brachial somites to the somatopleure between stages 13 and 18, and that by stage 18, the limb bud contains the progenitor cells of the entire musculature of the limb. These results are compatible with those of Chevallier (1978).

The results of the brachial somite transplants (Section 2) and, more particularly, of the single-somite transplants (Section 3) have shown that somites 16 - 21 give rise to the pectoralis major muscle and all other wing and shoulder muscles. This conclusion is based on the fact that when any or all of somites 16 - 21 are grafted from a quail to a chick embryo at stages 12 - 14, quail cells are found in some or all of these muscles at stage 35. Furthermore, similar grafts of somites above or below this level result in wing and shoulder muscles that are composed entirely of host cells.

In a recent publication, Chevallier (1979) concluded that the somites which gave rise to the pectoralis major muscle are somites 12 - 22 inclusive and that all other wing and thorax muscles arise from somites 12 - 20. This is a much larger region than my experiments indicate.

As in his previous studies, Chevallier (1979) grafted quail somites or somitic mesoderm to chick hosts at 2 days in ovo and analyzed the chimaeras between 4 and 11 days after the operation. His experiments consisted of the following series of orthotopic grafts: Somites 10 - 15, 12 - 17, 15 - 20, 17 - 22 and 19 - 26. When he grafted somites 10 - 15, the wing and thorax muscles were entirely composed of chick cells in some chimaeras while in others the muscles contained a mixture of chick and quail cells. When he grafted somites 12 - 17 or 15 - 20, all wing, shoulder and thorax muscles, including the pectoralis, were composed entirely of quail cells in all cases. These results led him to conclude that somites 12 - 20 give rise to all wing, shoulder and thorax muscles. When he grafted somites 19 - 26, no quail cells were found in any wing and shoulder muscles, but in a few cases there were quail cells in pectoralis. These results, and the results of grafting somites 12 - 17 and 15 - 20, led him to conclude that the pectoralis arises from somites 12 - 22. These results, however, do not warrant such a conclusion. If somites 12 - 20 all contribute myoblasts to the muscles of the wing, shoulder and thorax, a graft of somites 12 - 17 or of somites 15 - 20, which contain only a portion of this region, should result in a chimaera with at least some muscles composed of a mixture of chick and quail cells. Chevallier, however, found that all wing and shoulder muscles were composed entirely of quail. A graft of somites 19 - 26, which contains part of the region that gives rise to these muscles, should result in a chimaera with quail cells in at least some of the wing

muscles, but Chevallier found quail cells only in the pectoralis. A graft of somites 10 - 15 should also result in a mixture of quail and chick in at least some of the muscles; Chevallier had some chimaeras like this, but others had no quail cells in the pectoralis, and some had no quail cells in the wing muscles. The author makes no attempt to explain how all of the muscles can arise from a portion of the region that normally gives rise to them, nor why a group of chimaeras with apparently identical grafts do not all show a similar distribution of quail cells in the muscles.

The results of Chevallier's study can perhaps be understood when it is considered that his operations involved grafting unsegmented somitic mesoderm rather than fully formed somites. All of the chimaeras shown in his figure received grafts of unsegemented or partially segmented somitic mesoderm. This is acceptable when the investigator is interested in finding the approximate region that gives rise to the structures he is interested in, but in order to define precisely which somites are involved, it is necessary to graft fully segmented mesoderm. The entire length of unsegmented somitic mesoderm that lies between the last-formed somite and Hensen's node at the caudal extreme of the embryo is referred to as the segmental plate. The absolute length of the segmental plate increases as development progresses, and yet at each stage, it contains the potential to form approximately 12 somites (Packard and Jacobson, 1976). It is therefore difficult if not impossible to determine the precise position of any somite that has not yet become fully formed, with the possible exception of the most anterior prospective somite in the segmental plate. It is likely that in Chevallier's experiments, he miscalculated the positions of the more caudal prospective somites. This is particularly likely to occur when most or all of the grafted region is unsegmented. Moreover, in some cases, he grafted tissue from a donor that was not at the same stage of development as the host

(Figure 5, Chevallier, 1979). In such chimaeras, the donor tissue may not be developmentally equivalent to the host. Chevallier's results must therefore be interpreted with caution.

Contributions to the Current State of Knowledge Regarding the Origin of Skeletal Muscle

Transplants of quail brachial somites to chick embryos at stage 13 (Sections 2, 3, and 4) show that the musculature is derived from somites, while the connective tissue is derived from somatopleure. The evidence in favour of a somitic origin for all skeletal muscles in the chick is now extensive (Christ et al., 1974a, b; 1977; 1978a, b; Chevallier et al., 1977; Chevallier, 1978; 1979). The present study confirms this, and is in general agreement with this view; in addition, it shows that all muscles of the shoulder girdle including the pectoralis major are derived from the six brachial somites. In the case of the pectoralis, it explains the divergent opinions concerning the origin of this muscle. Both Seno (1961) and Pinot (1969) assumed that it arose at the thoracic level, since this is the area it occupies in the adult chicken. Seno's conclusion in favour of a somatopleural origin for the pectoralis was based on the fact that carbon particles placed in the somatopleure opposite thoracic somites 19 - 28 were subsequently found in the muscle. In all probability, however, he marked the source of the connective tissue of this muscle. Cells from the brachial somites migrate laterally, and later, caudally into the thoracic somatopleure during the development of this muscle. Pinot (1969) destroyed somites 21 - 25. When the muscle formed, she concluded that it arose in the somatopleure; it is likely, however, that it formed from the undamaged somites 16 - 20.

The present study confirms Sullivan's (1962) observation that the pectoralis develops at the wing level. All of the muscles considered in this study attach to the wing. All of them originate from somites at the wing level. Subsequent morphogenetic movements may carry the bulk of a muscle some distance caudal to the level of origin, but each one retains an anatomical attachment at the level of its embryonic origin.

The removal of brachial somites does not prevent formation of the wing and shoulder muscles (Section 2). Similar results were obtained by Chevallier et al. (1978). Although there are deficiencies at the site of the operation, more peripheral structures are apparently normal. These results could be interpreted to mean that somites are not required for the formation of peripheral muscles. This was the conclusion of Lewis (1910) and Detwiler (1936) in amphibian embryos. However, the evidence of quail somite and somatopleure grafts (Christ et al., 1974a, b; 1977; Chevallier et al., 1977; 1978; the present study), somite destruction (Chevallier et al., 1977), and the evidence of the ability of intact somites to take over the role of damaged somites (Section 3) all support the view that somites are the source of the skeletal musculature, but that development can be regulated to make up for their loss if need be. It is possible that the situation is similar in the amphibian embryo. The results of Lewis (1910) and Detwiler (1936) are not incompatible with this conclusion.

Transplants of the dorsal walls of primary somites show that this region is the source of the dermomyotome (Section 5). This confirms the prevailing view, and refutes the conclusion of Mestres and Hinrichsen (1976) that the myotome arises from the sclerotome.

The dermomyotome and limb-bud transplants at stage 17 indicate that by this time in development, the migration of myogenic cells from somites to somatopleure is complete (Section 5). These results suggest an explanation for the findings by Straus and Rawles (1953), who concluded that

ventral muscles arise from somatopleure. They marked the somites or somatopleure of 2.5 to 3 day in ovo chick embryos. Their results showed that somites formed only the dorsal muscles, while somatopleure formed the ventral muscles. But by 2.5 days in ovo, the migration of myogenic cells from somites to somatopleure is complete. There are indeed myogenic cells in the somatopleure, but they have previously migrated to this region from the somites. The somites still contain myogenic cells, but these cells remain in the somite and form the intervertebral muscles.

Similarly, other studies on embryos of other classes of vertebrates may lead to erroneous conclusions for the same reasons. The situation in the chick invites a re-evaluation of studies in other classes of vertebrates that employed methods and reasoning similar to those discussed above.

Possibilities for Future Studies

Somite and limb-bud transplants between quail and chick embryos have permitted the location of muscle primordia in 2 to 2.5 day in ovo embryos. Surgical manipulations of embryos this early in development is easier and more successful than at later stages, when embryos are enveloped in extra-embryonic membranes and have become highly vascularized. The information about muscle development that has been acquired during the course of this investigation could be used to study other interesting problems in development.

1. Environmental influences on the pathogenesis of muscular dystrophy

The original purpose of the present study was to define the region of the 2-day in ovo embryo that gives rise to the pectoralis major muscle. This information would make it possible to transplant this region between normal chick embryos and chick embryos carrying the gene for muscular dystrophy, in order to study the effects of early environmental influences

on the pathogenesis of this disease. In the normal embryo, the pectoralis major muscle derives from somites 16 - 21. However, before the study described above is carried out, it would be advisable to perform grafts of quail somites 16 - 21 into dystrophic embryos at 2 days in ovo, in order to determine whether this muscle is derived from the same region in the dystrophic as in the normal chick embryo. Although the probability that the pectoralis is derived from a different region in the dystrophic embryo may seem remote, it must be kept in mind that normal and dystrophic cells cannot be distinguished histologically by any means other than the phenotypic expression of dystrophic traits. Since both varieties belong to the same species, graft-derived cells in a dystrophic-normal chimaera will appear identical to host-derived cells unless one type is expressing features of dystrophy and the other is not. Moreover, since the initial abnormality in dystrophic birds is not known, the possibility of an abnormal site of origin of the affected muscles in the dystrophic animal cannot be ruled out.

If the region that gives rise to the pectoralis in the dystrophic embryo is the same as that in the normal embryo, it would be possible to transplant the primordium of the pectoralis major between normal and dystrophic embryos at 2 days in ovo. The muscle could be analyzed subsequently for the presence or absence of dystrophic characteristics.

Although differences between normal and dystrophic muscles have been found in embryos before hatching (Cosmos, 1964; Weinstock and Dju, 1967; Stewart et al., 1977) the most definitive differences develop after hatching when the muscles undergo functional maturation (Cosmos and Butler, 1967; Cosmos, 1970). Chimaeras made between normal and dystrophic embryos would have to be hatched in order to determine whether the definitive dystrophic characteristics of a muscle are imposed by the host environment or inherent

in the muscle itself. Unfortunately, it has proven to be exceedingly difficult to hatch embryos that have been subjected to grafting procedures at 2 days in ovo. It may not be possible to hatch sufficient numbers for such an analysis.

The results of the limb bud transplants suggest an alternative method of hatching chimaeras. Since the cells that will give rise to the pectoralis muscle are contained within the limb bud at stage 18, grafting limb buds between normal and dystrophic embryos at this stage is another means of transplanting the primordium of the pectoralis. Limb bud transplants are far less traumatic than somite transplants and are therefore more likely to permit the chimaera to hatch. If it is desirable to place the primordium of the muscle in a different host at stage 13 rather than stage 18, the somites could be transplanted at stage 13, and the resulting chimaeric limb bud could be grafted to a second host at stage 18. It would thus be possible to study almost the entire in ovo and ex ovo development of a muscle of one variety of bird in the natural environment of another.

2. Environmental influences on muscle fiber type

Grafting of quail somites 16 - 21 to a chick embryo at 2 days in ovo has been carried out in the present study in order to map the origins of the pectoralis major muscle. Similar grafts could be carried out for another purpose, i.e. to determine the influence of the environment on muscle fiber type. In the chick, the pectoralis major is a nearly homogenous muscle consisting of white fast-twitch glycolytic fibers (Cosmos, 1966; Cosmos and Butler, 1967; Cosmos, 1970). In the quail, the pectoralis consists of two fiber types: fast-twitch glycolytic and fast-twitch oxidative-glycolytic (E. Cosmos, personal communication). The latter fiber type can be distinguished from the former by appropriate histochemical staining procedures. It is well established that the innervation of a muscle fiber influences

its metabolic characteristics (Guth, 1968). In the chicken, the denervation of a fast-twitch glycolytic muscle and its subsequent re-innervation by a nerve that normally innervates a slow tonic oxidative muscle, if performed soon after hatching, will result in a conversion of at least some of the fast-twitch glycolytic fibers to slow tonic oxidative fibers (Mazliah, Cosmos and Butler, 1978). However, the fast-twitch oxidative-glycolytic fiber type seen in the quail is not present in the pectoralis of chickens (E. Cosmos, personal communication). If chick embryos that received grafts of quail brachial somites at 2 days in ovo could be hatched (as discussed above) and their muscles analyzed several weeks ex ovo, after metabolic maturation occurs, it would be possible to determine whether the quail oxidative-glycolytic fiber type could be expressed in the environment of a chick host. If the quail fiber type did appear in a quail muscle that was innervated by chick neurons, it would demonstrate that this fiber type is capable of expressing metabolic characteristics that are not dictated by its innervation. Similarly, if quail embryos that received grafts of chick brachial somites at 2 days in ovo could be hatched and their muscles analyzed several weeks later, it would be possible to determine whether a quail nerve could direct a chick muscle to express quail fiber type characteristics.

3. Cell lineage studies

Grafting of quail brachial somites at 2 days in ovo leads to the appearance of quail cells in the limb bud by stage 17. At later stages, these quail cells are invariably found in the muscles of the wing, while the cartilage is invariably of host origin. Although overt muscle and cartilage differentiation does not occur until approximately stages 25 - 27 (Sections 1 and 4; Caplan and Koutroupas, 1973), the segregation of prospective myogenic and chondrogenic cells is apparent by stage 23 (Section

4). This strongly suggests that the wing bud mesenchyme is not a homogeneous population of multipotent cells, as suggested by Searls and Janners (1969) and Caplan and Koutroupas (1973) (discussed in Section 4), but that from the time it first becomes visible, it is a heterogeneous population of prospective myogenic cells of somitic origin, and prospective cartilage and connective tissue cells derived from the lateral mesoderm.

This latter hypothesis has been suggested by previous investigators (Bienstman et al., 1974; Newman, 1977), but attempts to resolve the controversy have not been successful because prospective myogenic and chondrogenic cells cannot be distinguished prior to stages 24 - 25, when myogenic and chondrogenic regions of the unoperated chick limb become apparent. The chimaera limb bud could be a useful preparation for investigating this problem. Limb bud mesenchyme from a chimaera can be isolated from surrounding tissues as early as stage 20, and broken apart by gently pipetting in order to mix the cells thoroughly. This mesenchyme can then be treated in one of two ways: 1) it can be packed into a "jacket" of chick wing bud ectoderm and grafted to the flank of a chick host; in this situation, the limb mesenchyme is capable of differentiating into both muscle and cartilage (Zwilling, 1972); or 2) it can be placed into several small culture dishes; some of these cultures can then be placed into environmental conditions that are optimal for muscle differentiation, while the others can be placed into environmental conditions that are optimal for cartilage differentiation (Caplan and Koutroupas, 1973). In both cases (1 and 2) the experimental tissue can subsequently be analyzed to see whether the quail cells have participated in the formation of cartilage, and whether chick cells have been able to differentiate into muscle. If this has occurred, it would indicate that at the stage when the limb bud mesoderm was isolated, the cells had not become committed to either a myogenic or a chondrogenic lineage and that subsequent commitment to a particular lineage was dependent

on environmental conditions. However, if quail cells had formed only muscle and chick cells had formed only cartilage, it would suggest that at the time the limb mesoderm was isolated, quail cells were committed to a myogenic lineage and chick cells to a chondrogenic lineage.

4. Nerve - target specificity

One of the most intriguing problems facing neurobiologists is to identify the mechanisms that direct nerves to form connections with specific end-organs. Among individuals of the same species, a given structure invariably receives innervation from a specific spinal nerve or nerves. Several theories have been advanced to account for this remarkable regularity (reviewed by Landmesser and Morris, 1975; Frank, 1977). Conceivably, an excessive number of neurons could grow out randomly and establish connections with the nearest receptive target; inappropriate connections would subsequently be lost. Alternatively, a neuron may be programmed to seek out a specific target from the time it first extends outward from the spinal cord. A third possibility is that several mechanisms operating at different times and in different regions serve to guide each neuron to its appropriate target.

One preparation that has been used to study this problem is the hind limb of the chick embryo. Hind limb muscles are always innervated by nerves from specific segments, and these connections do not appear to be the result of random nerve outgrowth (Landmesser and Morris, 1975). Moreover, the nerves to a particular muscle are capable of locating their target prior to its cleavage from the dorsal or ventral myogenic mass within the limb bud. This indicates that nerves "recognize and respect pre-muscle boundaries" within the myogenic mass (Landmesser, 1978). The nature of this recognition phenomenon is not known.

Single-somite chimaeras may provide a new method for studying the relationship between a nerve and its target. In a chimaera that has received a graft of a single quail somite, all of the tissue derived from that somite can be identified and distinguished from tissues derived from other somites. It should therefore be possible to determine whether the muscle or skin derived from a particular segment is innervated by the spinal nerve associated with that segment. The data of Landmesser and Morris (1975) is not inconsistent with this hypothesis. These investigators have shown that each muscle in the chick hindlimb is innervated by nerves from two, three, or four adjacent segments. Single-somite experiments (Section 3) have shown that most muscles in the chick wing and shoulder girdle are derived from two, three or four adjacent somites. Single-somite transplants involving somites 26 - 32 would provide information that could be compared with the data of Landmesser and Morris (1975) to determine whether there is indeed a relationship between the muscles and nerves derived from the same segments.

REFERENCES

REFERENCES

Agnish, N.D. and Kochlar, D.M. (1977). The role of somites in the growth and early development of mouse limb buds. Devel. Biol. 56:174-183.

Asmundson, V.S. and Julian, L.M. (1956). Inherited muscle abnormality in the domestic fowl. J. Hered. 47:248-252.

Balfour, F.M. (1878). A monograph on the development of Elasmobranch fishes. London. Cited in Detwiler (1936).

Balfour, F.M. (1881). A treatise on comparative embryology. Macmillan and Co., London.

Bekoff, A. and Betz, W.J. (1976). Acetylcholine hot spots: Development on myotubes cultured from aneural limb buds. Science 193:915-917.

Beresford, B., Le Lievre, C., and Rathbone, M.P. (1978). Chimaera studies of the origin and formation of the pectoral musculature of the avian embryo. J. Exp. Zool. 205:321-326.

Bonner, P.H. (1978). Nerve-dependent changes in clonable myoblast populations. Devel. Biol. 66:207-219.

Bonner, P.H. and Hauschka, S.D. (1974). Clonal analysis of vertebrate myogenesis. I. Early developmental events in the chick limb. Devel. Biol. 37:317-328.

Byrnes, E.F. (1898). Experimental studies on the development of limb-muscles in Amphibia. J. Morph. 14:105-140. Cited in Nicholas (1955).

Caplan, A. (1970). Effects of the nicotinamide-sensitive teratogen 3-acetylpyridine on chick limb cells in culture. Exp. Cell Res. 62:341-355.

Caplan, A. (1972). The effects of the nicotinamide-sensitive teratogen 3-acetyl-pyridine on chick limb mesodermal cells in culture: Biochemical parameters. J. Exp. Zool. 180:351-362.

Caplan, A. and Koutroupas, S. (1973). The control of muscle and cartilage development in the chick limb; the role of differential vascularization. J. Embryol. exp. Morph. 29:571-583.

Chevallier, A. (1975). Rôle du mésoderme somitique dans le développment de la cage thoracique de l'embryon d'oiseau. I. Origine du segment sternal et mécanismes de la differenciation des côtes. J. Embryol. exp. Morph. 42:275-292.

Chevallier, A. (1978). Etude de la migration des cellules somitiques dans le mésoderme somatopleural de l'ébauche de l'aile. Wilhelm Roux's Arch. devel. Biol. 184:57-73.

Chevallier, A. (1979). Role of the somitic mesoderm in the development of the thorax in bird embryos. II. Origin of thoracic and appendicular musculature. J. Embryol. exp. Morph. 49:73-88.

Chevallier, A., Kieny, M. and Mauger, A. (1977). Limb-somite relationship: Origin of the limb musculature. J. Embryol. exp. Morph. 41:245-258.

Chevallier, A., Kieny, M. and Mauger, A. (1978). Limb-somite relationship: Effect of removal of somitic mesoderm on the wing musculature. J. Embryol. exp. Morph. 43:263-278.

Christ, B., Jacob, H.J. and Jacob, M. (1974a). Über den Ursprung der Flügelmuskulatur. Experimentelle Untersuchungen mit Wachtel- und Hühnerembryonen. Experientia 30:1446-1448.

Christ, B., Jacob, H.J. and Jacob, M. (1974b). Experimentelle Untersuchungen zur Entwicklung der Brustwand beim Hühnerembryo. Experientia 30:1449-1451.

Christ, B., Jacob, H.J. and Jacob, M. (1977). Experimental analysis of the origin of the wing musculature in avian embryos. Anat. Embryol. 150:171-186.

Christ, B., Jacob, H.J. and Jacob, M. (1978a). An experimental study on the relative distribution of the somitic and somatic plate mesoderm to the abdominal wall of avian embryos. Experientia 34:241-242.

Christ, B., Jacob, H.J. and Jacob, M. (1978b). On the formation of the myotomes in avian embryos. An experimental and scanning electron microscope study. Experientia 34:514-516.

Cohen, A.M. and Hay, E.D. (1971). Secretion of collagen by embryonic neuroepithelium at the time of spinal cord-somite interaction. Devel. Biol. 26:578-605.

Cosmos, E. (1964). Intracellular distribution of calcium in developing breast muscle of normal and dystrophic chickens. J. Cell Biol. 23:241-252.

Cosmos, E. (1966). Enzymatic activity of differentiating muscle fibers. I. Development of phosphorylase in muscles of the domestic fowl. Devel. Biol. 13:163-181.

Cosmos, E. (1970). Ontogeny of red and white muscles: The enzymic profile and lipid distribution of immature and mature muscles of normal and dystrophic chickens. In: The Physiology and Biochemistry of Muscle as a Food, 2. E.J. Briskey, R.G. Cassens, and B.B. Marsh, eds., University of Wisconsin Press, Madison, Wisc. pp 193-207

Cosmos, E. and Butler, J. (1967). Differentiation of fiber types in muscle of normal and dystrophic chickens. A quantitative and histochemical study of the ontogeny of muscle enzymes. In: Exploratory Concepts in Muscular Dystrophy and Related Disorders. A.T. Milhorat, ed. Excerpta Medica, Amsterdam. pp 197-204.

Detwiler, S.R. (1918). Experiments on the development of the shoulder girdle and the anterior limb of Amblystoma punctatum. J. Exp. Zool. 25:499. Cited in Detwiler (1936), Nicholas (1955).

Detwiler, S.R. (1920). Experiments on the transplantation of limbs in Amblystoma. The formation of nerve plexuses and the function of the limbs. J. Exp. Zool. 31:117. Cited in Detwiler (1936), Nicholas, (1955).

Detwiler, S.R. (1929). The development of the spinal cord in Amblystoma embryos following unilateral myotomectomy. J. Exp. Zool. 52: 325. Cited in Detwiler (1936), Nicholas (1955).

Detwiler, S.R. (1936). Neuroembryology - An Experimental Study. The MacMillan Company, New York.

Detwiler, S.R. (1955). Experiments on the origin of the ventrolateral trunk musculature in the urodele (Amblystoma). J. Exp. Zool. 129: 45-75.

Dienstman, S.R., Biehl, J., Holtzer, S. and Holtzer, H. (1974). Myogenic and chondrogenic lineages in developing limb buds grown in vitro. Devel. Biol. 39:83-95.

Feulgen, R. and Rossenbeck, H. (1924). Mikroskopisch-chemischer Nachweis einer Nucleinsäure vom Typus der Thymonucleinsäure und die darauf beruhende elektive Färbung von Zellkernen in mikroskopischen Präparaten. Hoppe-Seyler's Z. Physiol. Chem. 135:203-252.

Finch, R. and Zwilling, E. (1971). Cultural stability of the morphogenetic properties of chick limb bud mesoderm. J. Exp. Zool. 176: 397-408.

Frank, E. (1977). Formation and maintenance of neural connections. In: Function and Formation of Neural Systems. G.S. Stent, ed. Berlin: Dahlem Konferenzen.

George, J.C. and Berger, A.J. (1966). Avian Myology. Academic Press, New York, London.

Goldspink, G. (1974). Development of muscle. In: Differentiation and Growth of Cells in Vertebrate Tissues, G. Goldspink, ed. Chapman and Hall, London, p 69.

Goodrich, E.S. (1930). Studies on the structure and development of vertebrates. MacMillan and Company, London. Cited in Detwiler (1936).

Grim, M. (1971). Development of the primordia of the latissimus dorsi muscle of the chicken. Folia Morphologica 19:252-262.

Guth, L. (1968). "Trophic" influences of nerve on muscle. Physiol. Rev. 48:645-687.

Hamburger, V. (1938). Morphogenetic and axial self-differentiation of transplanted limb primordia in 2-day chick embryos. J. Exp. Zool. 77:379-399.

Hamburger, V. and Hamilton, H.L. (1951). A series of normal stages in the development of the chick embryo. J. Morphol 88:59-92.

Hanks, J.H. and Wallace, R.E. (1949). Relation of oxygen and temperature in the preservation of tissues by refrigeration. Proc. Soc. exp. Biol. Med. 71:196-200.

Harrison, R.G. (1895). Die Entwicklung der unpaaren und paarigen Flossen der Teleostier. Arch. f. mikr. Anat. 46:500-578. Cited in Detwiler (1936), Nicholas (1955).

Harrison, R.G. (1917). Transplantation of limbs. Proc. Nat. Acad. Sci. 3:245-250. Cited in Detwiler (1936), Nicholas (1955).

Harrison, R.G. (1918). Experiments on the development of the forelimb of Amblystoma, a self-differentiating equipotential system. J. Exp. Zool. 25:413-462. Cited in Detwiler (1936), Nicholas (1955).

Hay, E.P. and Meier, S. (1974). Glycosaminoglycan synthesis by embryonic inductors: Neural tube, notochord, and lens. J. Cell Biol. 42: 889-898.

Herrmann, H., Heywood, S.M. and Marchok, A.C. (1970). Reconstruction of muscle development as a sequence of macromolecular synthesis. Current Topics in Devel. Biol. 5:181-234.

His, W. (1868). Untersuchungen uber die erste Anlage des Wirbelthierleibes. Die erste Entwickelung des Hünchens im Ei. F.C.W. Vogel, Leipzig. Cited in Straus and Rawles (1953).

Holtzer, H. (1972). The cell cycle and myogenesis. In: Cell Differentiation, Scheide, O., and de Vellis, J., eds, Van Nostrand Reinhold, Princeton, N.J.

Holtzer, H. and Bischoff, R. (1970). Mitosis and myogenesis. In: The Physiology and Biochemistry of Muscle as a Food, v. 2. Briskey, E.J., Cassens, R.G. and Marsh, B.B., eds. The University of Wisconsin Press, Madison, Milwaukee, and London.

Jotereau, F.V. and Le Douarin, N.M. (1978). The developmental relationship between osteocytes and osteoclasts: A study using the quail - chick nuclear marker in endochondral ossification. Devel. Biol. 63: 253-265.

Katznelson, Z.S. (1934). Histogenesis of muscular tissue in amphibia. Anat. Rec. 61:109-130.

Kieny, M., Mauger, A. and Sengel, P. (1972). Early regionalization of the somitic mesoderm as studied by the development of the axial skeleton of the chick embryo. Devel. Biol. 28:142-161.

Koch, T. (1973). Anatomy of the Chicken and Domestic Birds. Iowa State University Press, Ames, Iowa.

Konigsberg, I.R. and Buckley, P.A. (1974). Regulation of the cell cycle and myogenesis by cell-medium interaction. In: Concepts of Development, J. Lash and J.R. Whittaker, eds. Sinauer Associates, Inc. Stamford, Connecticut, p 179.

Landmesser, L. (1978). The distribution of motoneurons supplying chick hind limb muscles. J. Physiol. 284:371-389.

Landmesser, L. and Morris, D. (1975). The development of functional innervation in the hind limb of the chick embryo. J. Physiol. 249: 301-326.

Lash, J. (1974). Tissue interactions and related subjects. In: Concepts of Development, Lash, J., and Whittaker, J.R., eds. Sinauer Associates, Inc. Stamford, Conn.

Le Douarin, N. (1969). Particularites du noyau interphasique chez la caille japonaise (Coturnix coturnix japonica). Utilisation de ces particularités comme "marquage biologique" dans les recherches sur les interactions tissulaires et les migrations cellulaires au cours de l'ontogenèse. Bull. Biol. Fr. Belg. 103:435-452.

Le Douarin, N. (1973). A biological cell labelling technique and its use in experimental embryology. Devel. Biol. 30:217-222.

Le Douarin, N.M. and Jotereau, F.V. (1975). Tracing of cells of the avian thymus through embryonic life in interspecific chimaeras. J. Exp. Med. 142:17-40.

Leidke, K.B. (1958). Experiments on the development of trunk muscles in anura (Rana pipiens). Anat. Rec. 131:97-118.

Le Lievre, C. and Le Douarin, N. (1975). Mesenchymal derivatives of avian neural crest: Analysis of chimaeric quail and chick embryos. J. Embryol. exp. Morph. 34:125-154.

Lewis, W. (1902). The development of the arm in man. Am. J. Anat. 1:145-184, Cited in Sullivan (1962).

Lewis, W. (1910). The relation of the myotomes to the ventro-lateral musculature and to the anterior limbs in Amblystoma. Anat. Rec. 4:183-190. Cited in Detwiler (1936); Nicholas (1955); Straus and Rawles (1953).

Lillie, F.R. (1908). The Development of the Chick. Henry Holt and Company, New York.

Marchok, A.C. and Herrmann, H. (1967). Studies of muscle development. I. Changes in cell proliferation. Devel. Biol. 15:129-155.

Mazliah, J., Cosmos, E. and Butler, J. (1978). Physiological and histochemical analyses of the early transformation of PLD fibers by the ALD nerve in normal and dystrophic birds. Proceedings of the IVth International Congress on Neuromuscular Diseases: 272.

Medoff, J. (1967). Enzymatic events during cartilage differentiation in the chick embryonic limb bud. Devel. Biol. 16:118-143.

von Mestres, P. and Hinrichsen, K. (1976). Zur Histogenese des Somiten beim Hühnchen. J. Embryol. Exp. Morph. 36:669-683.

Mollier, S. (1893). Die paarigen Extremitäten der Wirbeltiere. I. Das Ichthyopteryguim. Anat. Hefte 3:1. Cited in Detwiler (1936).

Morata, G. and Ripoll, P. (1975). Minutes:mutants of Drosophila autonomously affecting cell division rate. Devel. Biol. 42:211-221.

Newman, S.A. (1977). Lineage and pattern in the developing wing bud. In: Vertebrate Limb and Somite Morphogenesis. Third Symp. British Soc. Devel. Biol. Ede, D.A., Hinchcliffe, J.R. and Balls, M., eds. Cambridge University Press, Cambridge, London, New York, Melbourne.

Nicholas, J.S. (1955). Limb and girdle. In: Analysis of Development. B.H. Willier, P.A. Weiss, and V. Hamburger, eds. W.B. Saunders Co., Philadelphia and London.

Nickel, R., Schummer, A., Seiferle, E. and Siller, W.g. (1977). Anatomy of the Domestic Birds. Springer-Verlag, New York, Heidelberg, Berlin.

O'Hare, M.J. (1972a). Differentiation of chick embryo somites in chorio-allantoic culture. J. Embryol. exp. Morph. 27:215-228.

O'Hare, M.J. (1972b). Chondrogenesis in chick embryo somites grafted with adjacent and heterologous tissue. J. Embryol. exp. Morph. 27:229-234.

O'Hare, M.J. (1972c). Aspects of spinal cord induction of chondrogenesis in chick embryo somites. J. Embryol. exp. Morph. 27:235-243.

Packard, D.S., Jr., and Jacobson, A.G. (1976). The influence of axial structures on chick somite formation. Devel. Biol. 53:36-48.

Paterson, A.M. (1888). On the fate of the muscle-plate, and the development of the spinal nerves and plexuses in birds and mammals. Quart. J. Micr. Sci. 28:109-129. Cited in Williams (1910); Straus and Rawles (1953).

Peterson, A.C., Frair, P., Rayburn, H. and Cross, D. (1968). Development and disease of the neuromuscular system in muscular dystrophic <-> normal mouse chimaeras. In: Soc. Neurosci. Symp. v. IV. Society for Neuroscience, Bethesda, Maryland. In press.

Pinot, M. (1969). Etude expérimentale de la morphogenèse de la cage thoracique chez l'embryon de poulet: mécanismes et origin du matériel. J. Embryol. exp. Morph. 21:149-164.

Rathbone, M.P., Stewart, P.A. and Vetrano, F. (1975). Dystrophic spinal cord transplants induce abnormal thymidine kinase activity in normal muscles. Science 189:1106-1107.

Rathbone, M.P. and Stewart, P.A. (1978). Role of the neural tube in the pathogenesis of hereditary muscular dystrophy in the chicken: Studies with transplantation chimaeras. Ann. N.Y. Acad. Sci. 317:594-610.

Remak, R. (1855). Untersuchungen über die Entwickelung der Wirbelthiere. G. Reimer, Berlin. Cited in Straus and Rawles (1953).

Robinson, M.C. (1970). Laboratory Anatomy of the Domestic Chicken. W.C. Brown, Co., Dubuque, Iowa.

Roncali, L. (1970). The brachial plexus and the wing nerve pattern during early developmental phases in chicken embryos. Monitore Zool ital. (N5) 4:81-89.

Saunders, J.W. (1948). Do the somites contribute to the formation of the chick wing? Anat. Rec. 100:756.

Searls, R.L. and Janners, M.Y. (1969). The stabilization of cartilage properties in the cartilage-forming mesenchyme of the embryonic chick limb. J. Exp. Zool. 170:365-376.

Seno, T. (1961). An experimental study on the formation of the body wall in the chick. Acta Anat. 45:60-82.

Sewertzoff, A.N. (1907). Entwickl, d. Muskeln, d. Extremitäten d. niederen Tetrapoda. Bull. Soc. Imp. Nat. Moscow. Cited in Detwiler, (1936).

Stewart, P.A., Werstiuk, E.S., Vickers, J.D. and Rathbone, M.P. (1977). Elevated cholesterol in tissues of chicken embryos with hereditary myotonic muscular dystrophy. Exp. Neurol. 57:475-485.

Straus, W.L. and Rawles, M.E. (1953). An experimental study of the origin of the trunk musculature and ribs in the chick. Am. J. Anat. 92: 471-509.

Sullivan, G.E. (1962). Anatomy and embryology of the wing musculature of the domestic fowl (Gallus). Australian J. Zool. 10:458-518.

Vetrano, F. (1974). The role of the spinal cord in the pathogenesis of hereditary muscular dystrophy in the domestic fowl. Masters Thesis, McMaster University, Hamilton, Ontario.

Weinstock, I.M. and Dju, M.Y. (1967). Phosphorylation of thymidine in developing breast muscle of the normal and dystrophic chicken. Life Sci. 6:797-802.

Weston, J.A. (1970). The migration and differentiation of neural crest cells. Adv. in Morph. 8:41-114.

Williams, L.W. (1910). The somites of the chick. Am. J. Anat. 2:55-100.

Yaffe, D. (1969). Cellular aspects of muscle differentiation in vitro. Current Topics Devel. Biol. 4:37.

Zechel, G. (1924). Über Muskelknospen beim Meschen, ein Beitrag zur Lehre von der Differenzierung des Myotoms. Zeit. f. Anat. und Ent. 74:593. Cited in Detwiler (1936).

Zwilling, E. (1968). Morphogenetic phases in development. Devel. Biol. (Supplement) 2:184-207.

Zwilling, E. (1972). Limb morphogenesis. Devel. Biol. 28:1-11.

ADDENDUM

Christ, B., Jacob, H.J., Jacob, M.: Uber die Herkunft der Mm. pectoralis major et minor. Experimentelle Untersuchungen an Wachtelund Huhnerembryonen. Verh. Anat. Ges. 70:1007-1011 (1976).

Grim, M., Differentiation of myoblasts and the relationship between somites and the wing bud of the chick embryo. Z. Anat. Entwickl.-Gesch. 132:260-271 (1970).

Gumpel-Pinot, M. Contribution du mesoderme somitique a la genese du membre chez l'embryon d'Oiseau. C.R. Acad. Sci., Paris 279:1305-1308 (1974).

Jacob, M., Christ, B., Jacob, H.J. On the migration of myogenic stem cells into the prospective wing region of chick embryos. Anat.Embryol. 153: 179-193 (1978).

APPENDIX I

Summary of the results of experimental studies concerning the embryonic origin of skeletal muscle in the bird at the beginning of the present study (1975).

Author	Experimental Procedure	Age of Embryo	Region Studied	Conclusion
Hamburger (1938)	intracoelomic graft of limb buds	2-2.5 days	limb muscles	limb muscles arise in somatopleure
Saunders (1948)	carbon marking of brachial somites	2.5-4 days	limb muscles	limb muscles arise in somatopleure
Straus and Rawles (1953)	carbon marking of thoracic somites or somatopleure; intracoelomic grafts of somatopleure	2.5-3 days	trunk muscles	back muscles arise from somites; abdominal muscles arise from somatopleure
Seno (1961)	same as Straus and Rawles	2.0-2.5 days	trunk muscles, pectoralis	back muscles and abdominal muscles arise from somites; pectoralis arises from somatopleure
Pinot (1969)	intra-coelomic grafting or X-irradiation of thoracic somites	2.0-2.5 days	trunk muscles and pectoralis	abdominal and intercostal muscles arise from somites; pectoralis arises from somatopleure
Christ, Jacob and Jacob (1974 a, b)	grafts of quail somites to chick hosts	2.0 days	limb and thoracic muscles	limb and thoracic muscles, including pectoralis, arise from somites