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Studies on Development and Differentiation of Muscle V. A comparative Study on the Growth of the Hatching Muscle and the Other Muscles in Chick Embryo

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Summary

1. The growth and development of striated muscle was studied in the hatching muscle (*M. complexus*), the M. biceps femoris and the M. pectoralis of chick embryos using the light microscopic technique.
2. The mean fiber diameters of these muscles increased gradually until 12 days of incubation with no significant difference. From 12 until 20 days of incubation, the M. complexus enlarged and developed its fiber diameters more remarkably than the other muscles.
3. There is a rapid and statistically significant rise in the count of fiber numbers in these muscles from 10 until 16 days of incubation. However, during subsequent stage of development until hatching, there appears no further significant change in the number of muscle fibers. The increase-rate of muscle fibers is more remarkable in the M. biceps femoris than in the M. complexus.
4. The increase rate of the muscle fiber numbers in a given primary muscle fascicle is closely parallel to that of the total muscle fiber numbers in cross section. The M. complexus at the hatching time has a smaller number of the muscle fibers in a given primary muscle fascicle and also in total cross section than the other muscles.
5. There is no significant difference between the M. complexus and the M. biceps femoris in the number of the primary myotubes in a given primary muscle fascicle from 11 to 13 (IV) days of incubation.

Although it is well known, in vitro (1, 2, 3) as well as in vivo (4), that during development striated muscle fibers increase their number of nuclei by fusion with mononuclear myoblasts, there remains an argument on the mode of the increase of the new muscle fibers. There are two theories about the mode of increase of muscle fiber number. In the first, "*Unifiber*" theory, the larger fibers may split and bud longitudinally into two or more smaller fibers (5, 6, 7, 8, 9). In the second, "*Multifiber*" theory, the new generation myotubes arise from mononuclear myoblasts proliferated around the wall of the primary large fibers (10, 11, 12).

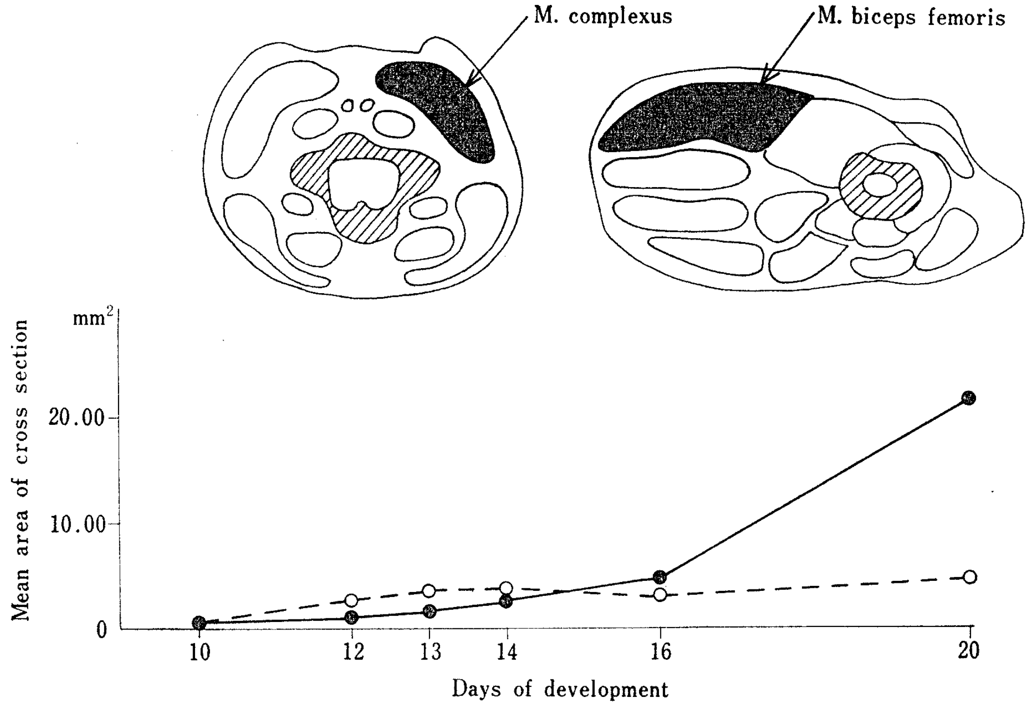

FIG. 1. Increase in the total cross-sectional area of the M. complexus (●——●) and the M. biceps femoris (○——○) with embryonic days.

During the last ten years, the electron microscopy has been applied in the research of this field. Fischman (1967) believed that the new generation myotubes arose from myoblast fusion process are destined to lateral fuse with primary myotube and form one large muscle fiber (13). Kelly and Zacks (1969) has arrived at a different view that, although the lateral fusion of undifferentiated, mononuclear cells with primary myotube occurred, there was no proof for the fusion of the successive generation myotube with the primary one (14). The mechanism of increase of the fiber number has been unsolved until now.

The following paper deals with the light microscopic observation on the growth of the chick hatching muscle which developes remarkably at the later stage of incubation and of the two other muscles selected as the common muscle tissue.

Materials and Methods

The fertilized eggs of White Leghorn were incubated at 37.8°C. The muscle tissues (*M. complexus, M. biceps femoris, M. pectoralis*) were sampled, immediately immersed in Locke's solution, and teared in small pieces under a dissecting microscope. The specimens were then fixed in Bouin's or Helly's solution and infiltrated with paraffin in the usual manner. When the muscles were sectioned, extreme care was taken to orient them so that the plane of sectioning would be perpendicular to the long axis of the muscle. Sections were made at 6μ and stained with PAS-Hematoxylin.

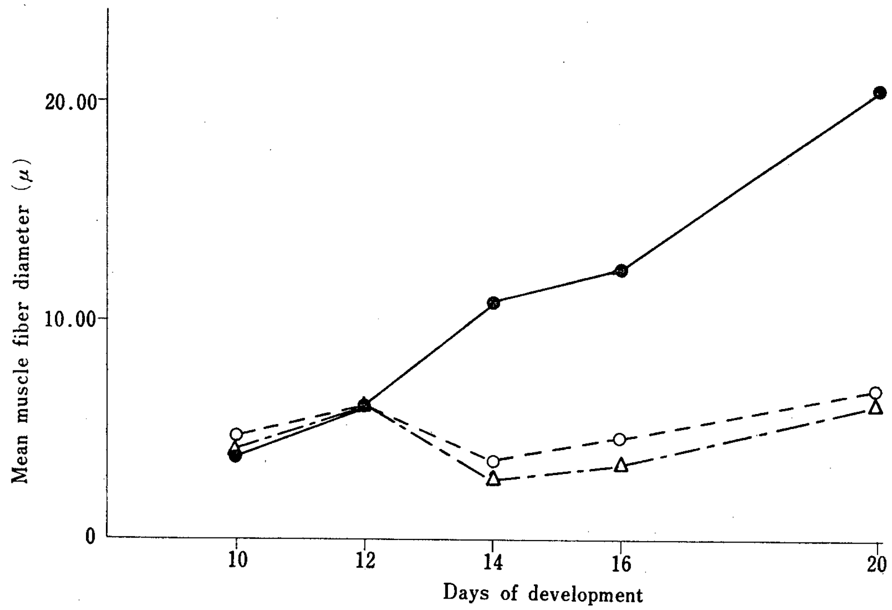

FIG. 2. Change in mean diameter of muscle fibers of the M. complexus (●——●), the M. biceps femoris (○——○) and the M. pectoralis (△——△) with embryonic days.

(1) *Total cross-sectional area of muscle.*

The M. complexus was sectioned through the middle of segments separated by tendinous conscriptions. The M. biceps femoris was sectioned through the middle of thigh. The outline of the entire section was drawn on the tracing paper using Abbe's drawing apparatus with a low power (4×) objective. The unit area of the tracing paper (1 mm^2) was weighted. The area of the cross section of the whole muscle was then calculated by dividing the weight of the cross-sectional area by that of unit area.

(2) *The distribution of the muscle fiber diameter.*

The cross-diameters of Helly-fixed muscle fibers were measured with an ocular micrometer and a high power (100×) objective. The fiber diameters were calculated only in the primary myotubes until 12 days of incubation. One hundred samples were taken, at random, for all the muscles so that they included fibers from all regions of the cross section.

(3) *Total number of muscle fibers in cross section.*

The number of individual fibers was counted in 0.01 mm^2 unit area until 14 days of incubation, and 0.063 mm^2 from 16 to 20 days of incubation. These fibers that were entirely within the unit area, and those that were partially within it at the upper and left line limits of the unit area outline were counted. Fibers which fell upon the right and lower line limits were omitted from the count. The average number of fiber per unit area was then determined. The total number of fibers in an entire cross section of muscle was calculated by dividing the total cross sectional area by the unit area and multiplying by the average number of fibers in the unit area.

(4) *The number of muscle fibers in a primary muscle fascicle.*

The muscle fibers are gathered in fascicles which consist of varying numbers of fibers and were surrounded by a layer of connective tissue. These fascicles, designated primary muscle fascicles, may again be aggregated, and may be gathered into larger fascicles. The primordia of such fascicles could be distinguished already in embryonic muscle tissues at the 10 days of incubation.

(5) *The number of primary myotubes in a primary muscle fascicle.*

The changes in the average number of primary myotubes in a given primary muscle fascicle were calculated in the M. complexus and the M. biceps femoris at 10. 12 (I, II, III, IV: 6 hrs interval), 13 (I, II, III, IV; 6 hrs interval), 14 (I, II, III, IV; 6 hrs interval) and 15, 16, 18, 20 days of incubation. The primary myotube was easily identified by its situation in a given group of myotubes, and by its central large nuclei and considerable accumulation of myofibrils at the periphery of the sarcoplasm by the use of PAS-Hematoxylin stain.

Results

Wet weight and % body weight of the paired M. complexus increase rapidly from 16 to 20 days of incubation. The major increase is observed between 18 and 20 days of incubation (Fig. 7). There is a marked decrease in these value for two or three days after hatching, followed by a gradual decline (15).

Between 10 and 20 days of incubation, there is a gradual enlargement of the mean area of cross section in the M. complexus and the M. biceps femoris closely corresponding to the muscle growth (Fig. 1). After 16 days of incubation, however, there is more remarkable enlargement in the M. complexus than in the M. biceps femoris. By 20 days of incubation, the area in the M. complexus had increased to 21.46 mm^2, while that in the M. biceps femoris to 4.56 mm^2

The values for the mean fiber diameter of the M. complexus, the M. biceps femoris and the M. pectoralis are given in Fig. 2. The mean fiber diameters increase gradually until 12 days of incubation with no significant difference. However, as growth progressed, differences emerged between the M. complexus and the other two muscles. As far as 14 days of incubation, the diameters of the M. complexus already attained to 10.83 μ, while those of the M. biceps femoris and the M. pectralis to 3.75 and to 2.89 μ. There is little difference in the diameter of the primary myotubes, but a wide difference in the diameter of the successive generation myotubes at 12 days of incubation between the M. complexus and the M. biceps femoris (Figs 8, 9). The muscle fiber diameters of the M. complexus in a given primary muscle fascicle are three times as much as those of the M. biceps

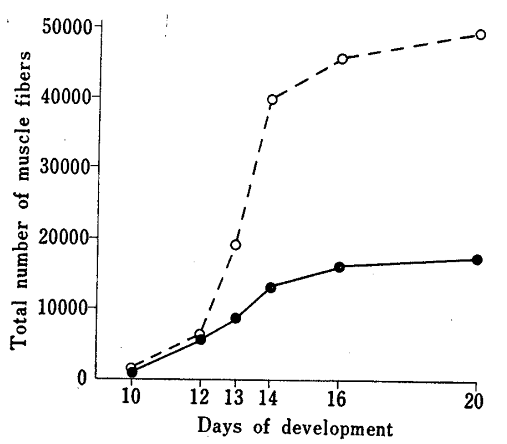

Fig. 3

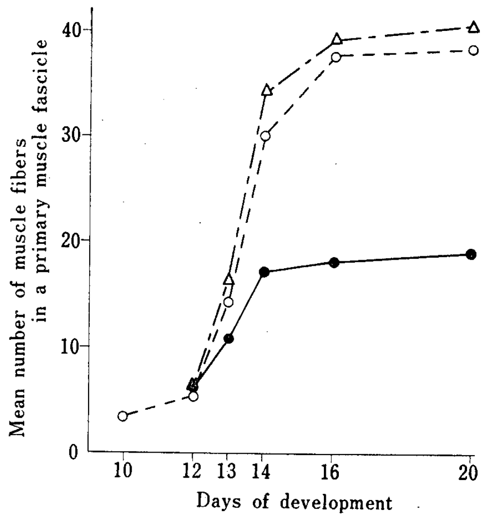

Fig. 4

FIG. 3. Change in the total number of muscle fibers in cross section of the M. complexus (●——●) and the M. biceps femoris (○——○) with embryonic days.

FIG. 4. Change in the number of muscle fibers in a primary muscle fascicle in the M. complexus (●——●), the M. biceps femoris (○——○) and the M. pectoralis (△——△) with embryonic days.

femoris at 20 days of incubation (Figs. 10, 11).

The counts of the total fiber number of the M. complexus and the M. biceps femoris were given in Fig. 3. There is a rapid and statistically significant rise in the counts in both muscles from 10 to 16 days of incubation. However, during subsequent stage of development until hatching, there appears no further significant change in the number of muscle fibers. In the rate of increase of muscle fibers, there is more remarkable increase in the M. biceps femoris than in the M. complexus. The total amounts of the muscle fibers in the cross section attained to 17725 in the M. complexus and 49595 in the M. biceps femoris at 20 days of incubation.

The counts of the fiber numbers in a given primary muscle fascicle are given in Fig. 4. While a differential increase-rate of total muscle fibers in cross section is evident, it appeared that the rate in a given primary muscle fascicle is closely parallel to that of total muscle fiber number in cross section. The coefficient of correlation between the total number of muscle fiber in cross section with the fiber number in a given primary muscle fascicle was calculated as shown in Fig. 5. The coeficients is 0.9702 in the M. complexus and 0.9784 in the M. biceps femoris. The regression line based on the following equations; $Y=936.50X-1083.05$ in the M. complexus and $Y=1336.07X-2089.23$ in the M. biceps femoris. As shown in Fig. 10 and Fig. 11, the mean value of fiber number in a given primary muscle fascicle at 20 days of incubation has attained to 19.45 in the M. complexus and to 38.78 in the M. biceps femoris.

The numbers of primary myotube in a given primary muscle fascicle were

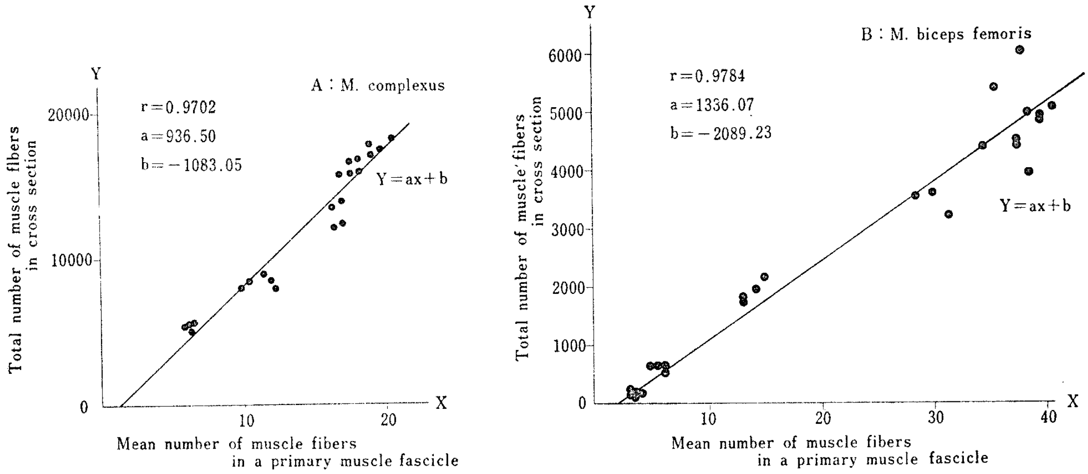

FIG. 5. The coefficient and the regression line between the total number of muscle fibers in cross section with the fiber number in a given primary muscle fascicle. The data of the M. complexus and the M. biceps femoris were shown in left figure (A) and in right figure (B).

calculated and shown in Fig. 7. There is no significant difference between the two muscles from 11 to 13 (IV) days of incubation. The primary myotubes cannot be distinguished, however, from the successive generation myotubes in the M. complexus after 14 (I) days of incubation. The primary muscle fascicles have smaller surrounding space between each primary myotubes in the M. complexus than in the M. biceps femoris (Figs. 8, 9).

Discussion

Little is known about the mode of the increase in the number of the muscle fibers and the factors that determine its number. MacCallum (1898) stated that hyperplasia in human striated muscle is completed early in foetal life (5). This statement was opposed by the work of Cuajunco (1942) that the increase of muscle fibers could not be detected after mid-gestation in the human biceps brachialis muscle (16). Tello (1917) and Montgomery (1962), on the other hand, deduced from the morphological appearances that new fibers might appear in human muscle up to the time of birth (17, 18). The increase of fiber number in rat muscles continued during the first two weeks of postnatal life in the radialis muscle (10), but during the first three weeks of postnatal life in the extensor carp radialis longus, soleus and plantaris muscle (19). Recently Bridge and Allbrook (1970) reported that the muscle fibers of the two leg muscles of quokka increased until proximately one hundred days of postnatal life (20).

From the discrepancy among those reports, it may be explained that there are difference in the increase rates of muscle fiber numbers between various foetal specimens and muscle specimens at term during foetal and early postnatal life.

It was examined in this report whether there were some differences in the

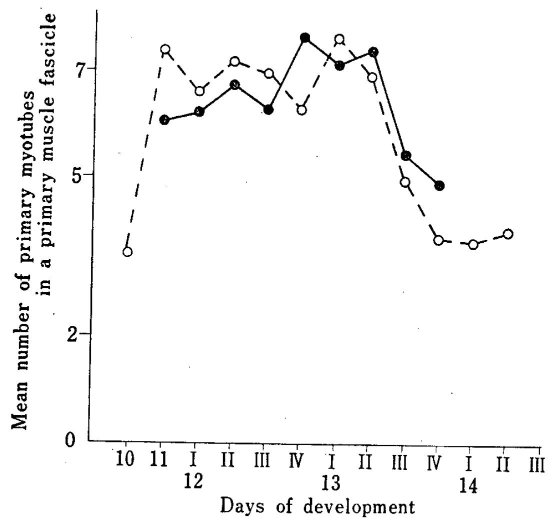

FIG. 6. Change in the number of primary myotubes in a primary muscle fascicle in the M. complexus (●——●) and the M. biceps femoris (○——○) with embryonic days

mode of increase in the number of muscle fibers between the M. complexus, the M. biceps femoris and the M. pectoralis of the chick embryos. The M. complexus, commonly called as "Hatching muscle", showed a remarkable growth for the breaking of egg shell at the hatching time (15, 21, 22). It was, therefore, strongly expected to be found any characteristic differences in the mode of increase in the fiber number between the M. complexus and the other two muscles.

Bridge and Allbrook (1970) suggested that muscle growth occurs in three successive stages. In stage 1, the original myotube grow rapidly both in size and functional capacity. In stage 2, a new population of small fibers appears, and the growth of the older fiber is temporarily suspended. In the third stage, no new fibers form, and all fibers grow together to reach the adult diameter (20). These three stages have also been described in the foetal sheep (23). The increase in the value of wet weight, percent body weight and the cross sectional area of the M. complexus involves both hypertrophy and hyperplasia of muscle fibers. This interests us with regard to the demonstration whether the growth in the M. complexus occurs in the three stages described above. At 12 days of incubation, there are no differences in the size of the primary myotubes formed originaly in the three muscles examined, but compared with the other muscles, there is a marked enlargement of the successive generation myotubes arose from the primary myotube in the M. complexus (24). These results suggested that the new small fibers appeared in stage 2 in the M. complexus began already to grow and remarkable enlarge before arrive at the maximum level of the muscle fiber number. It is difficult, therefore, to distinguish between stage 2 and stage 3 in accordance with Bridge and Allbrook's theory already given. The increase of fiber number continued progressively until 16 days of incubation in the M. complexus and the other muscles.

There were highly significant positive correlations between the total number of fibers in the cross section and the fiber number within the primary muscle fascicle; r=0.97 in the M. complexus and r=0.98 in the M. biceps femoris. This showed that it is an important factor for the study of the mode of increase of the total muscle fiber number in the cross section to calculate the increment of fiber number in a given primary muscle fascicle. In "*Multifiber*" theory, it is from some of these satellite cells that new fibers are added to the development muscle until the full number is achieved. Fischman (1969), however, recently reported that the all successive generation myotubes destined to fuse laterally to a given primary myotube and form a mature fibers (13). The diameter of the primary myotube is expected, if this opinion is true, to increase considerably by lateral fusion of the successive generation myotubes during foetal development and this increase should be more remarkable in the M. complexus than in the other muscles. Since there were no significant changes in the size and even in the number of the primary myotubes between the M. complexus and the M. biceps femoris, Fischman's assuption can not be supported by our present work.

Since the successive generation myotubes in the M. complexus develop and enlarge more rapidly than those in the M. biceps femoris, and are deprived of the space in which they increase their number and size, their numbers in the M. complexus remain smaller than those in the M. biceps femoris. This suggests that the number of the muscle fibers within a given primary muscle fascicle is determined by the surrounding space out of the primary myotubes

Acknowledgement

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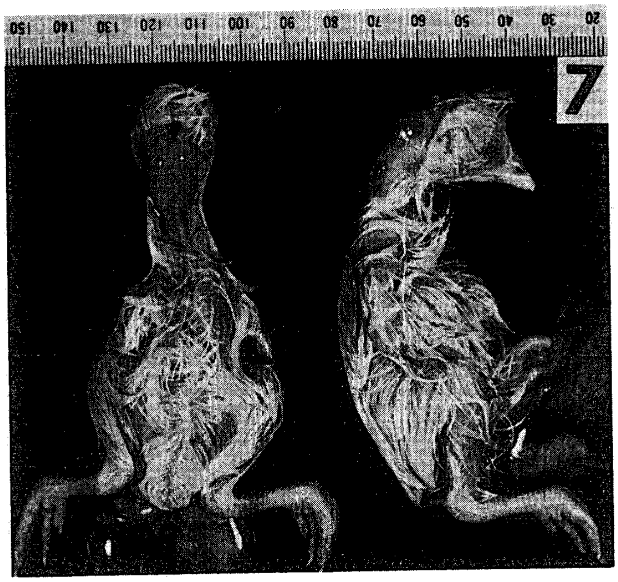
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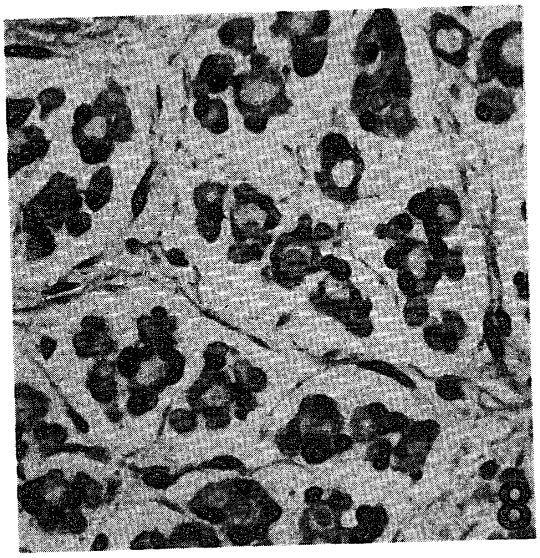
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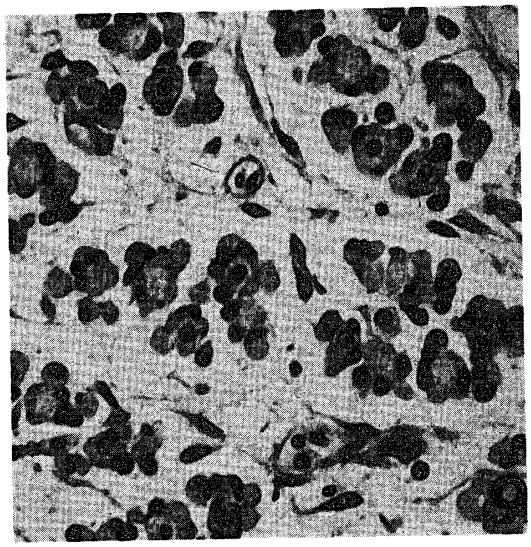

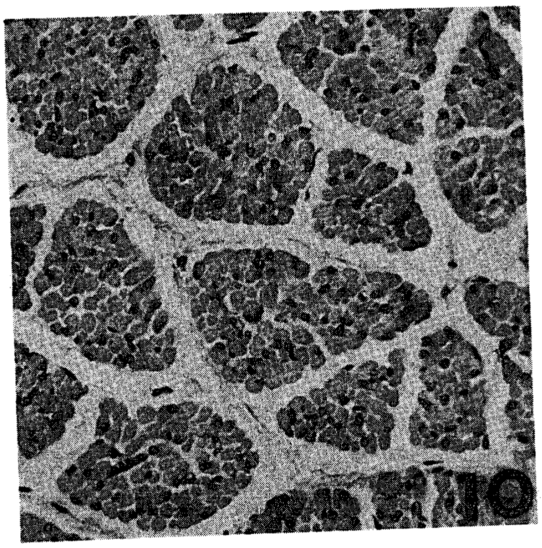
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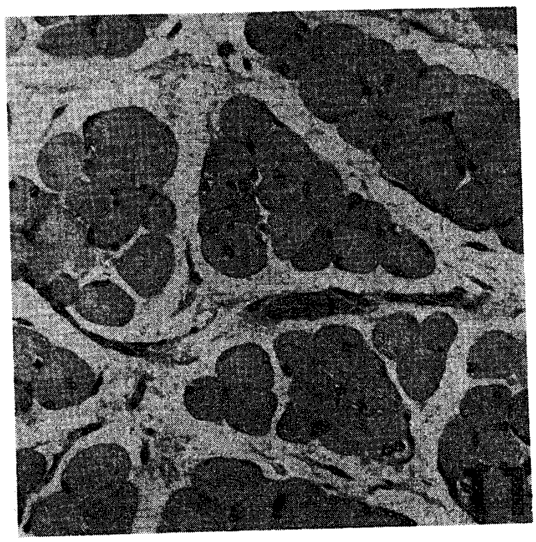
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Explanation of the Figures

FIG. 7. The M. complexus of chick at 20 days of incubation. It reached its maximum development at about this time and rapidly became smaller after hatching. This paired muscle overlying the M. semispinalis cervicis and the M. biventer cervicis originates from the neural spines of cervical vertebrae 2, 3, 4, 5 and from fascia overlying the deeper muscles.

FIG. 8. The primordia of the primary muscle fascicles in the M. biceps femoris at 12 days of incubation. PAS-Hematoxylin stain × 800

FIG. 9. The primordia of the primary muscle fascicles in the M. complexus at 12 days of incubation. Note the remarkable growth of the successive generation myotubes formed along the wall of the primary myotubes. PAS-Hematoxylin stain × 800

FIG. 10. The primary muscle fascicles in the M. biceps femoris at 20 days of incubation. PAS-Hematoxylin stain × 800

FIG. 11. The primary muscle fascicles in the M. complexus at 20 days of incubation. The diameters of muscle fibers are about three times as much as those of the M. biceps femoris, but the number of muscle fibers in a given primary muscle fascicle is smaller than those of the M. biceps femoris. PAS-Hematoxylin stain × 800