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First ultrastructural differentiation of myoblasts of chicken embryos: appearance of the initial filaments

R. González Santander, G. Martínez Cuadrado, J. Martínez Alonso and M.V. Toledo Lobo
Department of Cell Biology and Genetics, Division of Histology and General Embryology, School of Medicine, University of Alcalá de Henares, Madrid, Spain

Summary. A study is made of the structure and ultrastructure of myoblasts located in the myotome in chicken embryos, between stages 18 and 19, for electron microscope observation of the occurrence of the first myogenetic filamentous molecules. We suggest a hypothesis for the formation of initial filaments consisting of an initial synthesis of actin globular molecules, carried out in the centre of the myoblast, near the nucleus, with the participation of RNA and the ribosomes. These molecules accumulate peripherically in areas below the plasmatic membrane where they polymerize into actin filamentous molecules which form the initial filaments. These move towards the sharp ends of the myoblast, under the plasmatic membrane, and thence to the interior of the cytoplasm, where they are evenly distributed. This genesis of initial filaments is independent of the influence of the nervous system. We postulate the existence of a single type of myoblast, of fibrillar form, with a dark central area containing the nucleus and the cell organelles, and two sharp, light end zones which contain only the initial filaments in a very light cytoplasmic matrix.

Key words: Myogenesis, Myoblasts, Myotome, Chick embryo, Initial filaments, Muscular cell differentiation

Introduction

Heidenhain, in 1911, was the first to postulate the presence of submicroscopic particles in the cytoplasm, which might correspond to the initiation of the formation of the first mucular fibrils. In 1955, Moscona confirmed the presence of cytoplasmic granules in the myogenesis. From then on, particularly in the last three decades, the electron microscope has provided some details on myogenesis, histogenesis and muscular regeneration and embryonic development (Allbrook, 1962; Bergman, 1962; Hay, 1962, 1963; Price et al., 1964) particularly in the chicken embryo (Bennett and Porter, 1953; Ferris, 1959a,b; Lindner, 1960; Wainrach and Sotelo, 1961; Ogawa, 1962; Allen and Pepe, 1965; Dessouki and Hibbs, 1965; Obinata et al., 1966; Przybylski and Blumberg, 1966; Fischman, 1967; Shimada et al., 1967; Bonner and Hauschka, 1974; Bonner and Adams, 1982; Rutz et al., 1982; Bonner and Martin, 1984; Bonner, 1988).

The aim of this paper is to determine the ultrastructure of myoblasts and when their cytodifferentiation begins to elaborate the initial filaments or filamentous molecules. This makes it possible to identify them, with the electron microscope, inside the first configuration of the «myotome» of the chicken embryo, as genuine «myoblasts».

Materials and methods

We used white Leghorn chicken embryos incubated at 38 °C selecting several series from stages 18 and 19 of Hamburger and Hamilton (1951). The embryos were extracted immersed in Ringer solution at 38° C, isolated in a Millonig buffer and fixed in glutaraldehyde at 3% and 4 °C for three hours. A microdissection was then done by means of a transverse sectioning of the embryo trunk to obtain the part of the embryo trunk at the level of the upper member outline. Then three or four sections involving the neural tube, the notochord and the branchial somites were obtained. These sections were post-fixed in 1% osmic acid for an hour and half, at 4 °C, block stained with 2% uranyl acetate, and embedded in Araldite. Two types of cuts were done, all across the neural tube: semithin cuts, of extensive type, including the whole transverse cut of the embryo, which were stained with toluidine blue and observed under the optical microscope; and thin cuts, done after suitably recutting the embedding block so as to take only the

Offprint requests to: Prof. González Santander, C./ Berlín nº 8-6º B, 28028 Madrid, Spain

«somite», basically the myotome, which were stained with lead citrate, using Reynolds' method (1963), and observed under the transmission electron microscope.

Results

We were able to locate the dermatome and the myotome, following a precise tracking by combining the optical and electron microscope observations on the same cuts so as to be certain of the ultrastructure of the first myoblasts.

The myotome is formed by mononucleate myoblasts located immediately under the internal face of the dermatome (Fig. 1). In the transverse cut of the embryo, the myotome forms a highly stretched «S» which clearly begins at the dorsal part, together with the dorsal section of the dermatome, and extends diffusely as far as the root of the upper member outline. At the level of the central part of the myotome, the myoblasts exceed the dermatome as such and are seen to enter the member root and to locate themselves in the blastematic mass of the member itself, in its most proximal zone (Fig. 3).

The myotome is formed by stretched myoblasts in the form of a needle pointed at both ends, and grouped like a pallisade, forming, as a whole, a strip of three to five myoblasts thick. The longer axis of the myoblasts is parallel to the longitudinal axis of the neural tube. The dorsal part of the myotome strip does not reach the dorsal edge of the dermatome (Fig. 2). The ventral part of the myotome considerably exceeds the ventral edge of the dermatome. The front edge of the myotome is very indeterminate, since it is lost amongst the cells of the outline of the upper member root, where it is difficult to differentiate the myoblasts in isolation with the optical microscope, although this is relatively easy with the electron microscope (Figs. 3, 5).

The myoblasts can be distinguished with the optical microscope, as well as from their situation in the myotome, since they are mononucleate cells with a clear cytoplasm (Fig. 4). They have no affinity for a specific acid or basic stain, although the cytoplasm may be considered to be slightly acidophile. The nucleus of the myoblasts is interphasic, with very extensive chromatin, and there may be two very dense and intensely basophilous nucleoli. The nucleus is located in the centre of the myoblast and is ovoid in shape. Mitosis is not observed in these myoblasts in this stage of development. The myoblast plasmatic membrane is very clear and, at the limits of the myotome strip, it is coated with an extremely fine basal membrane which clearly defines the myotome of the dermatome and of the mesenchyma (Fig. 5). The myoblasts are never linked any type of union complex. They are completely free cells although, as a whole, they are delimited by a basal membrane which groups all the myoblasts.

The myoblasts can be perfectly distinguished with the electron microscope, since filaments appear in them of 5 to 7 nm thickness, of varying length (0.5 to 0.8 μm), and in a zig-zag arrangement, located in the cytoplasm in a very clear matrix. These filaments, which we call «initial filaments», appear in larger numbers in the sharp ends of the myoblast, where there is virtually no cellular organoid (Fig. 5). On the periphery of the myoblast cytoplasm, several accumulations of molecules smaller than ribosomes, near the plasmatic membrane, and from which some initial filaments begin, are observed.

In the immediately supra- and infra-nuclear parts of the cytoplasm, it is possible to see how the initial filaments are associated with ribosomes, and it is here that there are some, albeit few, cellular organoids. The nucleus is large and has a clearly differentiated nucleolus. The mitochondria are small, oval, with few crests and a dense matrix. There are very few Golgi complexes. The smooth endoplasmic reticulum is very slight. There are small profiles of rough endoplasmic reticulum, but there is a high concentration of ribosomes and free polyribosomes.

Discussion

When the ventro-medial part of the somite disappears (its cells making up the sclerotome in emigration), a fine cellular strip is formed (Fig. 1). This is reorganized from the dorsal edge of the dermatome, towards the dorso-ventral-lateral edge, by proliferation and cellular emigration. This will form the blastematic mass of the myotome.

In this first myoblast emigration, the neural tube plays no part (Fig. 1), as was shown in the immediately subsequent evolution by Butler et al. (1982), who state that «innervation is not necessary for myoblast migration». For Crow and Stockdale (1986), there is a second phase in the myogenesis (fetal phase), where the innervation is essential. McLennan (1983) calls this second phase «second generation of myoblasts» and for its differentiation, the influence of the nervous system is essential. We are able to assert that neither the neural tube nor its cellular prolongations (neuroblasts) make contact with the myotome as such in the chicken embryo in stages 18 and 19 (19-). This means that the first appearance of initial filaments described here in the myoblasts, and their emigration towards the outline of the upper member, is independent of the nervous system. The first contact between neuroblasts and myoblasts occurs much later, when the myoblast has already begun to differentiate. This was shown by Bonner (1988) using chicken embryo cell cultures. At the same time, neural induction is necessary for the myoblasts to finish differentiating (Bonner and Adams, 1982).

The myotomic strip is located under the medial or deep surface of the dermatome, and in close contact with it (Figs. 2, 4), separating it from the set of sclerotome cells in emigration. The migratory movements of the myotome were described by Williams in 1910, and the cytological aspects of myogenesis in chicken somites were examined by Weed in 1936.

The myoblast in this stage (19-) are mononucleated cells of elongated form, clearly delimited and individualized, with a large nucleole-nucleus, easily

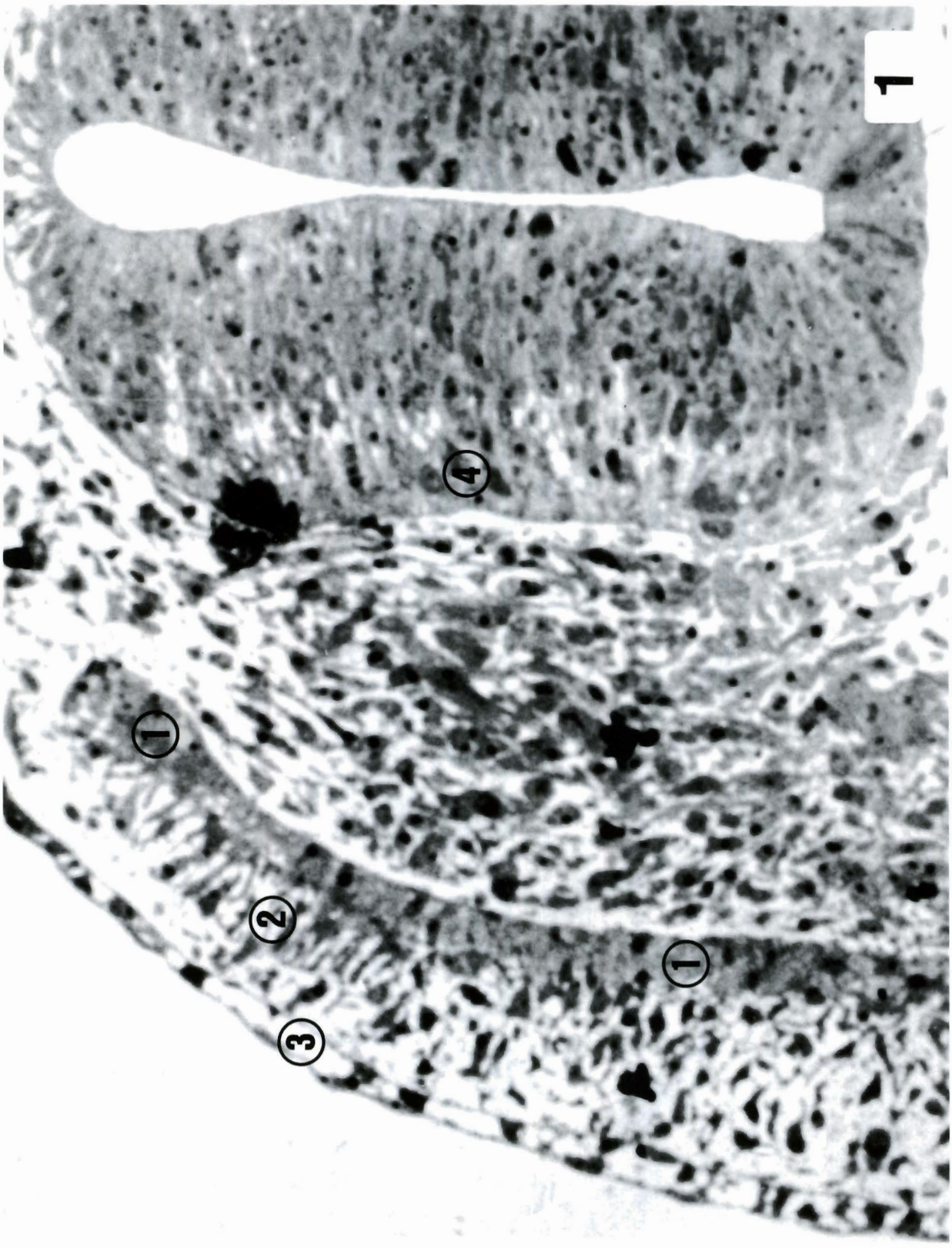

Fig. 1. Transverse cut of a chicken embryo (stage 19-). A semithin cut stained with toluidine blue. 1 = myotome; 2 = dermatome: 3 = epiblast; 4 = neural tube. × 100

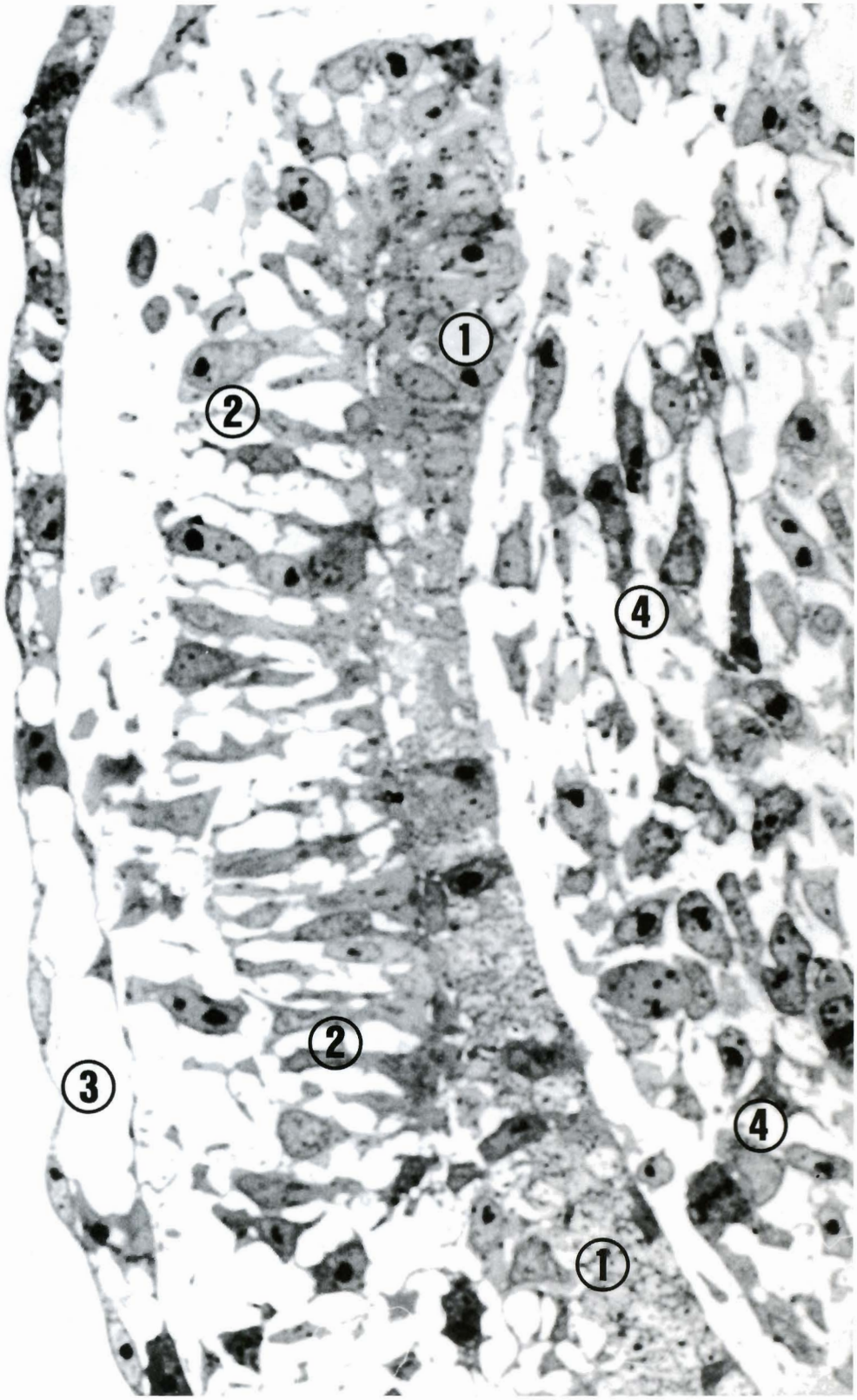

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Fig. 2. Transverse cut of a chicken embryo (stage 19-). A semithin cut stained with toluidine blue. 1 = myotome; 2 = dermatome; 3 = epiblast; 4 = sclerotome. × 400

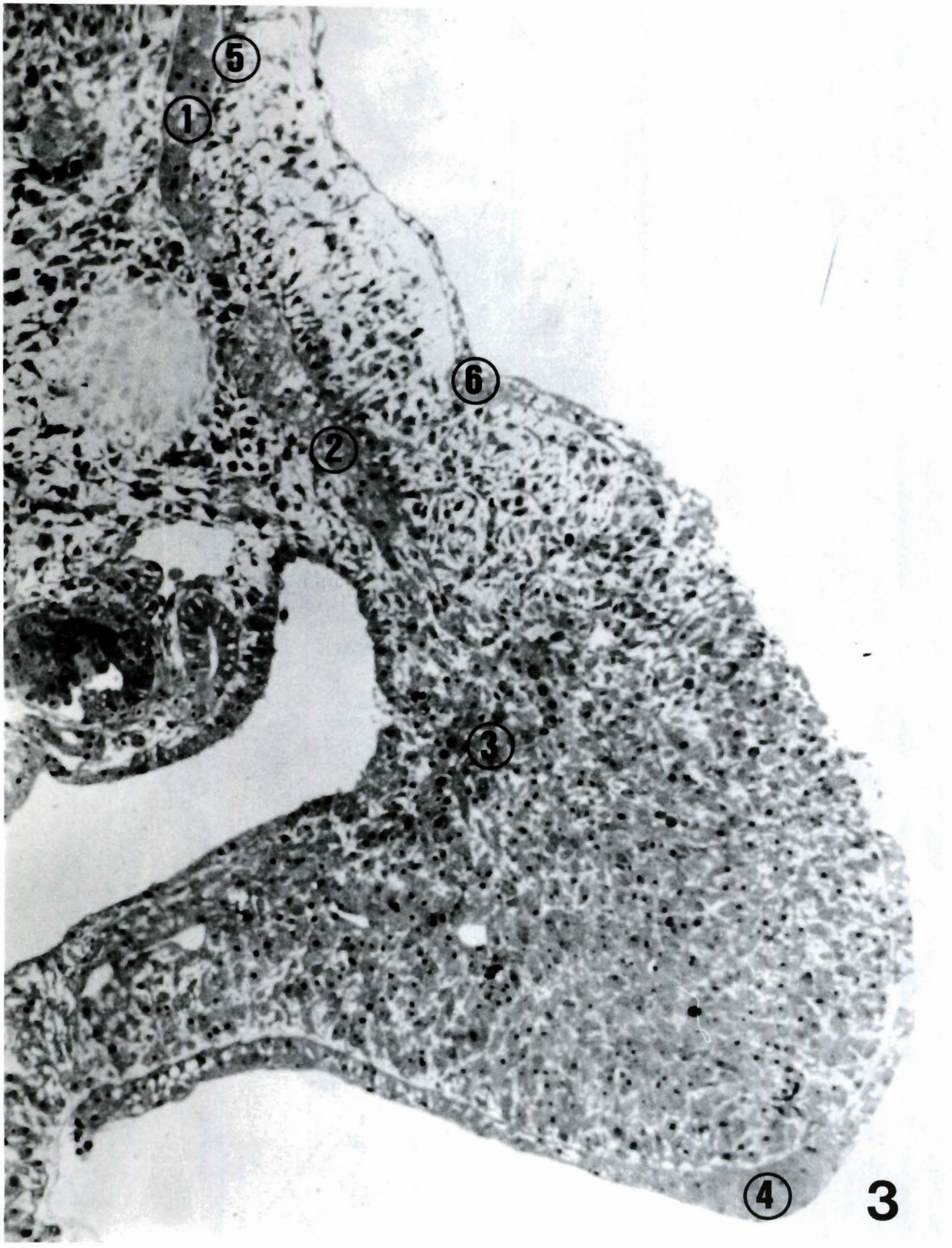

Fig. 3. Transverse cut of a chicken embryo (stage 19-). A semithin cut stained with toluidine blue. 1 = myotome; 2 = myoblast emigration to upper member; 3 = myoblasts in the blastema of the upper member outline; 4 = upper member apical crest; 5 = dermatome; 6 = epiblast. × 100

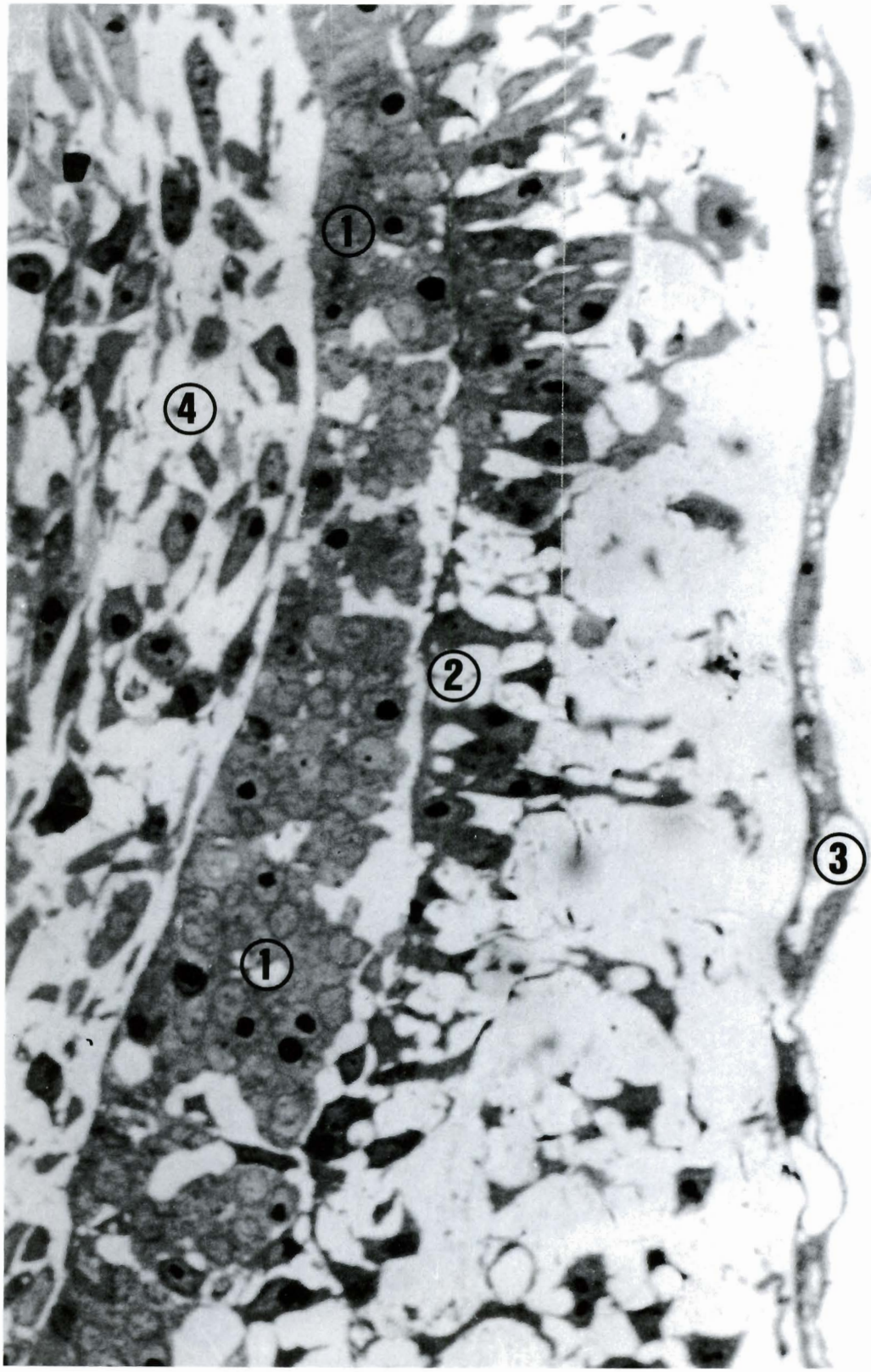

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Fig. 4. Transverse cut of a chicken embryo (stage 19-). A semithin cut stained with toluidine blue. 1 = myotome; 2 = dermatome; 3 = epiblast; 4 = sclerotome. × 400

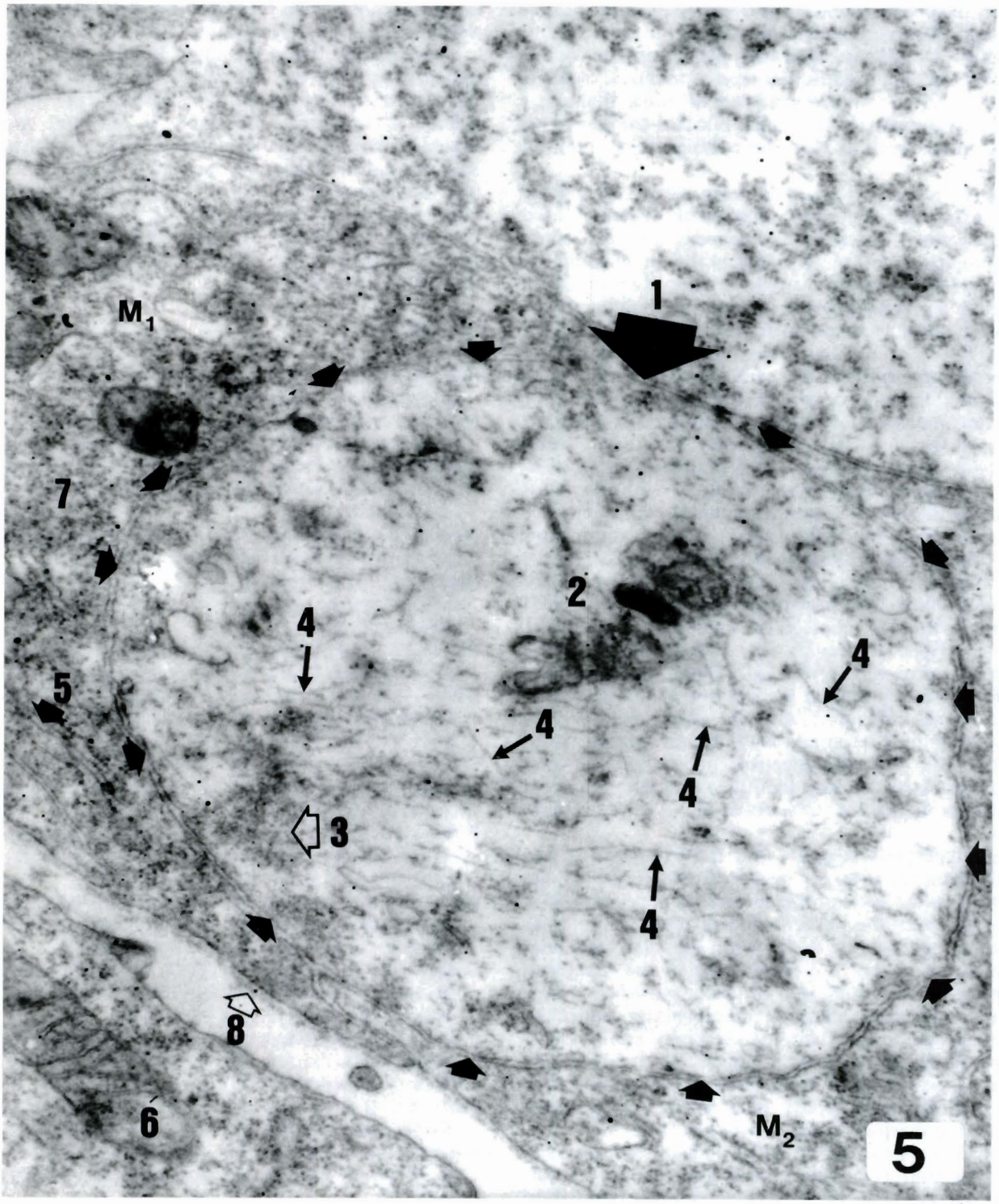

Fig. 5. Chicken embryo (stage 19-). Transverse cut of a sharp end of a myoblast (arrows) surrounded and clasped by two myoblasts (M_1 M_2). 1 = plasmatic membrane; 2 = centre, with cellular organelles; 3 = accumulations of globular molecules; 4 = initial filaments; 5 = smooth endoplasmic reticulum; 6 = mitochondria; 7 = ribosomes; 8 = basal membrane. × 12,000

stained and dense, round at the transverse cut and oval at the longitudinal cut, which is situated in the centre of the cell (Fig. 4). Ultrastructurally, the myoblasts would basically be like undifferentiated embryonic cells if they did not contain the synthesis of initial filaments. These are completely free cells, becuase they have no union complexes, so that their emigration potential is high.

The myoblast cytoplasm is very clear and slightly acidophile. The central part contains the nucleus, where the few cellular organelles are concentrated, e.g. some mitochondria, a few dictyosomes of the Golgi complex, plenty of free ribosomes, very little smooth endoplasmic reticulum and some polyribosomes. The peripheral part of the myoblast, located at the two sharp ends, is very light (Figs. 2, 4, 5), since it is almost free of cellular organelles, the centre of the cytoplasm may contain just one small mitochondria, or a piece of smooth endoplasmic reticulum membrane, while the remainder is occupied by initial filaments which are short, fine and evenly distributed (Fig. 5). Close to the plasmatic membrane, there are some zones where some accumulations of particles with smaller dimensions than the ribosomes are condensed (Fig. 5). These particle condensation zones are related to the initial filaments and may correspond to actin globular molecules. These organize the initial filaments which may, hypothetically, represent the first polymerization of molecules precursing contractile proteins. These molecules are, presumably, the ones which organize themselves into initial filaments and which then move toward the interior of the myoblast. These initial filaments are the first sign of the ultrastructural differentiation of the myoblasts, which can be seen with the electron microscope. Several researchers have already identified the fine filaments with the protein actin (Obinata et al., 1966; Fischman, 1967).

Our hypothesis suggests that the formation of the initial filaments (Fig. 5) begins in the centre of the myoblast, at the level of the nucleus, in the circanuclear cytoplasm, with the participation of the RNA and the ribosomes, giving rise to the actin globular molecules which move toward the sharp ends of the myoblast, grouping in accumulation of such particles, which continue on the periphery of the cytoplasm, under the plasmatic membrane. Then, in these peripheral accumulations, the initial filaments originate. These invade the centre of the myoblast in this phase of cytodifferentiation, in which they appear arranged in homogeneous form on the sharp ends of the myoblast (Fig. 5).

This first appearance of initial filaments in myogenesis is independent of the nervous system since, so far, there is no cell (neuroblast) or prolongation of the neural tube which comes into contact with the myoblasts (Figs. 1-4). This independence between the myogenesis and the nervous system has been shown in «in vitro» by Shimada et al. (1967), and by Phillips and Bennett (1984).

It appears that the presumed myoblasts (i.e. those that, as yet, have no initial filaments), are prepared by increasing the cytoplasmic RNA concentration, and this is most intense when the myotome strip adheres to the dermatome strip (Przybylsky and Blumberg, 1966).

In stage (19-) between stages 18 and 19, it does not appear that there are two types of myoblast - light and dark - as stated by Przxyblyski and Blumberg (1966), but rather, as we think, that there is only one type of myoblast which given the concentration of its organelles and ribosomes, is dark in the central perinuclear zone and, because of their absence, is light at the sharp ends, where the initial filaments gradually accumulate inside a very light cytoplasmic matrix. Our work gives only partial confirmation of the earlier research by Przybylski and Blumberg (1966).

References

Allbrook D. (1962). An electron microscope study of regenerating skeletal muscle. J. Anat. 96, 137-152.

Allen E.R. and Pepe F.A. (1965). Ultrastructure of developing muscle cells in the chick embryo. Amer. J. Anat. 166, 115-148.

Bennett H.S. and Porter K.R. (1953). An electron microscope study of sectioned breast muscle of the domestic fowl. Amer. J. Anat. 93, 61-105.

Bergman R.A. (1962). Observations on the morphogenesis of rat skeletal muscle. Bull. Hopkins Hosp. 110, 187-198.

Bonner P.H. (1988). Gap junctions form in culture between chick embryo neurons and skeletal muscle myoblast. Dev. Brain. Res. 38, 233-244.

Bonner P.H. and Adams T.R. (1962). Neural induction of chick myoblast differentiation in culture. Dev. Biol. 90, 175-184.

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

Bonner P.H. and Martin C.L. (1984). Electrotonic synapses between cultured neurons and mononucleated muscle cells. J. Cell Biol. 99, 24 a.

Butler J., Cosmos E. and Brierley J. (1982). Differentiation of muscle fiber types in aneurogenic branchial muscles of the chick embryo. J. Exp. Zool. 224, 65-80.

Crow M.T. and Stockdale F.E. (1986). Myosin expression and specialization among the earliest muscle fibers of the developing avian limb. Dev. Biol. 113, 238-255.

Dessouky D.A. and Hibbs R.G. (1965). An electron microscope study of the development of the somatic muscle of the chick embryo. Am. J. Anat. 116, 523-566.

Ferris W.E. (1959a). Electron microscopic observations of early myogenesis in the chick embryo. Ph.D. Thesis. Department of Zoology, University of Chicago.

Ferris W.E. (1959b). Electron microscope observations of the histogenesis of striated muscle. Anat. Rec. 133, 275-293.

Fischman D.A. (1967). An electron microscope study of myofibril formation in embryonic chick skeletal muscle. J. Cell Biol. 32, 557-575.

Hamburger V. and Hamilton H.L. (1951). A serie of normal stages in the development of the chick embryo. J. Morphol. 88, 49-92.

Hay E.D. (1962). Cytological studies of dedifferentiation and differentiation in regenerating amphibian limbs. In Regeneration. Rudnick D. (ed). New York. Ronald Press. Co. p 177.

Hay E.D. (1963). The fine structure of differentiating muscle in the salamander tail. Z. Zellfosch. 59, 6-34.

Heidenhain M. (1911). Handbuch der Anatomie des Menschen. Plasma und Zelle, L. 2. Jena Fischer-Verlag.

Lindner E. (1960). Myofibrils in the early development of chick embryo heart as observed with the electron microscope. Anat. Rec. 136, 234-235.

McLennan I.S. (1983). Neural dependence and independence of myotube production in chicken hindlimb muscles. Dev. Biol. 98, 287-294.

Moscona A. (1955). Cytoplasmic granules in myogenic cells. Exp. Cell Res. 9, 337-380.

Obinata T., Yamamoto M. and Maruyama K. (1966). The identification of randomly formed thin filaments in differentiating muscle cells of the chick embryo. Dev. Biol. 14, 192-204.

Ogawa Y. (1962). Synthesis of skeletal muscle proteins in early embryos and regenerating tissue of chick and triturus. Exp. Cell Res. 26, 269-274.

Phillips W.D. and Bennett M.R. (1984). Differentiation of fiber types in wing muscles during embryonic development: Effet of neural tube removal. Dev. Biol. 106, 457-468.

Price H.M., Howes E.L.,Jr. and Blumberg J.M. (1964). Ultrastructural alterations in skeletal muscle fibers injuried by cold. II. Cells of the sarcolemmal tubes: Observations on «discontinuous» regeneration and myofibril formation. Lab. Invest. 13, 1279-1302.

Przybylski R.J. and Blumberg J.M. (1966). Ultrastructural aspects of myogenesis in the chick. Lab. Invest. 15, 836-863.

Reynolds E.S. (1963). The use of lead citrate at high pH as an electronopaque stain in electron microscopy. J. Cell Biol. 17, 208-212.

Rutz R., Haney C. and Havschka S. (1982). Spatial analysis of limb bud myogenesis: a proximodistal gradient of muscle-colony-forming cells in chick embryo leg buds. Dev. Biol. 90, 399-411.

Shimada Y., Fischman D.A. and Moscona A.A. (1967). The fine structure of embryonic chick skeletal muscle cells differentiated in vitro. J. Cell Biol. 35, 445-453.

Wainrach S. and Sotelo J.R. (1961). Electron microscope study of the developing chick embryo heart. Z. Zellforsch. 55, 622-634.

Weed I.G. (1936). Cytological studies of developing muscle with special reference to myofibrils, mitochondria, Golgi material nuclei. Z. Zellforsch. Mikr. Anat. 25, 516-540.

Williams L.W. (1910). The somite of the chick. Am. J. Anat. 11, 55-100.

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