Myogenesis and Muscular Function in Frog and Rat Embryos[1]

By

Tomoji Aoto[2]

(Zoological Institute, Hokkaido University)

(With 1 Plate)

Embryonic development of skeletal and cardiac muscle has attracted the attention of many workers, and quite a few data have been assembled since the technique of electron microscopy and biochemical methods of extracting actomyosin have been developed during recent years. In 1940, Goss made an observation on the occurrence of contractility in the embryonic heart muscle of rat, using both fixed and fresh material, and came to the conclusion that "the specialized cytological structures, fibrillae and cross striations, are not elaborated until some hours after contractile activity is well established." Similar observations were made on cardiac and skeletal muscles of embryos of a wide variety of vertebrates by several authors : in urodeles by Copenhaver ('39); in anurans by Nicholas ('50): in the chick by Szepsenwol ('46), Patten ('49) and Baud ('54); and in the rat by Nicholas ('50). All of these authors are in agreement that contractility appears before visible differentiation of intracellular structures of the kind that "we are accustomed to think of as characteristic of muscular tissue" (Patten). However, in some cases the myofibrils show birefringence positively before contractility appears.

The present paper deals with the time relations between histogenesis and functional development of the muscle in frog and rat embryos.

Material and methods

For observation in the present study, fresh and fixed embryos of *Rana pipiens* (from St. 16 to St. 25) and of the rat (from St. 21 to St. 34, or 12-18 days old) were used. Developmental stages are designated in accordance with Witschi's standard stages of frog development ('56) and of rat development ('56 and '57). After removal of the darkly pigmented epidermis and the yolk endoderm, fresh frog tissues were placed on glass slides and mounted in glycerin. Some frog and rat embryos were fixed with 10% formalin, embedded in paraffin and cut sagittally or frontally at 7 μ. Mallory's triple

1) The present study was made at the Zoology Department, State University of Iowa, Iowa City, Iowa, U.S.A. It was aided by a grant from the Rockefeller Foundation.

2) Present address : Department of Zoology, State University of Iowa, Iowa City, Iowa, U.S.A.

Jour. Fac. Sci. Hokkaido Univ. Ser. VI, Zool. 13, 1957 (Prof. T. Uchida Jubilee Volume).

stain and Harris's hematoxylin were used for staining. Unstained preparations were observed under a Spencer polarization microscope.

Results

Frog: No double refraction was noted in the embryo at stages 16, 17 and 18, although segmentation of myotomes takes place in the upper part of the body as early as St. 14. The first sign of muscular movement is noticeable when a mechanical stimulus is applied to an embryo at St. 18–19. At St. 19, a few parallel, fibrillar, slightly birefringent elements appear in the upper myotomes. The number of myotomes containing birefringent materials increases as the embryo grows, and at St. 20 almost all trunk myotomes show birefringence. First signs of cross striation can be observed in the myofibrils at this stage (Fig. 1). The latter are few in number and still hard to recognize. Numbers of highly refractive granules are scattered throughout the embryonic cell bodies. The myoblasts are arranged in rows parallel to the body axis suggesting already fibrillar structure due to the double refraction on certain portions of the chain (Figs. 2 and 3). The scattered granules decrease rapidly in number during St. 21. Their almost complete disappearance at St. 22 renders detection of myofibrils easier. Striation of myofibrils appears first in upper myotomes, and then spreads posteriorly. At St. 22–23, all the myofibrils of the trunk are striated and have increased in size and number (Fig. 4). The birefringence is usually strongest near the intersegmental membrane of each myotome where myofibrils are also most dense (Fig. 5). This fact seems to suggest that the myofibril development starts at or near the intersegmental membrane, progressing toward the center of each myotome. Ventro-dorsal and median-lateral progression are also noticeable, though less clearly.

In later stages of development, birefringence of myofibrils becomes very brilliant, with fibers thicker. At St. 24–25, when the embryo completes yolk absorption and starts feeding, the fibers are so thick and tightly packed that little space remains between them (Fig. 6).

Rat: In the $12\frac{1}{2}$-day old rat embryo (St. 25), elongated myoblasts arrange themselves parallel to the long axis of the body. Proliferation of the sclerotomes is very active except in the tail somites. But at this stage, no myofibrils are formed. Refractive granules occur scattered in the somites. In the 14-day old embryo (St. 29), myotomes are clearly differentiated especially in the upper part of the body. The nuclei of myoblasts are strongly refractive. They are more elongated than before, the longest diameter being about two and a half times the length of the shortest. The cytoplasm also is stretching longitudinally and displays weak birefringence.

In the 15-day old embryo (about St. 32), the myofibrils appear clearly and noticeably birefringent, although not yet striated (Fig. 7). During later stages of development, the myofibrils increase in number and size, while the number of

refractive granules scattered through the myotomes decreases markedly (Fig. 8). The first sign of contractility may be elicited in the 16-day old embryos (St. 33) ; and the first sign of cross striation in the myofibrils is found in the neck region of the 17-day old embryos (St. 33/34). In the 18-day old embryos (St. 34), striation is found to exist in the myofibrils throughout the body (Fig. 9).

Discussion

Applying faradic shock directly to rat embryos, which were taken from the maternal uteri, Windle, Minear, Austin, and Orr ('35) found that the first responding muscles are located in the shoulder region, and that, during the course of development, excitability spreads "rostrad and caudad, as well as distad and ventrad, from this region." Our own observations show clearly that the morphologic differentiation of the myoblasts of frog embryos, in terms of birefringence and cross striation in myofibrils, begins in the upper myotomes and then proceeds downward during later stages of development. The same tendency of axial progression of myogenesis is noted in rat embryos. Fibrillar structure, birefringence and cross striation, each of the myoblasts, appear first in the myotomes of the neck region and later in those of the trunk. These facts seem to indicate that the development of muscular function is definitely related with that of changing cellular microstructure differentiation. The cytological findings show that the contractility appears, in both species, before the differentiation of specialized structure in that tissue.

Physiologically, muscluar activity develops in three steps, myogenic reaction, neuromotor reaction, and sensory-motor reaction, following closely one another. Myogenic reactions are established soon after the myoblasts become birefringent (frog embryos at St 19, rat embryos at St. 33). It is assumed that double refraction indicates an orderly arrangement of molecules in cells and tissues. This very same arrangement most likely is connected with contractility. It may be safely surmised, therefore, that birefringence is an expression of the molecular structure that is essentially allied with contractility. For further analysis enzymatic studies seem promising. Herrmann and Nicholas ('48) report a sharp rise of apyrase activity in rat muscle at the 16th day of development, when the embryonic muscular movements start. They were, however, very cautious in drawing inferences, saying that "it is possilbe.... that the simultaneous onset of muscle contraction and of apyrase activity.... is an accidental coincidence, indicating a more general readjustment in the activities and metabolism of the rat embryo at this developmental stage." Moreover, a submicroscopic study seems to be needed for clarifying the constitution of molecules, and the mechanism of embryonic contraction.

Summary

1. Development of muscular movement was studied by parallel observation of fresh whole mounts and stained or unstained preparations of frog (St. 16–25) and rat (St. 25–34) embryos under the light and the polarization microscope.

2. In both species, it was found that birefringence precedes contractility, which is followed by the appearance of cross striation in myofibrils.

3. The mechanism of embryonic contractility is discussed.

The present study was performed as a part of a more extensive investigation on this subject, now carried on by Dr. Emil Witschi and his collaborators. The writer wishes to express his cordial thanks to Professor Witschi for his encouragement and helpful criticism given to the writer throughout the course of this work.

Literature cited

Baud, C.A. 1954. Les premiers stades de la différenciation submicroscopique du cytoplasme dans les cellules embryonnaires. Excerpta Medica 8 (sect. 1) : 409 (abstr. no. 1716).

Copenhaver, W.M. 1939. Initiation of beat and intrinsic contraction rates in the different parts of the *Amblystoma* heart. Jour. Exp. Zool. 80 : 193-224.

Goss, C.M. 1940. First contractions of the heart without cytological differentiation. Anat. Rec. 76 : 19-38.

Herrmann, H., and J.S. Nicholas 1948. Enzymatic liberation of inorganic phosphate from adenosinetriphosphate in developing rat muscle. Jour. Exp. Zool. 107 : 177-181.

Nicholas, J.S. 1950. Development of contractility. Proc. Amer. Philosoph. Soc. 94 : 175-183.

Patten, B.M. 1949. Initiation and early changes in the character of the heart beat in vertebrate embryos. Physiol. Rev. 29 : 31-47.

Szepsenwol, J. 1946. A comparison of growth, differentiation, activity, and action currents of heart and skeletal muscle in tissue culture. Anat. Rec. 95 : 125-146.

Windle, W.F., W.L. Minear, M.F. Austin, and D.W. Orr 1935. The origin and early development of somatic behavior in the albino rat. Physiol. Zool. 8 : 156-185.

Witschi, E. 1956. Development of Vertebrates. W.B. Saunders Co., Philadelphia.

———— 1957. The Standard Stages of Rat Development. Mimeographed copy.

Explanation of Plate VII

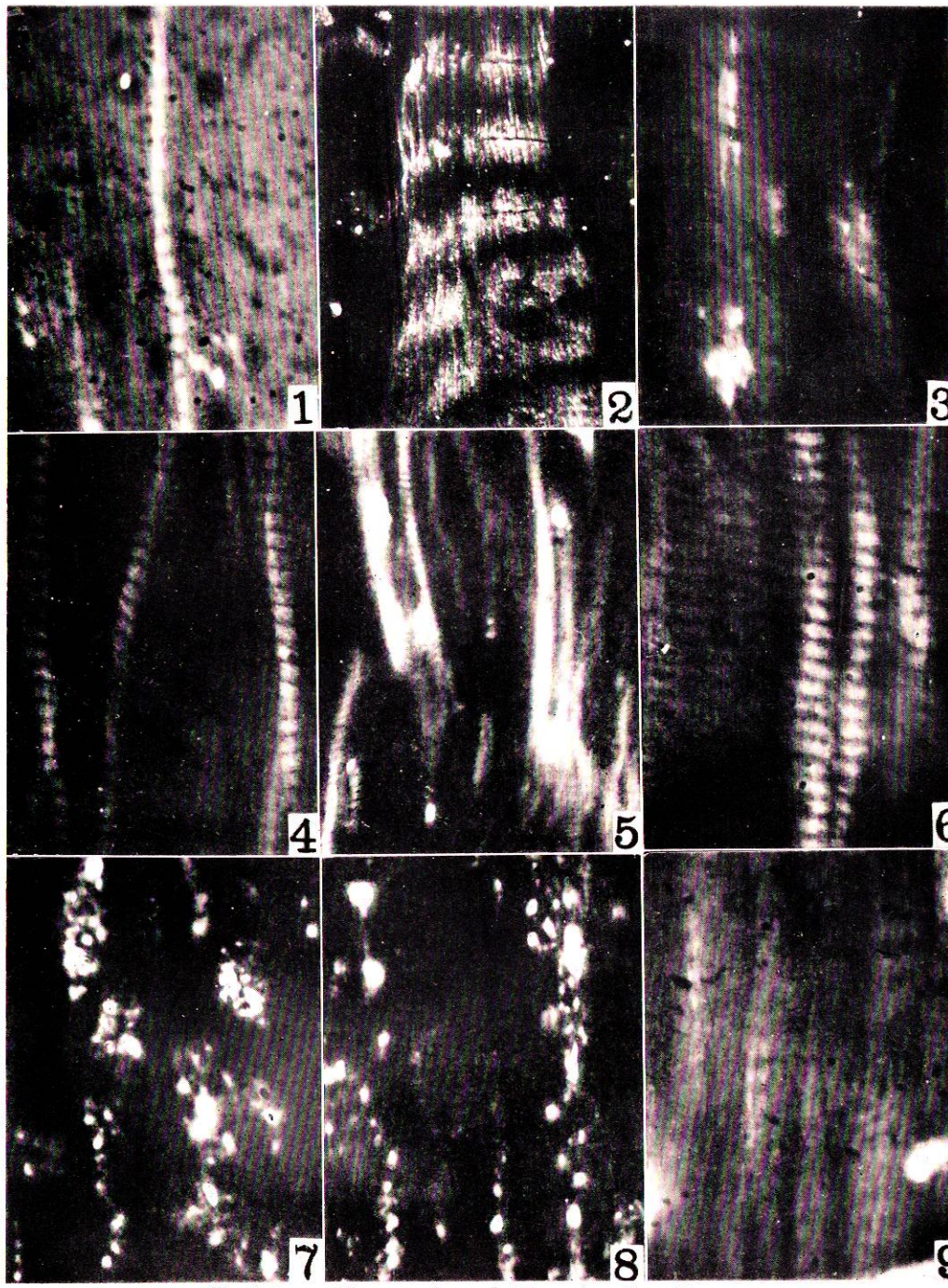
All photomicrographs illustrate birefringence of unstained myofibrils in frontal or sagittal sections.

Frog Embryos

1. Single myofibril at St. 20, showing cross striations. ×1900.
2. Myofibrils at St. 21, at low magnification. Note the stronger birefringence along the intersegmental membrane of myotomes. ×200.
3. Myofibrils at St. 21, at higher magnification. ×1900.
4. Myofibrils at St. 23. ×1900.
5. Myofibrils at St. 25, spreading on approach to slanting intersegmental membrane. ×800.
6. A higher magnification of the same specimen as shown in Fig. 5. The same pattern of cross striation is seen in neighboring fibers. ×1900.

Rat Embryos

7. Myofibrils in the neck region of a 15-day old embryo (about St. 32). The cells are arranged parallel. Myofibrils show weak birefringence. ×1900.
8. The neck region of a 17-day old embryo (St. 33/34). Myofibrils become longer, showing stronger birefringence. ×1900.
9. Myofibrils in the neck region of an 18-day old embryo (St. 34), showing distinct cross striations. ×1900.



T. Aoto : Myogenesis and Muscular Function, etc.