Histochemistry of the Chicken Skeletal Muscles II. Distribution and Diameter of Three Fiber Types

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II. Distribution and Diameter of Three Fiber Types.

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Summary

The distribution and diameter of three fiber types were examined in the *M. biventer cervicis*, *M. pectoralis thoracicus*, *M. iliotibialis lateralis*, and *M. flexor cruris medialis* of the chickens. The three fiver types were classified on the basis of the intensity of NADH-diaphorase and alkali-stable myosin ATPase activity. The type A fibers with a high activity of NADH-diaphorase and alkali-stable ATPase were generally smaller in diameter than the type B fibers with low NADH-diaphorase activity and high alkali-stable ATPase activity. The type C fibers with high NADH-diaphorase activity and low alkali-stable ATPase activity were substantially larger in diameter than the A and B fibers. The *pectoralis thoracicus* consisted almost entirely of the B fibers except for the limited, deepest region, which had A, B, and C fibers. In the *iliotibialis lateralis* the A fibers were fewer than the B fibers and C fibers were not found. The A fibers were greater in number than the B and C fibers in the *biventer cervicis* and *flexor cruris medialis*. The C fibers were distributed evenly throughout the *biventer cervicis* muscle. In the *flexor cruris medialis* they were greater in number in the superficial region and fewer in the deeper region.

Chicken skeletal muscle fibers have been classified into three and more fiber types on the basis of the differences in histochemical properties (1–4). In the previous paper (3), the three fiber types were designated as the type A, B, and C. The type A fibers are high in the activity of succinate dehydrogenase, NADH-diaphorase, and myosin ATPase, and moderate to high in phosphorylase activity. The type B fibers are low in the dehydrogenase activity and high in myosin ATPase and phosphorylase activity. The type C fibers are high in the dehydrogenase activity and low in myosin ATPase and phosphorylase activity. The purpose of the present study was to determine the distribution and proportion of the three fiber types in four muscles.

Materials and Methods

Six adult chickens (White Leghorn) and eight 6-week-old chickens (four White Leghorns and four commercial broilers) were used in this study. They were killed by bleeding. Muscle samples were quickly removed and frozen in a mixture of Dry ice and acetone. The muscles examined were *M. pectoralis thoracicus*, *M. iliotibialis lateralis*, *M. flexor cruris medialis*, and *M. biventer cervicis*. The nomenclature for the muscles followed that of Vanden Berge (5). In the previous paper (3), the *pectoralis superficialis*, *biceps femoris*, and *gracilis* are the *pectoralis thoracicus*, *iliotibialis lateralis*, and *flexor cruris medialis*, respectively. Muscle samples were taken from the belly or middle part of the *M. biventer cervicis* and *M. flexor cruris medialis*, from the superficial middle part of the *M. pectoralis thoracicus*, and from the posterior part of the *M. iliotibialis lateralis*. In the 6-week-old chickens, muscle samples were separated in the deepest, anterior part of the *M. pectoralis thoracicus* adjacent to the *M. supracoracoideus*. This part looked reddish (Fig. 1). They were also taken from the proximal and distal part of the *M. iliotibialis lateralis* and *M. flexor cruris medialis*.

Sections, 8 μm thick, were cut on a cryostat and stained with NADH-diaphorase (6) and with myosin ATPase (7). Acid- and alkali-stable ATPase was demonstrated by incubation with substrate at pH 9.4 after preincubation at pH 4.4. and at pH 10.6 (8, 9). The muscle fibers were classified into three fiber types (A, B, and C) in the same manner as in the previous paper (3). Each fiber type was counted and then expressed as percentage. One hundred fibers of each type were measured across their smallest diameter with a micrometer.

Results

The type A and B fibers showed a high activity of alkali-stable ATPase and a low activity of acid-stable ATPase. The type C fibers gave a low activity of alkali-stable ATPase and a high activity of acid-stable ATPase (Figs. 2 and 3).

The *biventer cervicis* muscle was composed of three fiber types, which were evenly distributed throughout the muscle. The A fibers were greater in number than the B and C fibers (Table 1). In the *iliotibialis lateralis* the A fibers were fewer than the B fibers and the C fibers were not found in the proximal, middle, and distal regions. The *pectoralis thoracicus* muscle was composed almost entirely of B fibers except for the limited, deepest region that contained all three fiber types (Figs. 2–5). In this region the C fibers accounted for about 30 percent and were greater in number than the B fibers; the A fibers were the greatest. The *flexor cruris medialis* muscle also contained all three fiber types. The A fibers were more numerous than the B and C fibers and distributed evenly throughout the muscles (Table 1). The B fibers were greater in number in the deeper region and fewer in the superficial region (Figs. 6 and 7). To the contrary, in the deeper region the C

fibers were fewer and greater in number in the superficial region. The distribution of the three fiber types hardly varied in the proximal and distal region.

The A fibers were generally smaller in diameter than the B fibers in the *biventer cervicis*, *iliotibialis lateralis*, and *flexor cruris medialis* (Table 2). The C fibers were larger in diameter than the B fibers. In the *pectoralis thoracicus* there was little difference in diameter among the three fiber types. The B fibers were substantially larger in diameter in the *iliotibialis lateralis* and *flexor cruris medialis* than in the *biventer cervicis* and *pectoralis thoracicus*.

TABLE 1. *Proportion in three fiber types in four muscles of chickens*

Muscle	Type A	Type B	Type C	N[a]
Biventer cervicis	58.0±5.3[b]	21.6±4.5	20.4±1.0	6
Pectoralis thoracicus				
superficial region	0.8±0.5	99.2±0.5	0	6
deepest region	54.3±2.8	16.7±3.4	29.4±1.3	8
Flexor cruris medialis				
superficial region	69.7±1.5	0.9±0.4	29.4±1.6	6
middle region	78.4±2.8	14.6±2.2	8.0±2.0	6
deep region	69.6±2.5	29.6±2.7	0.8±0.4	6
Iliotibialis lateralis	34.5±3.8	65.5±3.8	0	6

a) The N is the total number of individuals.
b) Mean±S.E.M.

TABLE 2. *Diameters of three fiber types*

Muscle	Type A	Type B	Type C	N[a]
Biventer cervicis	42.2±2.2[b]	44.5±3.0	56.8±3.2	6
Pectoralis thoracicus				
superficial region	—	43.7±1.2	—	6
deepest region	43.8±1.7	44.8±2.8	49.9±3.4	8
Flexor cruris medialis	42.7±1.6	56.0±2.3	68.1±2.2	6
Iliotibialis lateralis	43.3±1.3	61.7±2.2	—	6

a) The N is the total number of individuals.
b) Mean±S.E.M.

Discussion

The *pectoralis thoracicus* muscle of chickens without the ability for long-term flight has only very few red fibers or A fibers (1, 3) and is a contrast to that of the pigeon possessing an ability for cruising flight. The pigeon *pectoralis thoracicus* muscle contained many red fibers (10, 11). The red fibers are thought to play an important role in long-term locomotion rather than the white fibers corresponding to the B fibers. White fibers are believed to be adapted for only short-term intense activity. The muscle fiber corresponding to the C fibers has not been

found in the pigeon *pectoralis thoracicus* (12). The C fibers were found in the limited, deepest region of the chicken *pectoralis thoracicus*. This finding shows that the differences in fiber type composition in the *pectoralis thoracicus* exist between chickens and pigeons. The differences in fiber type composition seem to be caused by a functional differentiation in muscle.

The mammalian *soleus* muscles that are primarily involved in the maintenance of posture have numerous fibers corresponding to the C fibers (13–15). Therefore, the *flexor cruris medialis* and *biventer cervicis* muscles possessing C fibers are presumed to be involved in the maintenance of posture in addition to locomotion, whereas the *iliotibialis lateralis* muscle composed of A and B fibers appears to function largerly during locomotion.

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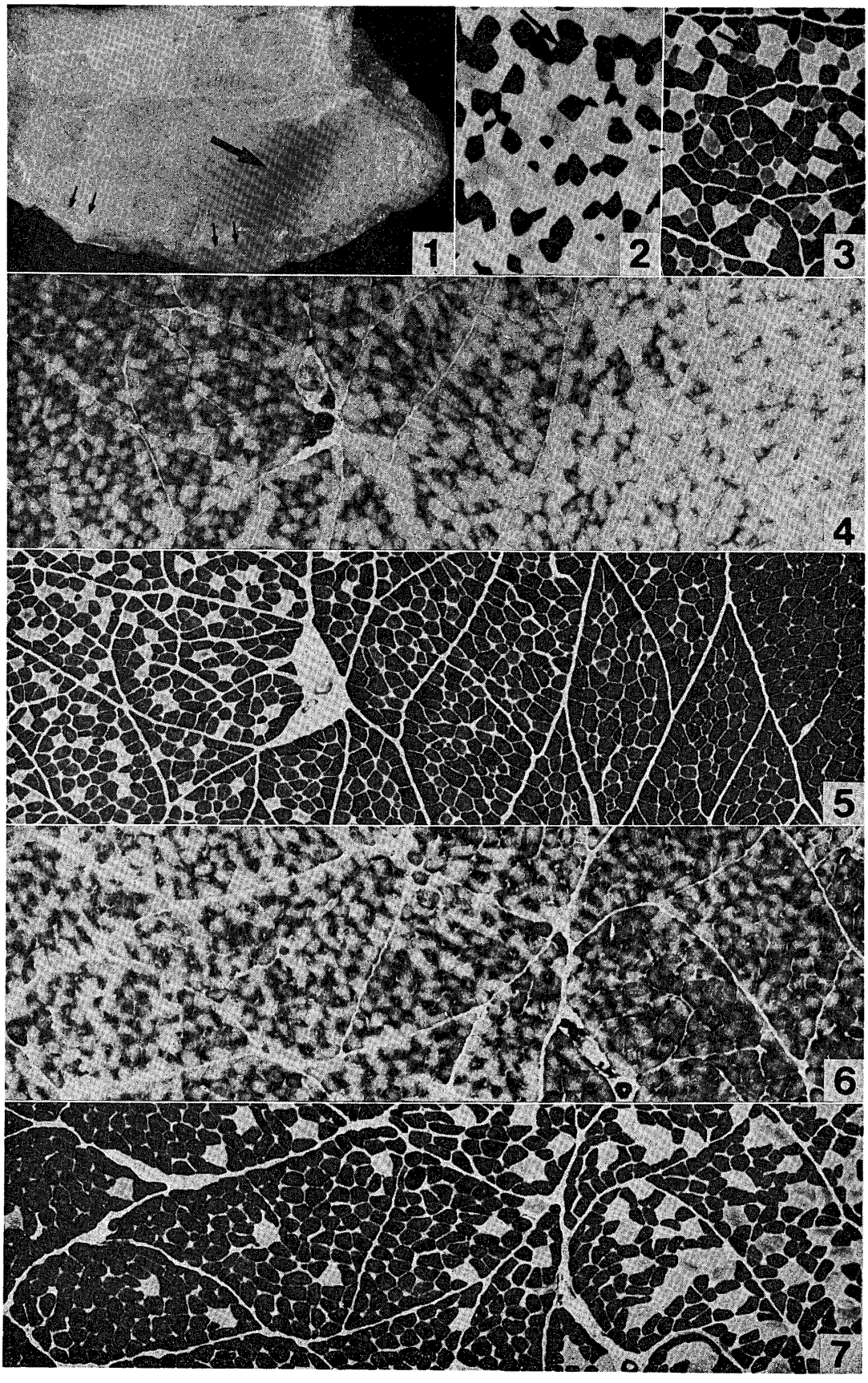
Explanation of Figures

Fig. 1. The *pectoralis thoracicus* muscle. Large arrow indicates the site from which muscle samples were removed. Small arrows indicate the part near the midline septum. The right of the figure is the anterior part.

Figs. 2–5. Cross sections in the deepest part of the *pectoralis thoracicus*. The regions of the figures are part of the site indicated in figure 1. Figs. 2 and 3. Serial cross sections. ATPase activity after preincubation at pH 4.4. (Fig. 2) and at pH 10.6 (Fig. 3). The C fibers with high acid-stable ATPase and low alkali-stable ATPase activity (arrow) exist in the limited, deepest part. Figs. 4 and 5. Serial cross sections. NADH-diaphorase activity (Fig. 4) and ATPase activity after preincubation at pH 10.6 (Fig. 5). The right of the figures is near the medial part and the left is the deepest part. The A fibers together with C fibers are abundantly distributed in this region. × 120.

Figs. 6 and 7. Serial cross sections of the *flexor cruris medialis* muscle. NADH-diaphorase (Fig. 6) and ATPase activity after preincubuation at pH 10.6 (Fig. 7). The right of the figures is the superficial part and the left is the deeper part. The B and C fibers are unevently distributed in this muscle. × 120.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