A radioautographic study of the development of the somite in the chick embryo

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INTRODUCTION

Considerable difference of opinion exists about the origin of the various components of the somite. According to Williams (1910), Hamilton (1952) and Boyd (1960), the cells of the myotome originate in the dorso-medial angle of the somite wall and migrate beneath the dermatome in ventro-lateral direction. A group of older investigators (Remak, 1855; His, 1888; Bardeen, 1900) state, however, that the myotome cells originate not only in the dorso-medial angle but also along the total length of the dorsal somite wall, formed by the dermatome. Similarly, in birds whether the myotome extends in ventro-lateral direction by growth and migration of existing cells (Engert, 1900; Williams, 1910) or by differentiation of locally found mesoderm cells into myoblasts (Straus & Rawles, 1953) remains even at present a controversial issue.

With regard to the formation of the sclerotome, Patten (1947), Hamilton (1952), Boyd (1960) and Trelstad, Hay & Revel (1967) believe that proliferation of the cells in the central cavity of the somite is of primary importance, while others suggest that cell formation for the sclerotome occurs only in the median and ventral walls of the somite.

Since our further studies on the differentiation of the somitic cells require a precise knowledge about the origin of the cells of the sclerotome and myotome, the purpose of this work is to examine the formation and initial morphological differentiation of the various somite components by means of tritiated thymidine and radioautographic techniques.

MATERIALS AND METHODS

Several hundred chick embryos, ranging in age from 20–96 h (stages 5–22, Hamburger & Hamilton, 1951), were treated with 5–10 μc tritiated thymidine (Schwarz BioResearch; sp.act. 1·9 c/mM) per embryo. The thymidine, dissolved in saline, was deposited on the embryos through an opening in the shell by means
of a small pipet or a 25-gauge needle. After an additional incubation period of 1–12 h, the embryos were fixed in Bouin’s and embedded in paraffin. Serial sections of 3–4 μ were mounted and stained with hematoxylin and ethyl eosin. They were then coated with Kodak NTB-3 emulsion (Kopriwa & Leblond, 1962). After 5–10 days exposure time, the sections were developed, fixed and mounted using regular histological techniques.

Thymidine, a specific precursor of DNA, is incorporated by the cell only when the nucleus duplicates its DNA content in preparation for cell division (the ‘S’ phase) (Cronkite et al. 1959; Lamerton & Fry, 1963). Upon completion of this phase, the cell enters the postduplication or ‘G2’ stage, a period of short duration before mitosis begins. After mitosis, the daughter cells enter the preduplication or ‘G1’ phase. Some of the cells never leave this phase; others proceed after variable time intervals to the S-phase in preparation for the next division. Though in mammals tritiated thymidine remains available for incorporation into DNA for approximately 30–60 min., in early chick embryos in which the vascular system has not yet fully developed we found it to remain available for 1–3 h. In control experiments we noticed that 10–15 min after application of the tritiated thymidine, labeled cells were present throughout the embryo, indicating that the thymidine had no difficulties in penetrating into the embryo.

Since the treatment with tritiated thymidine will give us information about the site of DNA synthesis, but not about the site of cell division, chick embryos, ranging in age from 24 to 48 h (stages 7–12), were also treated for 1–2 h with 2–5 mg of vincristine sulfate (Lilly), a drug acting similarly to colchicine in arresting mitosis in the metaphase (Cutts, 1964; Palmer et al. 1960; George, Journey & Goldstein, 1965).

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**Plate 1**

Fig. A. Transverse section through a 24 h chick embryo (stage 7), labeled with tritiated thymidine 1 h before fixation. Note that the labeled mesoderm nuclei are randomly distributed. ×384.

Fig. B. Transverse section through a 24 h chick embryo in the region of the developing somites. The mesoderm cells begin to show some organization and a small irregular cavity is visible in the center of the tissue. ×384.

Fig. C. Transverse section of a 48 h embryo labeled with tritiated thymidine for 1 h. Note the epithelial organization of paraxial mesoderm cells. The labeled nuclei are found at the periphery of the epithelium, while the unlabeled nuclei and mitotic figures (arrows) are adjacent to the central space. Note the labeled cells within the central cavity. ×640.

Fig. D. Similar section as in Fig. C 7 h after labeling with tritiated thymidine. The majority of the labeled nuclei are again seen at the periphery, but the dividing cells (arrow) adjacent to the central space are also labeled.

Fig. E. Transverse section through a 48 h embryo treated for 2 h with vincristine sulfate. Note that all arrested metaphases are adjacent to the central cavity. ×518.

Fig. F. Transverse section through a similar embryo as in fig. E labeled for 7 h with tritiated thymidine. Note the cells in the center of the somite undergoing degenerative changes (arrows). ×518.
Fig. A. Transverse section through a 60 h embryo. Note that both the dermatome and myotome extend in ventrolateral direction to the coelomic cavity. × 24.

Fig. B. Transverse section through a 72 h embryo. The myotome now consists of a long band of tissue characterized by an abundance of cytoplasm and many pale nuclei with a sharp nuclear membrane. The cells of the dermatome have migrated peripherally to form the dermis. Note the persistence of the most dorsal portion of the dermatome. × 24.
RESULTS

In a transverse section through a 24 h chick embryo just rostral to the node of Hensen (stage 7), the intraembryonic mesoderm forms a loosely woven sheet of tissue extending on either side of the notochord between the epiblast and hypoblast. The cells of the mesoderm are attached to each other as well as to the surrounding tissues by thin cytoplasmic extensions. When treated with tritiated thymidine at this stage of development, the majority of the blastoderm cells are found to be synthesizing DNA. The labeled mesoderm cells do not show any particular localization, but are randomly distributed throughout the tissue (Plate 1, fig. A).

During subsequent development the number of mesoderm cells adjacent to the neural wall increases rapidly and the paraxial mesoderm can be distinguished. Some organization becomes then gradually evident: at first a vaguely defined, irregular cavity appears among the cells of the paraxial mesoderm (Plate 1, fig. B), and shortly later the cells become arranged as an epithelium surrounding a central space (Plate 1, fig. C). In some areas an internal limiting membrane seems to be present, while in other areas cytoplasmic extensions protrude into the central space. After treatment with tritiated thymidine for 1–2 h the labeled nuclei are found predominantly at the periphery of the epithelium; the unlabeled nuclei and mitotic figures are adjacent to the central space. A few labeled, as well as unlabeled, cells are usually present within the central space (Plate 1, fig. C). The latter cells, contained entirely within the central cavity, do not have any epithelial characteristics. When similar embryos are sacrificed 3–7 h after treatment with tritiated thymidine, many of the mitotic figures are labeled (Plate 1, fig. D). Since dividing cells are never seen at the periphery of the epithelium, the DNA-synthesizing cells observed 1–2 h after treatment must have moved towards the central space to undergo division. Additional proof that mitosis occurs exclusively adjacent to the central cavity is found in embryos treated

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**Plate 2**

Fig. A. Transverse section through a 48 h chick embryo labeled with tritiated thymidine 1 h before fixation. The label is located primarily over the peripheral nuclei of the epithelial somite walls while only a few of the loosely connected cells in the center are labeled. × 518.

Fig. B. Transverse section through a 48 h chick embryo. The ventral and major portion of the medial walls of the somite have lost their epithelial continuity and the sclerotome cells begin to move in the direction of the notochord. Note the position of the dividing cells in the dermatome (arrow). × 384.

Fig. C. Transverse section through a similar embryo as in Fig. B labeled with tritiated thymidine 1 h before fixation. All the cells of the dermatome including the mitotic figures (arrows) are labeled, while the cells of the myotome remain entirely unlabeled. Note that the great majority of the sclerotome cells are now actively synthesizing DNA. × 384.

Fig. D. Transverse section through a 48 h chick embryo labeled with tritiated thymidine 10 h before fixation. Note the random distribution of both labeled and unlabeled cells in the myotome. × 518.
with vincristine sulfate. Arrested metaphases are seen adjacent to the central space and not at the periphery of the epithelial wall (Plate 1, fig. E). Since 10 h after treatment the mitotic figures are unlabeled and the majority of the labeled nuclei are again at the periphery, it is concluded that the nuclei undergo inter-kinetic migrations, similar to those observed in the wall of the neural tube: the nuclei synthesize DNA at the periphery of the somite wall, move towards the central region to divide and then return to the periphery. Whether at this stage of development all the daughter cells return to the periphery or whether some remain in the center of the somite is not certain. Many of the cells initially present within the central space show degenerative changes (Plate 1, fig. F).

During further development of the somite the ventral wall and a major portion of the medial wall lose their pseudo-stratified appearance and consist of a single row of cuboidal cells. When labeled with tritiated thymidine for 1 h, DNA synthesis is found to occur mainly in the cuboidal cells, but seldom in the rapidly increasing, loosely connected cells in the center of the somite (Plate 2, fig A). Since throughout development DNA synthesis and mitosis occur primarily within the epithelial walls, the cells in the center of the somite must be the products of cell division in the somite walls. With a further increase in the number of cells in the center of the somite, the ventral wall and a major portion of the medial wall gradually lose their epithelial continuity. The original cuboidal cells then intermingle with those of the central region and together they form a loosely woven tissue of homogeneous appearance, referred to as 'the sclerotome' (Plate 2, fig. B).

During formation of the sclerotome the dorsal somite wall, now referred to as 'the dermatome', and a variable portion of the medial wall maintain their epithelial structure. Shortly after, a new layer, consisting of cells with a pale nucleus and a darkly stained nucleolus, begins to appear directly in contact with the inner surface of the dermatome (Plate 2, fig. C). This layer is referred to as the myotome. Mitotic figures are observed in the dermatome but not in the myotome (Plate 2, figs. B, C).

To determine the origin of the cells of the myotome, 48 h embryos (stage 13) were treated with tritiated thymidine and sacrificed at hourly intervals. During the first 3 h after treatment the cells of the dermatome are labeled, but none of the myotome (Plate 2, fig. C). After treatment for 4–5 h, labeled cells appear in the myotome and after 10 h they are found randomly distributed throughout the myotome layer (Plate 2, fig. D). Since neither DNA synthesis nor cell division is ever observed in the cells of the myotome, and rarely in the dorso-medial lip, the myotome cells must have originated from the cells of the overlying dermatome.

During further development the most distinctive feature of the original somite is formed by the dermatome, which now extends towards the coelomic cavity (Plate 3, fig. A). After subsequent formation of the myotome layer along its inner surface, the cells of the dermatome lose their epithelial continuity and move under the surface ectoderm to form the dermis. The most dorsal portion of the
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dermatome persists temporarily (Plate 3, fig. B). The cells of the myotome now form a band of tissue, characterized by an abundance of cytoplasm and a large number of round nuclei with a sharp nuclear membrane. While mitotic figures and DNA-synthesizing cells are abundant among the cells of the dermatome and sclerotome, at no time during development are they observed among the cells of the myotome.

DISCUSSION

Considerable information is available on the differentiation of the somitic cells. When Holtzer & Detwiler (1954) grafted tissue strips of four somites obtained from salamanders into similarly aged hosts, the somites failed to differentiate. When, however, spinal cord was included in the graft, the somite cells formed cartilage and muscle. In additional experiments the notochord was likewise found to influence both the skeletogenous activity and muscle differentiation of the somite cells (Avery, Chow & Holtzer, 1955). Though in the chick, as well as in the mouse embryo, similar observations were made about the inductive influence of the spinal cord, the notochord was found to influence only cartilage formation (Grobstein & Holtzer, 1955; Lash, Holtzer & Holtzer, 1957). According to these authors, the influence of the spinal cord appears to be mediated by a factor which is transmissible through mesenchyme, muscle tissue and millipore filters, while the notochord acts more locally and its influence appears to be associated with the notochordal sheath cells. Hence, in the vertebrate embryo the differentiation of the somitic cells is in all probability influenced by the spinal cord and notochord.

Although the ‘somite centers’ (Spratt, 1955), Hensen’s node (Fraser, 1954) and the neural tissue (Grünewald, 1936; Fraser, 1960) have been considered to cause undifferentiated mesoderm to form somites, Bellairs (1963) has questioned the validity of these theories since extirpation of the ‘inductive tissues’ did not prevent the formation of somites. In her opinion the regression movements (Pasteels, 1937) play an important role in somite formation. To examine whether some factor passes from the cranial end of the embryo in caudal direction, Deuchar & Burgess (1967) performed in amphibian embryos a number of experiments designed to eliminate any stimulus or controlling influence passing from the occipital region caudalwards. No such influence was found in any of the deletion or reversal experiments. The possibility was considered that the closure of neural folds in cranial-caudal sequence might cause some passive vertical stretching of the mesoderm cells and subsequently influence somite formation. In this regard we noticed that during stages 5–7 the mesoderm cells bounded by the wall of the neural groove, the notochord and the adjacent hypoblast and epiblast are polymorphic. They are, however, frequently observed to be connected to each other, as well as to the surrounding tissues, by thin cytoplasmic extensions. According to Abercrombie & Heaysman (1954), Abercrombie & Ambrose (1958, 1962), and Weiss (1958, 1961), intercellular
connexions may serve either as attachment zones, by which cells can pull themselves forward, or as areas of contact inhibition, which prevent further movement in a given direction. Trelstad et al. (1967) noted that the mesoderm cells are connected to those of the hypoblast and epiblast by 'close' or focal tight junctions. Based on observations of the migration of mesoderm cells in lateral direction, they suggest that the junctions serve temporarily as anchors by which cellular movement can occur. In our opinion it is not unlikely that these junctions also play an important role in the arrangement of the unorganized mesoderm cells into the epithelial somite structure. During the neural-plate stage the distance between the hypoblast and the opposing neural wall and epiblast is relatively small. With formation of the neural groove and subsequent closure, the distance between the hypoblast and epiblast increases continuously (Plate 1, figs. A–C). During the same period of development the original polymorphous mesoderm cells assume a polarized form. Since the basal ends of the mesoderm cells are attached to the epiblast and hypoblast, and the apical poles are firmly attached more centrally to each other, the tension caused by the separation of the hypoblast and epiblast may lead to the polarization of the mesoderm cells. Once the cells have assumed a polarized form, the tight junctions at the apical ends of the cells are gradually replaced by more definitive attachments in the form of terminal bars (Trelstad et al. 1967). Hence, the intercellular connexions between the mesoderm cells and the surrounding tissues are thought to play an important role in the organization of the paraxial mesoderm into the somite structure.

During the formation of the somite the polarized cells enclose a central space, which frequently contains a few irregularly shaped cells. These cells, according to Patten (1947), originate in the intermediate mesoderm. Trelstad et al. (1967), however, suggest that, during the formation of the somite, a few cells become 'trapped' in the central space and that proliferation of these cells results in the great increase of loosely connected cells later seen in this region. We agree with the suggestion that a few cells, lacking connexions with the surrounding tissues, become trapped in the center of the somite, due to the polarization of the the adjacent mesoderm cells. In our opinion, however, it is not likely that these cells are the primary source of the great number of cells which later occupy the central region. Upon labeling with tritiated thymidine, DNA synthesis was found to occur in the cells of the ventral and medial somite walls, but only rarely in the centrally located cells. Hence, cell proliferation in the pseudo-stratified epithelium of the ventral and medial walls must give rise to the majority of the cells in the central region. With continuous increase in the number of these cells, the medial and ventral walls expand in the direction of the notochord and become gradually thinner. Finally, possibly as a result of the increasing pressure of cells in the central region, the expanded somite walls lose their epithelial continuity and blend with the central cells to form the sclerotome (Plate 2, figs. A, B).

From our work it is evident that the somite walls consist of a pseudo-
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stratified epithelium and that the nuclei undergo inter-kinetic migration. Since, according to Trelstad et al. (1967), the epithelial cells are attached to each other by terminal bars at their apical ends, the wall of the somite greatly resembles that of the primitive neural tube (Langman, Guerrant & Freeman, 1966). In contrast, however, to the neural tube, in which the products of cell division migrate peripherally to form neuroblasts, the products of division in the somite move centrally. As the majority of the cells entering the central space lose their ability to synthesize DNA, the question arises whether at this time they begin to synthesize specific proteins, such as collagen.

According to Williams (1910), Patten (1947), Hamilton (1952) and Boyd (1960), the cells of the myotome originate in the dorso-medial ‘lip’ (the variable portion of the medial wall remaining after formation of the sclerotome (Plate 2, fig. B), and then extend beneath the dermatome or dorsal somite wall. Our studies with tritiated thymidine indicate, however, that the cells of the myotome originate primarily in the dermatome. If, as generally accepted, the dorso-median ‘lip’ is the primary source of the myotome, we would expect that in embryos labeled for 8 h (approximately the duration of the cell cycle), the myotome cells adjacent to the ‘lip’ would be labeled. With further time intervals a stream of labeled myotome cells would be seen to extend gradually in ventro-lateral direction. This was not the case. Our labeling experiments showed that for 4–8 h after treatment, labeled cells appeared randomly distributed throughout the myotome. This observation indicates that the cells of the myotome in all probability do not originate in the dorso-medial lip, but rather throughout the length of the overlying dermatome. Further support for this concept is found in the observation that (1) the dermatome does not increase in thickness despite its high proliferative activity (Plate 2, fig. C), and (2) mitotic figures are frequently seen on the inner surface of the dermatome and hardly ever in the dorso-medial ‘lip’ (Plate 2, figs. B, C).

As soon as the myotome plate extends from the dorso-median ‘lip’ to the coelomic cavity, the cells of the dermatome, with the exception of the most dorsal portion, lose their epithelial structure and begin to form the dermis.

SUMMARY

Chick embryos ranging in age from 24 to 72 h were treated with $^3$H-thymidine to examine proliferation, formation and differentiation of the various somite components. The embryos were fixed 1–10 h after treatment, sectioned at 4 $\mu$ and processed for radioautography. The following observations were made:

1. Before the formation of the paraxial mesoderm, the DNA-synthesizing mesoderm cells are randomly distributed throughout the tissue. When the paraxial mesoderm cells become arranged in epithelial fashion, DNA synthesis occurs predominantly in the nuclei at the periphery of the epithelium, while the unlabeled nuclei and the mitotic figures are adjacent to the central space.
Since 3 h after labeling the mitotic figures are labeled and 5 h later the labeled nuclei are again at the periphery, it is concluded that the nuclei in the walls of the somite undergo interkinetic migration.

2. The cells formed by the ventral and major portion of the medial somite walls are deposited in the central cavity of the somite, where they lose their epithelial shape and become mesenchyme-like. They rarely synthesize DNA. With further increase in the number of the cells in the center of the somite, the ventral and medial walls lose their epithelial continuity and blend with the centrally located cells. Together they form a loosely woven tissue, referred to as the sclerotome.

3. After formation of the sclerotome, the dorsal somite wall, now referred to as the dermatome, is the main source of a layer of cells with pale nuclei and darkly stained nucleoli. This layer, which is directly in contact with the inner surface of the dermatome, is referred to as the myotome. DNA synthesis is never observed in the cells of this layer.

4. After the myotome has extended in a ventro-lateral direction to the coelomic cavity, the dermatome, with the exception of its most dorsal portion, loses its epithelial structure and the cells begin to form the dermis.

**RESUME**

_Etude autoradiographique du developpement des somites chez l'embryon de poulet_

Des embryons de poulet, d'un age compris entre 24 et 72 h, ont ete traites a la $^3$H-thymidine pour l'examen de la proliferation, de la formation et de la differentiation des divers composants du somite. Ces embryons ont ete fixes de 1 a 10 h apres le traitement, debites en coupes de $4/\mu$ et preparees pour l'autoradiographie par la methode du revetement.

Les observations suivantes ont ete faites:

1. Avant la formation du mésoderme paraxial, les cellules mésodermiques qui synthetisent l'ADN sont reparties au hasard dans tout le tissu. Quand les cellules du mésoderme paraxial se disposent selon le mode epithelial, la synthèse d'ADN a lieu essentiellement dans les noyaux a la peripherie de l'epithélium tandis que les noyaux non marques et les figures de mitose sont adjacents a l'espace central. Etant donne que les figures de mitose sont marquées 3 h apres le marquage, et que les noyaux marques sont de nouveau situes a la periherie 5 h plus tard, on conclut que les noyaux des parois du somite subissent une migration intercinetique.

2. Les cellules formées par la paroi ventrale et la plus grande partie de la paroi de la région moyenne du somite s'accumulent dans la cavité centrale de celui-ci, où elles perdent leur forme épithéliale et deviennent semblables à des éléments mésenchymateux. Elles synthétisent rarement de l'ADN. Avec un nouvel accroissement du nombre de cellules dans le centre du somite, les parois ventrale et médiane perdent leur continuité épithéliale et se mélangent aux
cellules localisées au centre. Elles forment ensemble un tissu lâche, qui est le sclérotome.

3. Après la formation du sclérotome, la paroi dorsale du somite, désignée maintenant comme étant le dermatome, est la principale origine d'une assise de cellules à noyaux pâles et à nucléoles fortement colorés. Cette assise, directement en contact avec la face interne du dermatome, est désignée comme étant le myotome. On n'observe jamais de synthèse d'ADN dans les cellules de cette assise.

4. Après que le myotome s'est étendu en direction ventro-latérale vers la cavité coelomique, le dermatome, à l'exception de sa partie la plus dorsale, perd sa structure épithéliale et les cellules commencent à former le derme.

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REFERENCES


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