The Sequential Replacement of Myosin Subunit Isoforms during Muscle Type Transformation Induced by Long Term Electrical Stimulation*

Wendy E. Brown, Stanley Salmons§, and Robert G. Whalen§†

From the Department of Anatomy, University of Birmingham, Birmingham B15 2TJ, United Kingdom and the §Département de Biologie Moléculaire, Institut Pasteur, 25 Rue du Dr. Roux, 75724, Paris

Fiber type transformation was induced in fast skeletal muscles of the rabbit by chronic electrical stimulation of the motor nerve. The accompanying changes in myosin subunit composition were examined in order to establish the precise sequence of events during this transition. Replacement of the fast by the slow isoforms of the individual light and heavy chains of the native myosin did not occur synchronously. The resultant changes in the stoichiometry of the different classes of light chains are described in relation to possible mechanisms regulating the expression of the corresponding genes. The neonatal form of myosin could not be detected at any stage of transformation, suggesting that the changes in myosin synthesis do not recapitulate the developmental sequence.

Mammalian skeletal muscles are capable of responding adaptively to new patterns of use. For example, when a continuous pattern of activity is imposed on fast muscles of the rabbit by electrical stimulation of the motor nerve over a period of several weeks, they acquire the physiological and biochemical characteristics of slow muscles (for reviews see Salmons and Henriksson, 1981; Jolesz and Sr6ter, 1981). In particular, this transformation of type includes changes in myosin, a major contractile protein that exists in polymorphic forms.

That both the heavy and light chains of myosin are involved has been shown by gel electrophoresis (Sr6ter et al., 1973, 1974, 1982; Pette et al., 1976), immunocytochemistry (Rubinstein et al., 1978), amino acid analysis (Sr6ter et al., 1975) and myosin paracrystal formation (Sr6ter et al., 1974). This experimental model therefore provides an opportunity for examining how synthesis of the individual subunits of myosin is regulated.

In a normal muscle, the myosin subunits are maintained in fixed equimolar ratios, the native molecule comprising 2 heavy chains and 4 light chains (Lowey and Risby, 1971). The light chains can be subdivided into two classes, present in equimolar amounts in native myosin, of which only one is capable of being phosphorylated. Within each of these classes, the light chains are chemically related, as shown by amino acid sequence data (Weeds, 1976 and references there). In the present study, these stoichiometric relationships have been examined during the process of fiber type transformation.

In addition, myosin prepared from chronically stimulated muscles has been analyzed for those myosin heavy chain isoforms that are present in developing muscles (Whalen et al., 1979, 1981; Hoh and Yeoh, 1979) in order to test the hypothesis that the fast-to-slow conversion of myosin in mature muscles proceeds via intermediate developmental forms.

**MATERIALS AND METHODS**

**Stimulation**—Stimulation experiments were carried out using adult New Zealand White rabbits of either sex. Animals were prepared for surgery with a premedication of atropine sulphate (3 mg kg−1) and diazepam (10 mg kg−1) administered subcutaneously, followed after 15 min by an intramuscular injection of fentanyl citrate and fluanisone (Hynorm, Janssen Pharmaceutica; 0.3 mg kg−1). Stimulators were implanted under aseptic conditions such that the TA and extensor digitorum longus muscles were subjected to chronic stimulation via the common peroneal nerve (for method see Eisenberg and Salmons, 1981). In some experiments, the electrodes were arranged close to the tendinous origin of the soleus muscle so as to stimulate this muscle as well, via the corresponding branch of the tibial nerve. Periods of stimulation less than 10 days were achieved with a different type of stimulator (Brown and Salmons, 1981) which was activated remotely 2 to 3 days after implantation. In this way, the immediate effects of the operative procedure could be dissociated from those of stimulation itself. In all cases, the pattern imposed on the nerve was a continuous train of pulses at a frequency of 10 Hz. At the end of the experiment, the animals were operated under urethane and sodium pentobarbitone anaesthesia. The muscles were removed, weighed, frozen in liquid nitrogen, and stored at −50 °C pending subsequent analysis.

Fast muscle myosin was prepared from TA and pesos, and slow myosin from soleus muscles, as described below. Neonatal myosin was prepared from hind limb muscles, excluding soleus, that were pooled from five 3-day-old rabbits.

**Preparation of Myosin**—Muscles, representing 2–3 g wet weight, were thawed, chopped finely, and homogenized in 2 volumes of cold extraction buffer (0.4 M NaCl, 50 mM Tris-HCl, pH 6.8, 1% 2-mercaptoethanol). An aliquot was taken for isoelectric focusing analysis and then an additional volume of extraction buffer was added and extraction continued for a further 20 min on ice with occasional agitation. The residue was pelleted at 20,000 × g for 20 min at 4 °C. The supernatant was diluted by the gradual addition of 10 volumes of 10 mM Tris-HCl, pH 6.8 and kept at 4 °C overnight to allow the precipitate to settle. The clear supernatant was decanted and the precipitate centrifuged at 8,000 × g for 5 min. The pellet was resuspended by gentle stirring in 0.6 M NaCl, 50 mM Tris-HCl, pH 6.8, and made up to 5 ml, regardless of the initial size of the muscle. Magnesium chloride and ATP were added to final concentrations of 10 and 6 mM, respectively, and the solution stirred on ice for 30 min to dissociate the actomyosin. Following centrifugation at 160,000 × g for 3 h at 4 °C, the supernatant was diluted with an equal volume of

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1 The abbreviations used are: TA, tibialis anterior; SDS, sodium dodecyl sulfate; LC, light chain.

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50 mM NaCl, 30 mM Tris-HCl, pH 6.8, and dialyzed overnight at 4 °C against this buffer. The precipitate was collected at 12,000 × g for 10 min and dissolved in an equal volume of phosphate buffer (0.5 M NaCl, 0.02 M sodium phosphate, pH 6.6) by gentle stirring. Myosin was purified from this solution by gel filtration on a column (1.5 × 50 cm) containing Sephadex G25 (Pharmacia), which was equilibrated and eluted with column buffer (0.5 M NaCl, 0.02 M sodium phosphate, pH 6.6, 0.1 mM MgCl₂, 1 mM ATP). The myosin peak was collected, concentrated to approximately 2 mg/ml in a dialysis sac packed in Sephadex G200 (Pharmacia), and denatured by the addition of SDS to 0.5% (w/v). Samples were stored at a temperature of −20 °C or below.

One-dimensional Gel Electrophoresis—One-dimensional SDS-polyacrylamide gel electrophoresis was carried out as described by Laemmli (1970), with acrylamide and methylenebisacrylamide concentrations of 12.5% (w/v) and 0.1% (w/v), respectively, in the separating gel, and 5% (w/v) and 0.13% (w/v), respectively, in the stacking gel. Samples for electrophoresis were mixed with half their volume of dissociating buffer (3% (w/v) SDS, 0.18 M Tris-HCl, pH 6.8, 15% (v/v) 2-mercaptoethanol) and heated in a boiling water bath for 2 min before being applied to the gel. Gels were stained in 0.25% Coomassie blue R.250 (Sigma) in methanol-water-acetic acid (6:5:1) and destained in the same solution at 4 °C (Whalen and Ecob, 1980).

For the nomenclature of the stained slab gels was carried out with an LKB 2202 Ultrascan and the peaks integrated by a Hewlett Packard Integrator 3390A. The proportions of the various light chains in each muscle sample were calculated from the results of densitometry after correcting the staining intensity for differences arising from molecular weight only. For this purpose, the molecular weights, determined by reference to molecular weight marker proteins (Pharmacia), were as follows: LC₁,F, 27,000; LC₂,F, 26,100; LC₁,S, 19,700; LC₂,F, 24,200; LC₂,S, 18,200; LC₃,F, 15,500. Repeated scans of the same gel showed that the error in the areas of the peaks contributed by the densitometric procedure was approximately ±3% corresponding to an error of ±2.5% in the light chain ratio. It has been found convenient to adopt the following nomenclature: the light chains LC₁,F, LC₂,F, LC₁,S, and LC₂,S are referred to as A ("alkali") light chains; LC₂,F and LC₂,S are referred to as P ("phosphorylatable") light chains; the term "fast A light chains" is used to refer to LC₁,F and LC₂,F collectively and "slow A light chains" to LC₁,S and LC₂,S collectively.

Proteolytic Cleavage of Myosin—Chymotryptic cleavage of myosin was performed essentially as described by Whalen et al. (1979). For one-dimensional gel electrophoresis, the 40 μl reaction mixture contained 16 μg of myosin and 2.2 μg of chymotrypsin (Worthington). After incubation at 37 °C for 90 min, proteolysis was stopped as described (Whalen et al., 1979) and the reaction mixture used directly for one-dimensional gel analysis. For two-dimensional gel electrophoresis, the 60 μl reaction mixture contained 60 μg of myosin and 6 μg of chymotrypsin. After incubation as above, samples were snap-frozen in liquid nitrogen, freeze-dried, and resuspended in 60 μl of solution A (9.5 M urea, 2% (v/v) Nonidet P-40, 2% ampholines (LKB) pH 5–7, 5% (v/v) 2-mercaptoethanol).

Two-dimensional Gel Electrophoresis—Two-dimensional gel electrophoresis (O’Farrell, 1975) was carried out essentially as described by Whalen and Ecob (1982). The isoelectric focusing gels were 20 cm in length and contained pH 5–7 ampholines. The acrylamide and methylenebisacrylamide concentrations used in the second dimension were the same as those used in the one-dimensional gels described above.

RESULTS

Analysis of Myosin Light Chains—Purified myosin, prepared from TA muscles that had been stimulated at 10 Hz for periods of 2 days to 10 weeks, was examined using SDS-polyacrylamide gel electrophoresis. The staining patterns were quantitated by densitometric scanning followed by integration of the individual light chain peaks. Fig. 1 shows an overall change in electrophoretic pattern from a typically fast to a typically slow muscle profile, confirming results described previously (Sriber et al., 1973, 1973). In the present study, the use of densitometry together with a more detailed time course has revealed further features of the transformation.

In Fig. 2, the relative proportions of the light chains over various periods of stimulation are shown. From two weeks, there was a progressive increase in the proportion of slow light chains (LC₂,S, LC₁,S, and LC₃,F) with a complementary decline in fast light chain components (LC₁,F, LC₂,F, and LC₂,S). Conversion of the LC₂,F forms preceded that of the LC₁,F forms. By 10 weeks, the fast light chains comprised only 11% of the total light chain fraction.

In some experiments, the electrodes were implanted such that the soleus, as well as the TA and extensor digitorum longus, was stimulated via its motor nerve. In one unstimulated contralateral soleus muscle, fast light chains represented approximately 4.5% of the total light chain fraction. In soleus muscles stimulated for 10 weeks or more, no fast light chain component could be detected. Histochemical analysis (results not shown) confirmed that the soleus muscle in the rabbit normally contains a population of fibers of the slow-oxidative-glycolytic subtype, type IIA. In contrast, the soleus muscle that had been stimulated for 10 weeks was composed entirely of the slow-oxidative type of fiber, type I. Myosin prepared from the 10-week-stimulated soleus muscle therefore provided a homogeneous slow muscle standard that served as a basis for comparison in subsequent experiments.

Light Chain Stoichiometry—Table I shows the changes induced by stimulation in 1) the ratio of the fast A to the fast P light chains, 2) the ratio of the slow A to the slow P light chains, and 3) the ratio of the total (fast-plus-slow) A to P light chains.

During stimulation, the proportions of the total A and P light chains remained substantially unchanged from their normal, approximately equinomil, ratio (0.94 ± 0.13). If, however, the fast and slow components are considered separately, the normal ratio of A to P light chains is seen to have been markedly disturbed. It is evident from Fig. 2 that this is a consequence of the different rates at which light chains of the A and P type were replaced.

The absolute amounts of fast light chains present in control soleus muscles were small; the ratio of the fast A to the fast P light chains was therefore subject to considerable variation, and the figure given in Table I should not be taken as indicating a significant departure from the 1:1 ratio normally observed for these light chains in control fast muscles. During the course of 10 weeks of stimulation, this small proportion of fast muscle light chains declined to undetectable levels.
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DURATION OF STIMULATION (wk)

**FIG. 2.** Proportions of individual myosin light chains as a percentage of the total light chains in myosin purified from TA and soleus muscles after various periods of stimulation.

### Table I

| Time of stimulation | Fast | Slow | Total |
|---------------------|------|------|-------|
|                     | \( \text{LC}_{1p} + \text{LC}_{2p} \) | \( \text{LC}_{1s} + \text{LC}_{3s} \) | \( \text{LC}_{1p} + \text{LC}_{2p} + \text{LC}_{1s} + \text{LC}_{3s} \) |
| Control TA muscle   | 0.98 | 0    | 0.98  |
| 2 days              | 1.02 | 0    | 1.02  |
| 7 days              | 1.04 | 0    | 0.94  |
| 10 days             | 0.91 | 0    | 0.82  |
| 12 days             | 0.91 | 0    | 0.81  |
| 2 weeks             | 0.88 | 0    | 0.79  |
| 3 weeks             | 1.17 | 0.44 | 0.99  |
| 4 weeks             | 1.42 | 0.38 | 0.89  |
| 5 weeks             | 1.71 | 0.42 | 0.80  |
| 7 weeks             | 13.49| 0.70 | 1.09  |
| 8 weeks             | 6.23 | 0.84 | 1.19  |
| 10 weeks            | 1.02 | 0.97 | 0.98  |
| Mean ± S.D.         | 2.57 ± 3.75 | 0.38 ± 0.37 | 0.94 ± 0.13 |

Control soleus muscle: 1.73 ± 1.07 ± 1.09

However, the slow light chains remained in approximately equimolar proportions throughout.

**Analysis of Myosin Heavy Chains**—Analysis of myosin heavy chains was accomplished by polypeptide mapping. In these experiments, purified myosin was denatured with SDS and then digested with chymotrypsin such that the proteolytic products were of a size range that could be analyzed conveniently by standard SDS-gel electrophoresis. Fig. 3 shows the polypeptide patterns after various periods of stimulation. Twelve days after the onset of stimulation, TA myosin still gave a polypeptide pattern like that of myosin of the fast muscle type. At 14 days, polypeptides of the type specific to slow muscle could be detected, becoming a significant, although still a minor, component at 21 days. After a further 7 days, the slow-specific peptides formed the major component; this interval (21–28 days) therefore represented the period of most marked transformation. Fast-specific peptides could still be detected after 5 weeks but were undetectable by 7 weeks of stimulation. At this stage, the chymotryptic digest showed an electrophoretic pattern indistinguishable from that of

**FIG. 3.** SDS-polyacrylamide gel electrophoresis of myosin heavy chains after partial digestion with chymotrypsin. Myosin was prepared from TA after various periods of stimulation. neo, neonatal myosin prepared from 3-day-old rabbit leg muscle. 70sol, myosin prepared from adult soleus muscle subjected to stimulation for 10 weeks.
myosin prepared from 10-week-stimulated soleus muscle.

Myosin prepared from pooled 3-day-old rabbit leg muscles was included in the analysis in order to evaluate the possibility that the neonatal form of myosin (see Whalen et al., 1981) was synthesized at some stage during the course of muscle transformation. The light chain pattern of neonatal myosin was indistinguishable from that of adult fast myosin (Fig. 1), but the heavy chain digest pattern showed distinct differences

FIG. 4. Two-dimensional polyacrylamide gel electrophoresis of myosin heavy chains after partial digestion with chymotrypsin. a, adult control TA; b, neonatal rabbit leg muscle (arrows indicate peptides characteristic of neonatal myosin); c, adult control soleus; d, TA, 2-day stimulation; e, TA, 4-week stimulation; f, TA, 8-week stimulation.
from both fast and slow adult myosins (Fig. 3). At no stage were the peptides characteristic of neonatal myosin heavy chain detected in myosin heavy chain digests prepared from stimulated adult TA muscles.

Heavy chain polypeptide cleavage products from the same myosin preparations were further analysed using two-dimensional gel electrophoresis (Whalen et al., 1979, 1981). With the greater resolution that this technique made available, the neonatal myosin pattern was readily distinguishable from that of either adult TA or adult soleus (Fig. 4, a–c), and could not be identified in myosin prepared at any time during the stimulation-induced transition from the fast to the slow isoform (Figs. 4, d–f). After 2 days of stimulation, the polypeptide pattern of myosin from TA muscle (Fig. 5b) remained very similar to that obtained from psoas (Fig. 5a), a muscle composed almost entirely of type IIb fibers. After an intermediate (12 days) period of stimulation, the polypeptide patterns were still unchanged qualitatively (see Fig. 3), but the second dimension revealed that several polypeptide species which were minor components at 2 days (up arrows in Fig. 5b) became major components in myosin after 12 days stimulation (up arrows in Fig. 5c). Conversely, other polypeptides became less abundant during the same period of stimulation (down arrows in Fig. 5, b and c). By 4 weeks of stimulation, many new polypeptides could be detected (horizontal arrows in Fig. 5d). These corresponded to the predominant polypeptides in myosin prepared from the slow muscle (Fig. 5e). After 8 weeks of stimulation, the heavy chain cleavage pattern consisted exclusively of polypeptides of the slow type (compare Fig. 4, c and f).

These results reinforce those from the one-dimensional analysis of the myosin heavy chain, i.e. the slow myosin heavy chain was the predominant form after 4 weeks of stimulation, and there was no evidence to indicate that the neonatal myosin heavy chain was generated during the course of type transformation. In addition, the two-dimensional analysis provided evidence of changes in the type of fast myosin heavy chain during the first 12 days of stimulation.

**DISCUSSION**

This study was concerned with the transformation of myosin types in a predominantly fast muscle, TA, in response to indirect, long term stimulation at 10 Hz. Changes in the myosin light and heavy chain complement due to electrical stimulation were first reported by Sréter et al., (1973), and their findings have subsequently been confirmed and extended (Sréter et al., 1974, 1982; Pette et al., 1976; Pette and Schnez, 1977; Roy et al., 1979). In the present work, the time course of these changes was examined for evidence of coordination in the expression of the several genes involved. We also sought evidence for the accumulation, at an intermediate stage, of myosin isoforms of an embryonic or neonatal character during the course of a muscle type transformation that begins and ends with adult myosin isoforms.

In broad terms, transformation involved the replacement of fast myosin isoforms by their slow counterparts, with changes in the P light chains proceeding in step with changes in the heavy chains and in advance of changes in the A light chains. This sequence of events, which was reflected by the composition of the myosin in all the stimulated muscles examined, forms the principal focus of this discussion; the precise time scale on which these events occurred may have been modulated to some extent by differences between animals in the latency of the response to muscle stimulation. The transformation showed a number of interesting features which will now be considered individually in more detail.

Two-dimensional analysis of the products of proteolytic

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**Fig. 5. Two-dimensional analysis of myosin heavy chain digests.** Enlargements of areas corresponding to the rectangle in Fig. 4. a, adult psoas; b, TA, 2-day stimulation; c, TA, 12-day stimulation; d, TA, 4 week stimulation; e, adult control soleus.
cleavage revealed that some changes in the myosin heavy chains took place during the first 12 days of stimulation. During this period, changes were observed in the relative amounts of several heavy chain polypeptides which were unrelated to the subsequent appearance of slow myosin-specific polypeptides. This suggests that the myosin heavy chain component of TA is composed of two subtypes and that a shift in their relative proportions took place in response to stimulation. The existence of two fast heavy chain types, denoted IIA and IIB, is borne out by other protein chemical data on the heavy chains (Starr and Offer, 1973; Billeter et al., 1982) and by histochemical evidence for two degrees of alkali lability in the myofibrillar ATPase of fast muscle fibers (Brooke and Kaiser, 1970; Tunell and Hart, 1977). The histochemical technique shows rabbit TA to be composed predominantly of type IIB fibers (58%) with smaller contributions from type IIA (38%) and type I (4%) fibers (Walker, 1982). The polypeptide species that declined between 2 and 12 days of stimulation (down arrows in Fig. 4, a and b) were those which predominated in the proteolytic map of myosin prepared from psoas (Fig. 4e), and since this muscle is composed almost exclusively of type IIB fibers, the results would be consistent with a loss of type IIB myosin in the early stages of stimulation. Significant amounts of slow (type I) myosin appeared only after longer periods of stimulation. The results therefore suggest that accumulation of type IIA myosin takes place at an intermediate stage in the replacement of type IIB by type I myosin.

With prolonged stimulation, the fast light chains were replaced progressively by slow light chains (Fig. 2). A changeover point could be defined as the time at which the slow isoforms became the dominant species. For the LC2 light chains, the changeover point occurred just before 4 weeks, almost 2 weeks before the changeover point for the LC1 light chains. It was during the same period (3 to 4 weeks) that the changeover of the myosin heavy chains took place (Fig. 3). This observation is suggestive of a degree of coordination between the replacement of the myosin heavy chain and the LC2 light chain. Little information is available about the turnover of these two proteins even under normal conditions. In a study of the adult rat myocardium, Zak et al. (1977) showed that the rates of turnover for the A and P myosin light chains were the same, and that both were less than the rate for the myosin heavy chain. On this basis, the simultaneous replacement of the heavy and the light chains in our experiments would require a rather complex sequence of regulatory events at the gene level. An alternative explanation emerges if it is borne in mind that our studies were carried out on purified myosin, and that the results refer to the subunit composition of the assembled myosin, not the total intracellular proportions of the individual components. Thus, coordination could have been imposed at the level of assembly of the subunits to form whole myosin molecules. Such an explanation would require that some of the light chains, in particular the slow A light chains, would be synthesized during muscle transformation but would remain, at least temporarily, unbound to heavy chains. This possibility is by no means excluded; indeed there is already evidence for the existence of free light chains in both normal skeletal (Hovarth and Gaetjens, 1972) and cardiac (Zak et al., 1977) muscle.

The functional consequences of exchanging fast for slow isoforms of the P light chains cannot readily be predicted. In invertebrates, LC2 is responsible for calcium regulation, but since this function is largely, if not entirely, taken over by the troponin complex in higher organisms, light chain replacement is unlikely to be significant in this context. Neither would a change in the LC2 isoform be expected to modulate the enzymic activity of myosin, in view of the observation by Malhotra et al. (1979) that the cardiac and fast skeletal forms of LC2 may be interchanged without affecting the myosin ATPase. It has been suggested, however, that LC2 may be involved in myosin filament formation (Pinsent-Harström and Whalen, 1979). Such a role would favor coordination of assembly of a given type of myosin heavy chain with its corresponding LC2 light chain.

The situation in regard to heavy chains of myosin is better understood. Replacement of the fast by the slow type of heavy chain would be expected to result in a significant reduction in the actin-activated myosin ATPase, on the basis of the different hydrolytic activities of the corresponding whole myosins (Seidel et al., 1964; Bárány et al., 1985) and evidence that these activities are determined primarily by the heavy chain components (Wagner and Weeds, 1977; Wagner, 1981). This in turn should be reflected in a reduced intrinsic speed of shortening of the muscle, in accordance with the relationship established by Bárány (1967). There is already adequate evidence for these effects; calcium-activated myosin ATPase declines progressively after three weeks of stimulation (Srûter et al., 1974), and at least part of the reduction in isometric contractile speed which is observed during this period (Salmons and Vrbová, 1969) is attributable to the loss of type IIB myosin in the myofibrillar ATPase of fast muscle fibers which predominated in the proteolytic map of myosin (Brooke and Kaiser, 1970; Tunell and Hart, 1977). The polypeptide species that declined between 2 and 12 days of stimulation (Fig. 4e), and since this muscle is composed almost exclusively of type IIB fibers, the results would be consistent with a loss of type IIB myosin in the early stages of stimulation. Such an explanation would require that some of the light chains, in particular the slow A light chains, would be synthesized during muscle transformation but would remain, at least temporarily, unbound to heavy chains. This possibility is by no means excluded; indeed there is already evidence for the existence of free light chains in both normal skeletal (Hovarth and Gaetjens, 1972) and cardiac (Zak et al., 1977) muscle.

In control TA or soleus muscle, the fast and slow types of myosin are sequestered in separate fibers. In TA muscle undergoing type transformation in response to stimulation, on the other hand, fast and slow type light chains can be present within the same fibers (Pette and Schnez, 1977; Srûter et al., 1982). Although asynchronous replacement of the A and P light chains markedly disturbed the individual A-P light chain ratios during this period (Table I), the total (fast-plus-slow) A-P light chain ratio remained relatively constant throughout (0.94 ± 0.13). These observations point to the presence of hybrid molecules of otherwise normal subunit structure within the myosin fraction in these fibers. This is best illustrated by the results obtained after 7–10 weeks of stimulation. At this stage, the myosin heavy chain was of the slow type; the fast heavy chain was undetectable (Fig. 4f). The P light chain was represented almost exclusively by LC2, but the A light chain comprised a mixture of LC1a, LC1b, LC1c, and LC1d (Fig. 2). Similar observations concerning the maximum speed of shortening (Al-Amood et al., 1973).

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Developing mammalian muscle has been shown to contain a neonatal form of myosin, distinct from the major adult types (Hoh and Yeoh, 1979; Whalen et al., 1981). It was therefore of interest to determine whether this isoform was re-expressed during transformation of an adult fast muscle. This was investigated by examining chymotryptic digests of myosin heavy chains which predominated in the proteolytic map of myosin (Brooke and Kaiser, 1970; Tunell and Hart, 1977). The simplest interpretation of these results is that stimulation

1 R. G. Whalen, unpublished observations.
brought about the direct replacement of adult fast myosin by the adult slow type. We cannot, however, exclude the possibility that neonatal myosin may have been expressed briefly but asynchronously within individual fibers of the muscle. There is evidence (reviewed by Salmons and Henriksson, 1981) that some fiber type transformation can take place in muscle under physiological and pathological conditions. The present study suggests that such transformation may be accomplished without the generation of transitional forms of myosin.

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