REGENERATION OF PIGEON FAST AND SLOW

MUSCLE FIBER TYPES

AFTER PARTIAL EXCISION AND MINCING

ROBERT S. HIKIDA and JOSEPH A. LOMBARDO

From the Department of Zoology and Microbiology, Ohio University, Athens, Ohio 45701

ABSTRACT

The pigeon's metapatagialis muscles, containing fast fibers in two slips and slow fibers in another slip, were excised for a third of their length, minced, and replaced into their previous sites. After regeneration, the pattern of fiber types and their ATPase and oxidative enzymes were examined histochemically. Ultrastructural examination was carried out on the fast fibers. After 4–17 wk the muscles had regenerated into patterns histochemically similar to the controls only within the slip containing fast fibers. The slow slip was much less regenerated, and had a histochemically embryonic composition. Fiber types were characterized and their cross-sectional areas measured, and the degree of atrophy was greatest in the large fast fibers and the slow fibers. Ultrastructural studies revealed a number of alterations of the mitochondria, including dense and light areas in the matrix and an altered pattern of the cristae into parallel tubular or vesicular aggregations. Other changes included dilated sarcoplasmic reticulum, myofibril disorganization, and a compaction of filaments. The slow fibers were thought to be slower in their regeneration rates because of the pattern of multiple innervation's producing a more complex regenerative pattern.

INTRODUCTION

Regeneration of skeletal muscle after mincing of a partially or completely excised muscle has been reported by Carlson (1968 b) and a number of Russian investigators (reviewed by Carlson, 1968 a, 1973). These studies have demonstrated that muscles are able to regenerate, but very little work has been carried out to determine the effect of regeneration on the fiber types in the muscle. Very recently, Snow (1973) has reported on some histochemical characteristics of regenerating muscles in the rat, and Carlson and Gutmann (1972) have investigated the contractile properties of rat regenerating muscles.

Regeneration of mammalian and anuran skeletal muscle after mincing has been studied by Carlson (1968 b), however, avian muscle has not been as well studied. Furthermore, the differential ability of different fiber types to undergo regeneration after mincing, and the characterization of these fiber types in the regenerate has not been studied. This investigation was made to determine (a) whether avian fast and slow muscles regenerate after mincing; (b) whether the original fiber type pattern is regenerated; (c) whether regeneration rates or properties differ between fast and slow muscle fibers; and (d) the ultrastructural characteristics of regenerating fibers.

The pigeon's metapatagialis muscle contains three slips: the anterior two slips contain fast-twitch fibers and are pink when viewed grossly;

the posterior slip contains predominantly slow fibers, and is white in gross appearance (Hikida and Bock, in preparation; Hikida, 1973). The presence of fast-twitch and tonus fibers in adjacent slips of a single muscle makes this muscle an excellent model for comparative studies, and in this case, the comparative study of the differential ability of various fiber types to regenerate.

MATERIALS AND METHODS

The serratus superficialis metapatagialis muscles of adult domestic pigeons, Columba livia, Carneaux breed, were used in all the studies. The birds were anesthetized with Nembutal (intravenous), and the feathers cleared from the side of the body. An incision of the skin was made just above the hip joint, and this incision was extended for about 6 cm toward the base of the wing, exposing the muscle. The following procedures were carried out on different birds: (a) in 13 birds, the proximal third of the muscle (about 3 cm) was dissected free, removed, and minced, and the minced fragments were replaced between the cut distal portion and the previous origin: (b) in two birds, the proximal third of the muscle was removed and discarded; (c) in two birds, the muscle was separated from its origin by cutting the proximal attachment. The contralateral muscle was used as a control in each of the birds.

The mincing procedure involved putting the muscle on a tongue depressor containing a couple of drops of either avian saline, Krebs solution, or 0.1 M sucrose in phosphate buffer, and this proximal third of the muscle (containing both the anterior and posterior slips together) was minced very finely with a razor blade into pieces less than 1-mm square. The entire mince was then replaced into the region between the remaining two-thirds of the muscle and the muscle's previous origin. The skin was sutured closed and the muscles were allowed to regenerate for 4–17 wk.

The muscles were investigated either histochemically or ultrastructurally, or both. For the histochemistry, the muscles were removed, frozen in methyl butane at −60°C, sectioned at 8 μm, and stained for the myofibrillar ATPase (Guth and Samaha, 1969), succinic dehydrogenase (SDH) (Lillie, 1964), or nicotinamide adenine dinucleotide-tetrazolium reductase (NADH-TR) (Engel and Brooke, 1966). In each case the experimental and control muscles were mounted on the same cover glass and processed together.

The muscles which had been minced and prepared for electron microscopy were usually divided into the minced regenerated portion (that region which had regenerated from the mince), the nonminced regenerated portion (that region of the experimental muscle which was not minced, and separated by

scar tissue from the minced region), and the contralateral control. They were removed from the birds, fixed in either 2.5% glutaraldehyde buffered to pH 7.3 with 0.1 M phosphate, or in Karnovsky's fixative (Karnovsky, 1965). After an hour, the muscles were diced, allowed to fix 1–3 h longer, rinsed in phosphate-buffered sucrose (0.1 M), and postfixed in 1% phosphate-buffered osmium tetroxide. After dehydration in an acetone series, and rinsing in propylene oxide, they were embedded in Epon-Araldite, sectioned on a Reichert Om2 ultramicrotome (C. Reichert, sold by American Optical Corp., Buffalo, N. Y.), contrasted with uranyl acetate and lead citrate, and examined with a Siemens Elmiskop I.

For fiber measurements, a number of fasciculi were selected from various parts of the muscle, and all of the fibers in these fasciculi were characterized for their histochemical type, traced at X 400 magnification using a Wild M-20 microscope with a drawing tube, and the tracings were measured for their cross-sectional area using a Keuffel and Esser compensating polar planimeter (Keuffel & Esser Co., Morristown, N. J.).

RESULTS

Normal Structure

The normal structure of this muscle is to be described[1] but a brief description has been presented (Hikida, 1973). The muscle originates from three adjacent ribs, and merges into a common slip until it inserts on the skin at the base of the wing. The portion originating from the posterior rib is the most dorsal slip, and is white in color; it is referred to as the posterior slip of the serratus superficialis metapatagialis (PSSM). The other two slips are pink in gross color and are referred to as the anterior slips (ASSM).

Each slip contains distinct populations of fibers and there is little mixing of fiber types between slips. The ASSM contains two types of fibers (Fig. 1), both of which are presumably fast in contractile speed: a small fiber which stains darkly with ATPase and SDH or NADH-TR, and a larger fiber which stains intermediate to dark with ATPase and lightly with NADH-TR or SDH. The PSSM has two types of fibers (Fig. 2), both apparently slowly contracting: the predominant type is lightly staining after ATPase, and the other stains slightly darker, but not as darkly as the larger type of fiber in the ASSM. Both fiber types in the PSSM stain homogeneously intermediate with SDH or NADH-TR.

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[1] Hikida and Bock, in preparation.

When the slips are investigated with the electron microscope, the fibers of the ASSM have all the characteristics of fast-twitch muscle fibers, with a regular pattern of myofibrils, well-developed sarcotubular system (Hikida, 1972), Z lines which have a lattice pattern in cross section, and a well-ordered pattern of striation. The fibers of the PSSM have the appearance of slow fibers, with an afibrillar pattern, reduced sarcotubular system, Z lines which have no lattice pattern in cross section, and a poorly organized or irregular striation pattern.

Regenerated Muscle

GROSS MORPHOLOGY AND FIBER SIZES: Of the 13 muscles on which the mincing procedure was carried out, 12 demonstrated varying degrees of regeneration, while 1 did not regenerate. In all cases in which the muscle did regenerate, pink and white slips were present and their normal origins onto the ribs were reestablished. In most of the minced muscles, the line of demarcation between the regenerated portion and the non-minced portion of the muscle was observed; a line of scar tissue marked this junction. All of the regenerated muscles displayed varying degrees of atrophy when compared to the control.

In a control series (two birds) in which 2–3 cm of the muscle was excised without replacement, there was no regeneration to the origin, and the muscle was attached to the connective tissue at the point it was cut.

In normal control muscles, the fast ASSM consists of two types of fibers: large fibers on the surface of the fasciculus, which stains with intermediate density with ATPase; and small fibers which stain darkly with ATPase, and lie either inside, or at the periphery, of the fasciculus. These two fiber types are distinct in both their staining (Fig. 1) and their size (Table I). The slow PSSM has two fiber types, but one stains slightly darker than the other with ATPase staining, and the fiber size is variable between these two types. The two types are distributed randomly within the fasciculi.

The regenerated muscle of the anterior slip resembled closely the control muscle in fiber integrity at all stages of regeneration. However, with the exception of the small, darkly staining fibers of the ASSM, the sizes of the regenerated fibers usually showed considerable and random deviations from the contralateral control. Variability was also noted among the fiber sizes of the controls between different birds, but the relative sizes of different fiber types remained constant (Table I). The measurements of the fiber cross-sectional areas demonstrate the atrophy of most fibers in the regenerated muscles. Generally, the large "white" fibers of the ASSM were atrophied to a much greater degree than the small "red" fibers (Table I).

HISTOCHEMISTRY: After alkaline preincubation and ATP incubation, the regenerated ASSM displayed small dark fibers and large intermediate-staining fibers in some muscles (Fig. 3). In most, the small and large fibers were both darkly staining for their ATPase activity, although the large fibers sometimes stained lighter than the small.

After acid preincubation and ATP incubation, the normal staining pattern was exhibited in the regenerated ASSM. However, the small fibers were either intermediate or dark in their staining while

FIGURE 1 A control pink slip, stained for ATPase activity after alkaline preincubation. Note the small dark fibers and the larger light fibers. × 180.

FIGURE 2 Control white slip, made up of slow fibers, stained for ATPase activity after alkaline preincubation. Two fiber types are distinguished here. × 180.

FIGURE 3 Regenerated pink slip 15 wk after mincing shows fibers which have a staining pattern similar to the control. ATPase activity after alkaline preincubation. × 180.

FIGURE 4 Regenerated white slip 11 wk after mincing, shows fibers which have undergone atrophy, disorientation, and varied staining with ATPase. × 180.

FIGURE 5 Border between white and pink slips, 15 wk after mincing, showing that the two slips again appear normal after ATPase staining. × 180.

FIGURE 6 Regenerated white slip 36 days after mincing, stained for ATPase activity after acid preincubation. There are some very dark fibers, but the majority stain lightly, unlike the controls. × 180.
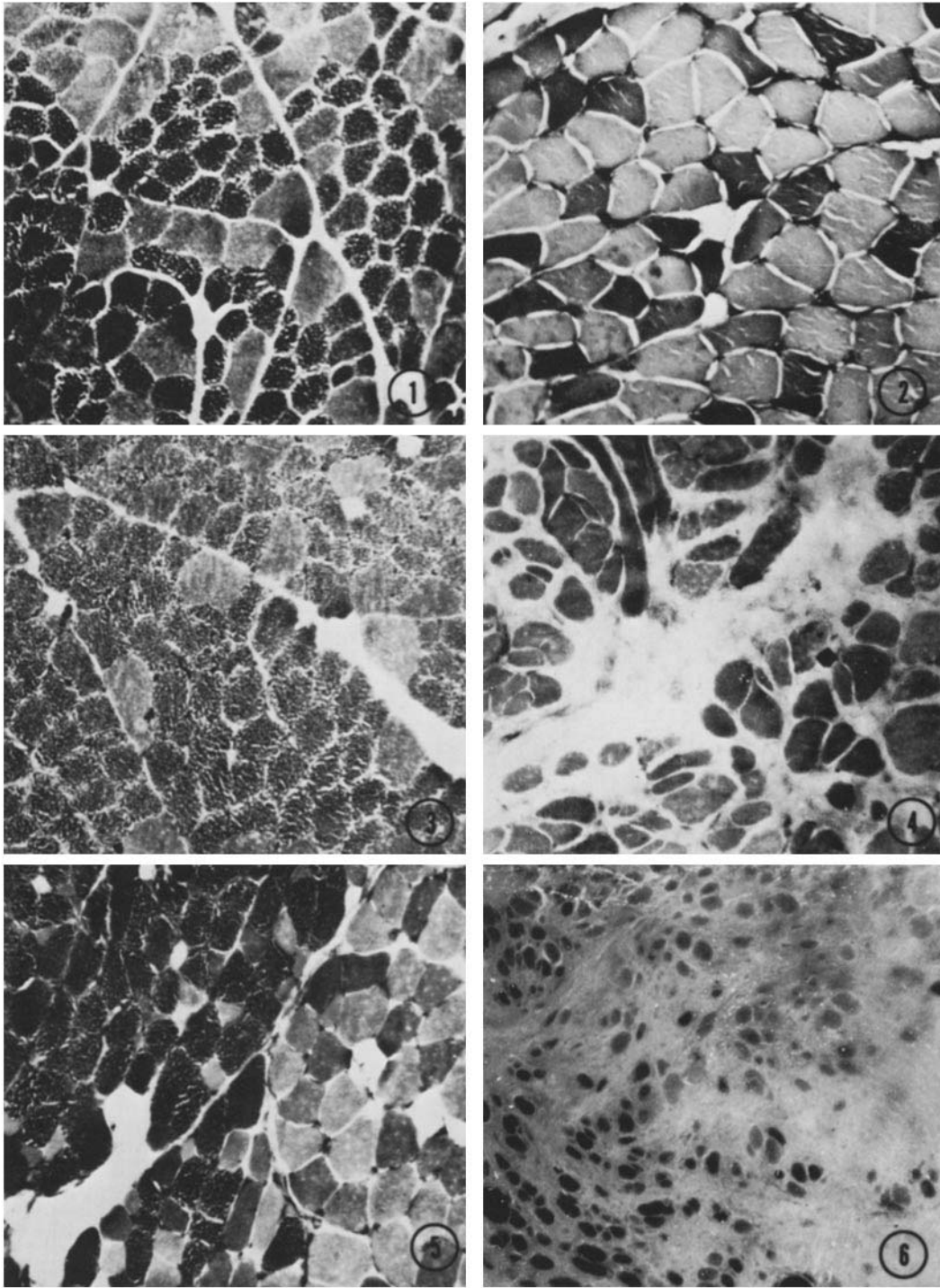
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TABLE I

*Mean Fiber Sizes and Percentages in Control and Regenerated Muscles**

	Experimental			Control		
Fiber type‡	Mean area ± SE	(number)	%	Mean area ± SE	(number)	%
Bird 110 (11-wk regeneration)						
PSSM, L-I	1,197 ± 129	(27)	36	1,040 ± 53	(22)	35
PSSM, L	846 ± 79	(47)	64	1,198 ± 87	(41)	65
ASSM, D	669 ± 31	(99)	95	565 ± 23	(106)	82
ASSM, I	375 ± 115	(5)	5	1,328 ± 60	(24)	18
Bird 102 (15-wk regeneration)						
PSSM, L-I	1,135 ± 56	(24)	30	Not analyzed		
PSSM, L	1,171 ± 28	(56)	70	Not analyzed		
ASSM, D	620 ± 30	(80)	81	423 ± 18	(81)	80
ASSM, I	1,632 ± 101	(19)	19	925 ± 86	(20)	20
Bird 107 (15-wk regeneration)						
PSSM, L-I	405 ± 43	(27)	28	1,481 ± 64	(27)	33
PSSM, L	952 ± 59	(69)	72	1,792 ± 54	(56)	67
ASSM, D	625 ± 27	(75)	80	603 ± 29	(74)	80
ASSM, I	842 ± 57	(19)	20	1,621 ± 71	(19)	20
Bird 101 (17-wk regeneration, minced portion)						
PSSM, L-I	361 ± 90	(8)	10	1,609 ± 78	(16)	20
PSSM, L	843 ± 37	(76)	90	1,482 ± 54	(65)	80
ASSM, D	541 ± 21	(94)	85	659 ± 24	(98)	82
ASSM, I	1,078 ± 76	(16)	15	1,272 ± 88	(22)	18
Bird 101 (17-wk regeneration, nonminced portion)						
PSSM, L-I	858 ± 98	(11)	13	See control above		
PSSM, L	1,175 ± 37	(73)	87	See control above		
ASSM, D	472 ± 19	(79)	79	See control above		
ASSM, I	869 ± 55	(21)	21	See control above		

* Fiber sizes are given as the area of a cross-sectioned fiber, in μm^2.

‡ Fiber types L, I, L-I, and D refer to the staining of the fibers after alkaline preincubation and ATPase incubation: light, intermediate, between intermediate and light, and dark, respectively.

the large fibers were light. Therefore, the usual reversal of staining seen in the controls was not present in the small fibers. In staining for oxidative enzymes, SDH or NADH-TR, the regenerated ASSM had light and dark fibers as in the controls. Again, the larger fibers stained lightly, as with the controls, and the small fibers were densely staining.

When compared to the anterior slips, the regenerated posterior slip exhibited great differences from the control muscle. The regenerated fibers of the PSSM were surrounded by excessive amounts of connective tissue, showed little or no fascicular organization, and frequently were irregular in size and shape (Fig. 4). Groups of small, apparently regenerating fibers were not uncommon. Unlike the ASSM, which showed little difference in the degree of regeneration with time in the period studied, the PSSM displayed progressive regenera-

tion (Fig. 5). However, even after 140 days, the developmental conditions persisted to some degree.

Most striking was the difference between the PSSM and ASSM after 36 days of regeneration. At this stage, the PSSM contained primarily dark fibers after alkaline preincubation and ATP incubation (Fig. 6). Many of these fibers were widely scattered throughout the matrix of connective tissue. In addition, the fibers were present in the greatest numbers near the point of junction of the PSSM with the ASSM; in regions of the PSSM more distant from this zone, fibers were scarce and connective tissue was the primary component of the muscle. The ASSM displayed nearly normal muscle integrity at this time, except that some fiber atrophy was evident.

Histochemically, the PSSM generally displayed the staining pattern found in the controls for ATPase activity after alkaline preincubation. In
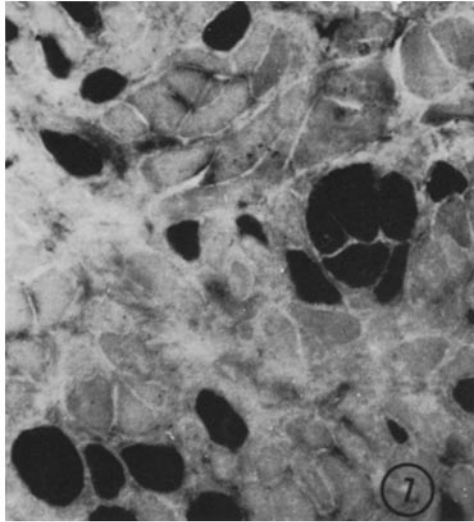
FIGURE 7 The regenerated white slip after 5 wk consists primarily of small fibers scattered throughout a matrix of connective tissue. Note the intense staining for ATPase after alkaline preincubation. × 190.

contrast to the 36-day regenerate, most of the regenerates were stained somewhat less intensely than the control, although occasional dark fibers were present. ATPase activity after acid preincubation resulted in a variety of staining patterns. In some early regenerates a number of fibers stained as after alkaline preincubation (Fig. 7). In other regenerates, light and dark were present, or intermediate and intermediate-light fibers. In these latter, it was often difficult to resolve the two types, as the relative stain intensities were nearly identical. In addition, it was not unusual to find a continuum of staining intensities from light to intermediate.

The staining for NADH-TR or SDH was similar to the control PSSM, with most of the fibers staining with intermediate intensity; but a few dark fibers, corresponding to the dark fibers after alkaline preincubation, were present. The dark fibers were most numerous in the 36-day regenerate.

The nonminced portion of the regenerated muscles were histochemically similar to the controls. Grossly, this portion displayed some atrophy, and the fiber cross-sectional areas supported this observation; but the overall appearance approached that of the normal muscle. The PSSM in the nonminced portion displayed some degenerative changes, but these were slight when

compared to the regenerated minced portion of the PSSM.

After complete excision of the metapatagialis, usually no regeneration of muscle fibers occurred, although in one bird after 15 wk, three muscle fibers were found embedded in the connective tissue. Similarly, after partial excision of the metapatagialis, in which the excised portion was discarded, no regeneration was apparent at the site of extirpation after 8 and 20 wk. The ASSM of the remaining portion of the muscle was relatively unaffected by the operation, which was similar to a tenotomy in these muscles with a very short tendon; the PSSM underwent massive degeneration. To investigate this occurrence further, an actual tenotomy was performed, and the muscle was observed to be reattached to its normal origin, and both ASSM and PSSM were normal in their appearance.

ELECTRON MICROSCOPY: Muscles from seven birds were examined with the electron microscope. These regenerating muscles were examined from 4 to 17 wk after mincing. In four of the birds the nonminced portion was examined, and in the other three the minced portion was investigated ultrastructurally. Only the fast ASSM is described here.

After 4 wk of regeneration, most of the fibers in the minced portion were abnormal in their appearance, although a few were normal in structure (Fig. 8). The structure of the nonminced region of the regenerated muscle appeared unaffected by the treatment (Fig. 9). Among the early changes occurring in response to the mincing and regeneration were swelling of the sarcoplasmic reticulum and cristae of the mitochondria. Formation of large vesicles, containing amorphous material and sometimes mitochondria, was prominent (Fig. 8). A common occurrence was a compact packing of filaments to form a large amorphous mass of muscle filaments. The fibers contained dilated vesicles of rough endoplasmic reticulum, and satellite cells were present. Some of these characteristics resemble denervation atrophy (Hikida and Bock, 1972), which is reasonable since his region lacks its innervation (see Discussion).

Later stages of regeneration showed fibers having a number of unique alterations of the mitochondria. (a) The mitochondrial structure typical for this muscle was one with a dense matrix and narrow, closely packed cristae. (b) This type of mitochondrion was usually larger, had an electron-lucent
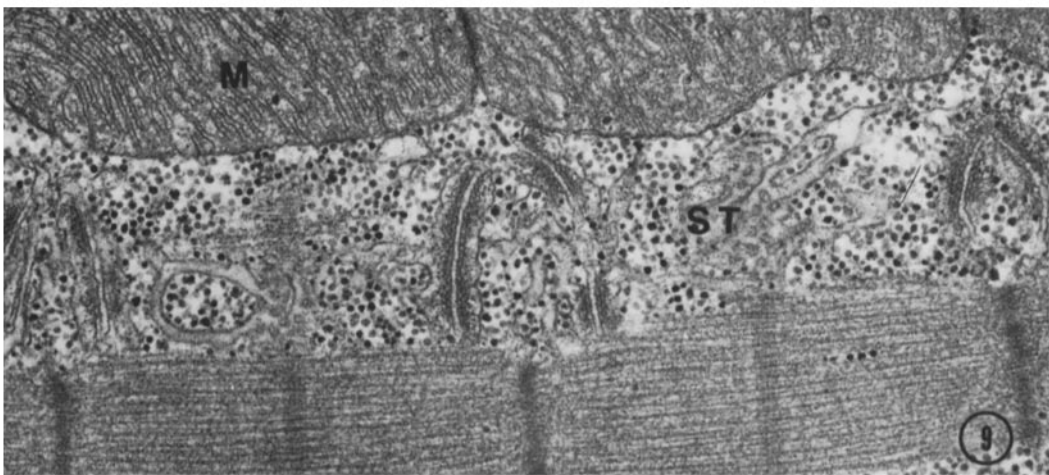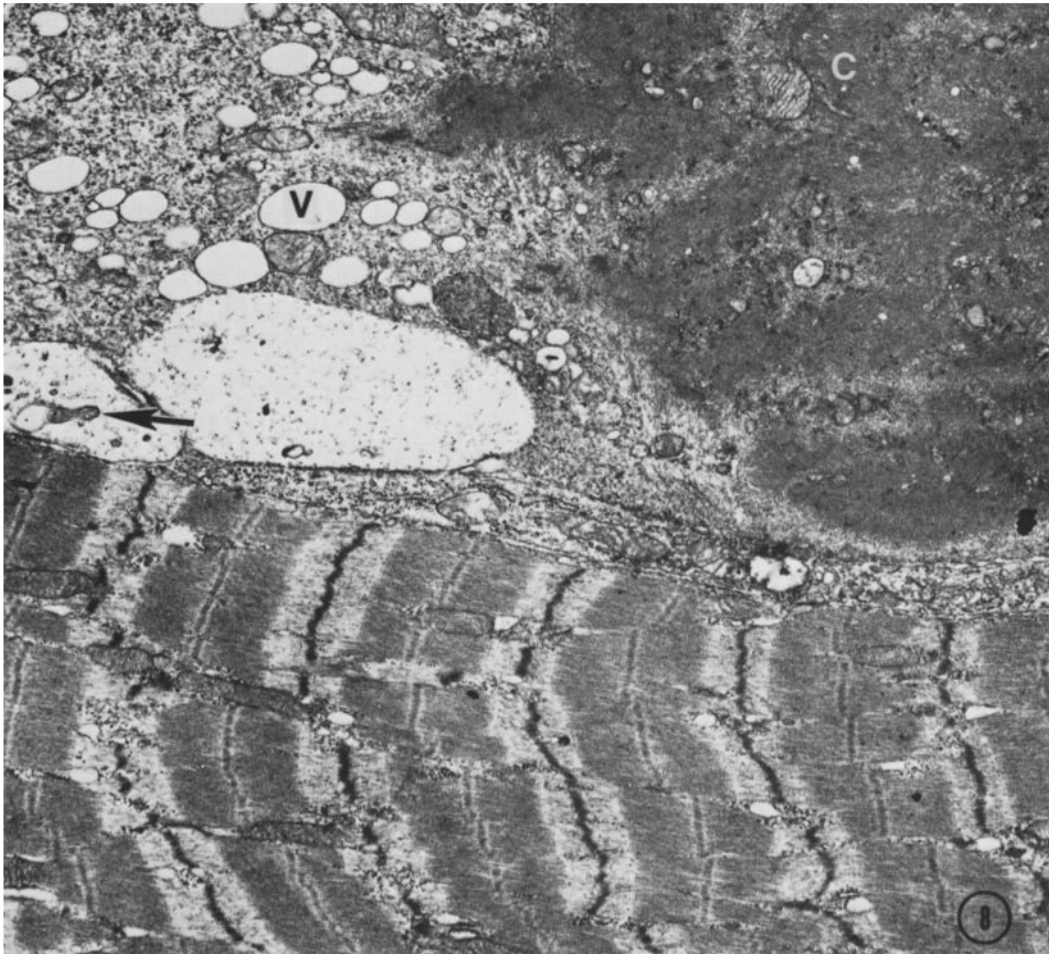
FIGURE 8   Regenerating fibers from the minced portion after 4 wk. One fiber is relatively normal, while the other has a compaction of its filaments (C), enlarged vesicles (V), and a large vacuole containing a mitochondrion (arrow) plus other membrane elements. × 11,400.

FIGURE 9   Nonminced portion of the regenerating muscle after 4 wk. The sarcotubular system (ST), mitochondria (M), and myofibrils are all normal. × 36,600.

matrix, and dark cristae, which were not packed as tightly as in the normal type (type 1). These first two types were found in the same muscle, and were seen at times to lie adjacent to one another (Fig. 10). (c) Electron-lucent spaces were seen in some mitochondria, usually those having the dense matrix (Fig. 11). (d) Other mitochondria had electron-dense sites which were more opaque than the matrix (Fig. 12). These were too large and irregularly shaped to be the previously described intramitochondrial granules. (e) The most unusual alteration of the mitochondrion was so extensive that these mitochondria might not have been recognized as such except for the observation of intermediate structures. In this modification, the mitochondrion appeared to lose its outer membrane, and the cristae became arranged into a series of parallel tubular or vesicular components (Fig. 13). These were seen in many fibers from a number of birds, and all showed the same arrangement. The basis for describing this as a mitochondrial alteration is that in a study of regeneration of slow muscle (Hikida, unpublished observations), an intermediate form between the structure seen in the metapatagialis and a normal mitochondrion was noted (Fig. 14). Here, a long organelle was present, and one end was recognized as a modified mitochondrion, while the other end had a structure similar to that seen in the present study.

Although the sarcoplasmic reticulum was swollen in the regenerating muscle, the transverse tubule was not abnormal (Fig. 15). Other conditions seen in the regenerating muscles were the presence of lysosomes and varying degrees of disorganization of striation patterns. Some of the fibers resembled embryonic muscle in the earliest stages of regeneration studied. In these, small myofibrils were isolated in wide areas of sarcoplasm (Fig. 16), and these fibrils were oriented randomly to one another. Motor end plates were seen in three of the muscles, and these terminations were small, and were multiple along the fibers (Fig. 17).

DISCUSSION

This investigation has suggested that there is specificity of regeneration of fast and slow avian skeletal muscle fibers after mincing and replacement, and slow fibers regenerate more slowly than the fast in the metapatagialis muscle. Snow (1973) has investigated some of the histochemical properties of muscles degenerating and regenerating after mincing, but did not discuss specific fiber type regeneration. Carlson (1972) reports that Snow has been able to distinguish red and white fibers after 6 wk, using SDH and periodic acid-Schiff reactions.

This study has shown that in the nonminced portion of the regenerated muscle, the structure is almost normal. When only a third of the muscle is excised and minced, the remainder of the muscle will be tenotomized, and should undergo atrophy and some pathological changes (Hikida and Bock, 1970); there may be a denervation reaction in the minced portion of the muscle, as well as severe trauma after mincing. It is of interest that few signs of denervation or tenotomy occurred in either the fast or slow slips. The only obvious indication of degeneration was severe atrophy in certain parts of the muscle. Tenotomy of the nonminced portion of a partially minced muscle is a transient condition because the regenerated minced portions invariably regain their normal origin. This may account for the lack of pathological changes in regenerated minced muscles because in Hikida and Bock's study (1970), tenotomy alone did not reestablish the normal origin, but the muscle attached to the underlying connective tissue. When the metapatagialis was subjected to an ordinary tenotomy, it regained its former origin and closely resembled normal muscle, but when 2-3 cm of the proximal third was removed, the remaining portion attached to underlying connective tissue and extensive degenerative changes occurred (Hikida and Lombardo, unpublished observations).

It is well established that neuromuscular interaction is essential to the regeneration of minced muscle fragments. The fact that all regenerates had differentiated into their characteristic white and pink slips indicates that the specific type of nerve supply to the various portions of the minced muscle might have some influence on their specific differentiation. Since the fast fibers making up the ASSM displayed a more rapid regenerative capacity than the slow PSSM, and these fast and slow fiber types have different patterns of innervation, this again suggests that neuromuscular interaction may be important in the regenerative process. However, when considering regeneration as related to neurotrophism, the rate at which reinnervation is established, and not necessarily the inherent nature of the nerve supply, may be the
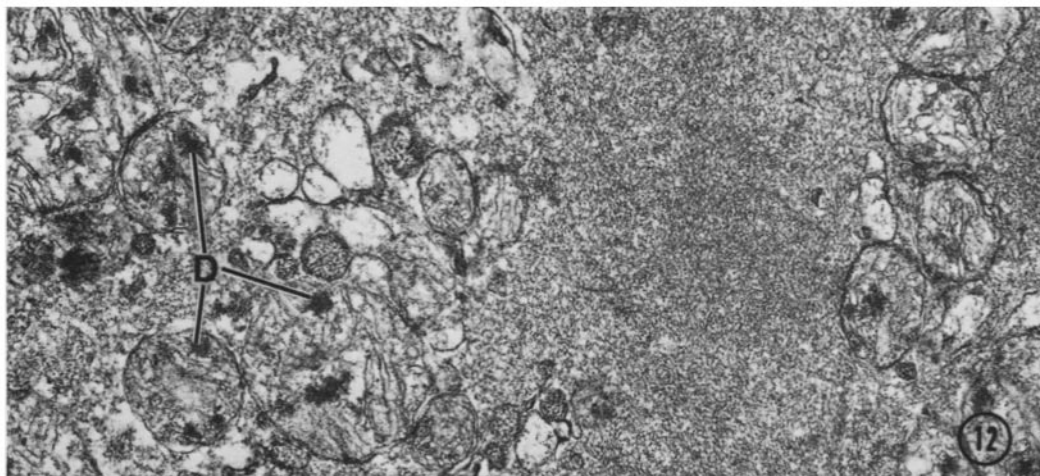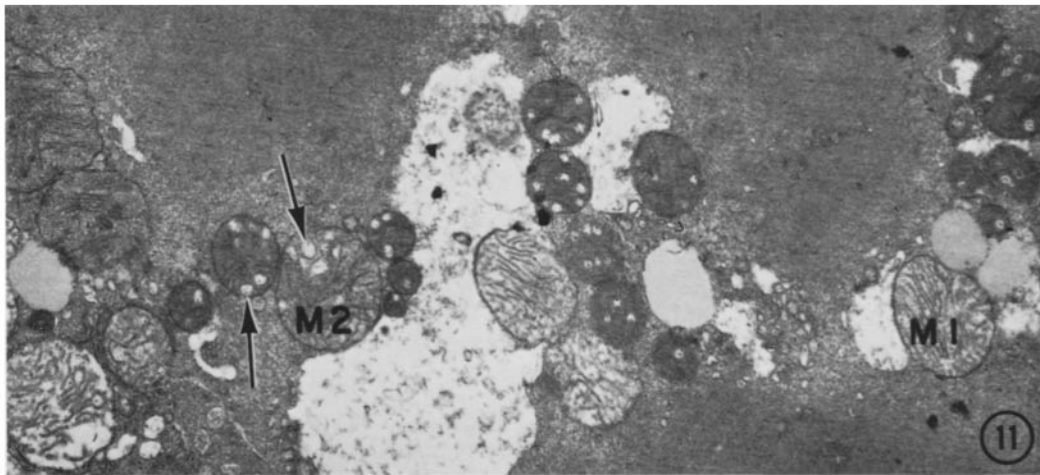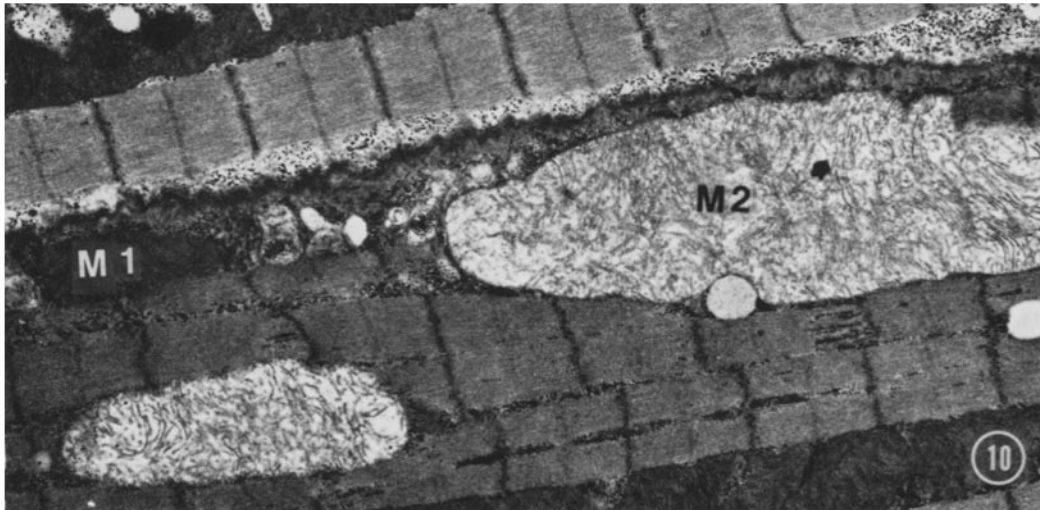
FIGURE 10 Regenerated muscle after 15 wk, demonstrating two types of mitochondria: the normal (M1), and one enlarged with a light matrix (M2). Other parts of the fibers are normal. × 8,700.

FIGURE 11 A regenerating fiber after 17 wk has light regions (arrows) within the matrix of both normal mitochondria (M1), and the altered type (M2). A large vacuole containing various elements is also noted. × 15,300.

FIGURE 12 Mitochondria having dark granular deposits (D) are seen in this fiber from the 4-wk regenerate. × 36,600.
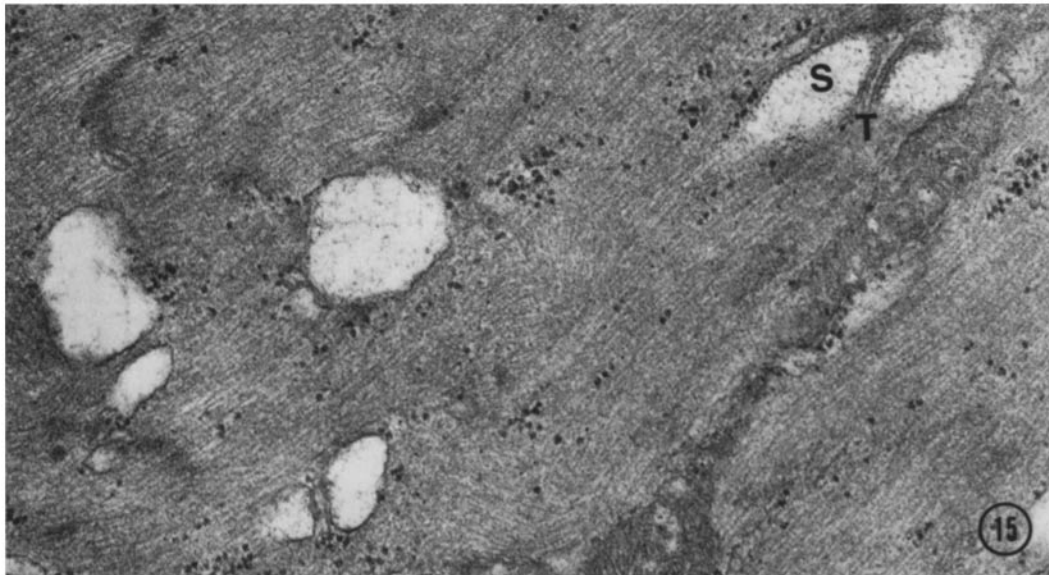
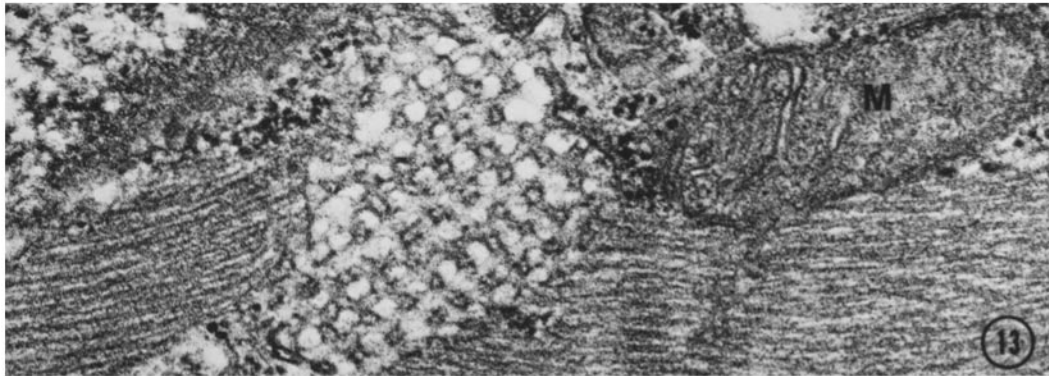FIGURE 13 A peculiar mitochondrial structure lies adjacent to a more normal mitochondrion (M) in this fiber from a 4-wk regenerate. Note the apparent absence of the outer membrane in the abnormal mitochondrion. × 68,100.

FIGURE 14 Another modified mitochondrion similar to that seen in Fig. 13. This is from the slow fiber of the anterior latissimus dorsi, after 8 mo of regeneration. The mitochondrial structure is recognizable at the arrow, but other parts resemble the structure seen in Fig. 13. × 36,600.

FIGURE 15 A portion of a fiber from the 15-wk regenerate, showing triads (T) in which the terminal cisternal portion of the sarcoplasmic reticulum (S) is dilated. × 52,500.
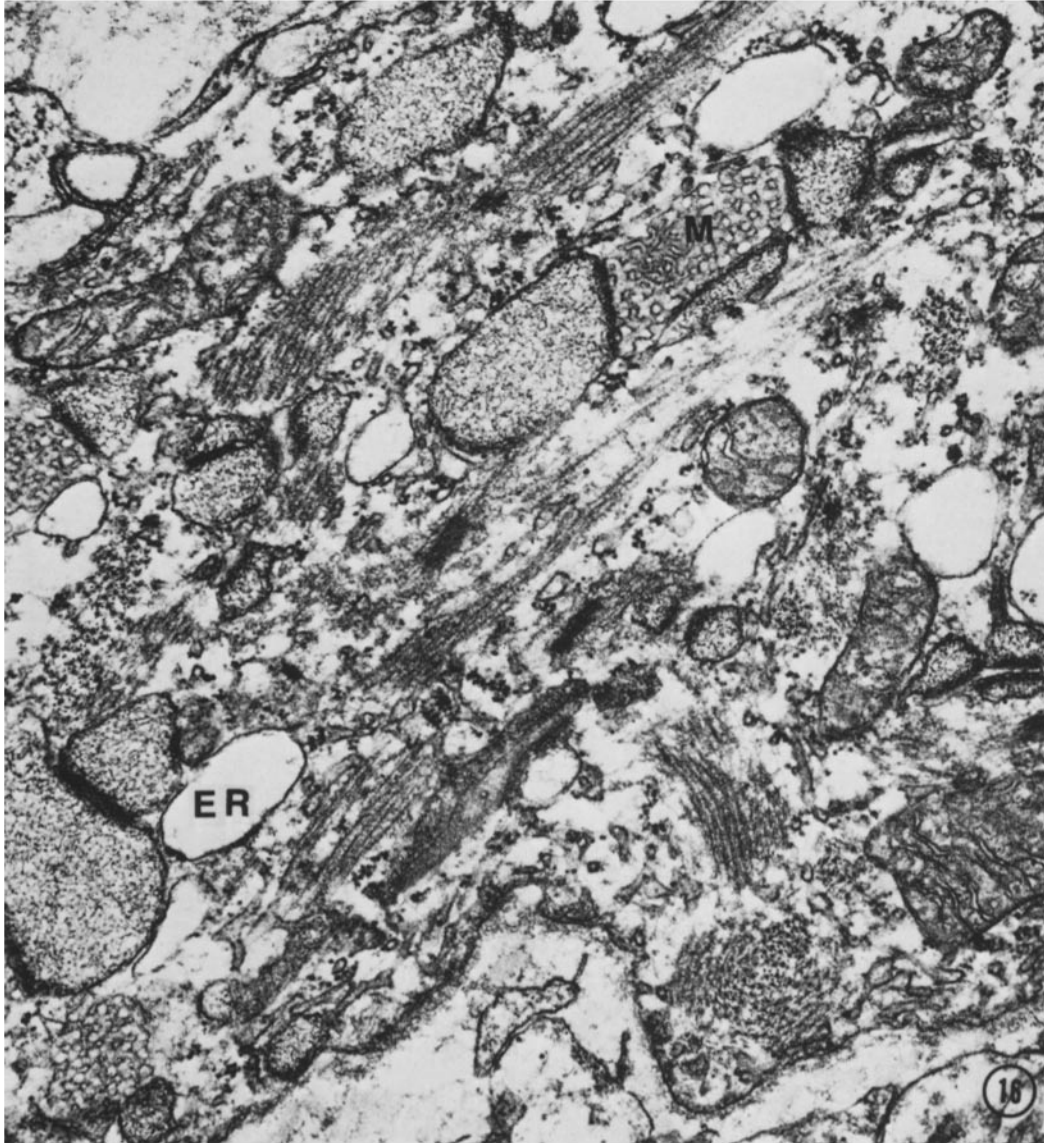
FIGURE 16 A fiber from the 4-wk regenerate showing properties of embryonic muscle, with disorientation of fibrils, dilated rough endoplasmic reticulum (ER), and two types of modified mitochondria (M). × 36,600.

prime factor in determining the extent and rate of regeneration; this does not say, however, that the nature of the nerve supply in itself may not affect the rate of regeneration. The ASSM apparently regains its nerve supply before the PSSM. The fast fibers of the ASSM usually have a single innervation per fiber, and in most cases the termination remained intact because the region of the mince was proximal to the point

of innervation. Regenerating fast fibers, therefore, needed only to reestablish continuity of their sarcolemma with that of the nonminced portion of the muscle to achieve the effects of innervation. The slow tonus fibers of the PSSM are innervated throughout their length (Hikida, 1973), and mincing destroys the terminations in the minced portion. Therefore the nerve, end plates, and sarcolemmal continuity must be restored to

FIGURE 17 Fiber from the 15-wk regenerate showing a normal structure, and with an innervation consisting of multiple terminations (P). × 12,000.

achieve the same result as the fast fibers. Thus it is likely that the slower rate of regeneration in the PSSM is due to the greater complexity of the regenerative process, which, in turn, is a consequence of its pattern of innervation.

Some instances of multiple terminations within one area of a fast fiber of the ASSM have been observed (Hikida and Bock, in preparation). These differ from the slow muscle terminations by being found only in one or two small groups in

the ASSM, as opposed to the groups being dispersed along much of the length of the fiber. This may account for the two terminations seen in Fig. 17, from the ASSM.

Guth and Samaha (1972) have shown that developing muscle fibers stain more intensely for their ATPase activity than was shown by the actual biochemical assay of the activity. Therefore this phenomenon might be expected to occur in the regenerating fibers of the metapatagialis,

and especially the PSSM, which displayed signs of regeneration throughout the stages investigated for this study This did occur, with the earlier stages of regeneration having more small, darkly staining fibers than the later stages.

Some aspects of the ultrastructural studies are worth noting. The peculiar structures that were described as modified mitochondria can be either mitochondria or modified sarcotubular system. It is suggested that these are mitochondria because similar structures were observed in a regenerating avian muscle different from that described in this report; but in this other muscle, an earlier stage of the modification was seen, and this structure resembled a mitochondrion in part of its structure, and the greatly modified structure in another portion. Similar structures have been reported in cultured muscle (Ishikawa, 1968), but these were shown to be complex networks of transverse tubules based on ferritin diffusion into the lumen of these membranes. Mitochondrial structures somewhat similar to these have also been reported in the giant amoeba (Pappas and Brandt, 1959).

This study has raised a number of questions concerning the regenerative properties of avian fast and slow muscles, among which are: What is the relative importance of the nerves in the regeneration of specific fiber types? Can the same fiber type patterns be regenerated in completely excised and minced muscles? Are the contractile properties of these muscles altered? Experiments are being conducted within this laboratory to attempt to answer these questions.

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