The Expression of Myosin Heavy Chain Isoforms in Normal and Hypertrophied Chicken Slow Muscle

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Abstract. Hypertrophy was produced in the anterior latissimus dorsi (ALD) muscle of 5-wk-old chickens by application of a load to the humerus. After 4 wk, hypertrophied ALD muscles were >2.5 times heavier than contralateral control ALD muscles. Two iso-myosins are distinguishable in normal ALD muscles by their different electrophoretic mobilities. It is shown here that the faster migrating SM-1 isomyosin decreases in abundance with age and that the application of an overload enhances both the rate and extent of this process. Monoclonal antibodies were selected by an immunotransfer technique that were specific for the heavy chains associated with either SM-1 or SM-2, or cross-reacted with both isoforms. The cellular distribution of the SM-1 and SM-2 isomyosins was analyzed by immunofluorescent technique using these antibodies. Anti–SM-1 and anti–SM-2 antibodies reacted with separate populations of cells, whereas the third antibody reacted with all myocytes in the normal ALD muscle. These data suggest that there is an exclusive cellular distribution of myosin heavy chains associated with SM-1 and SM-2 proteins. Immunofluorescent analysis of hypertrophied muscle showed the anti–SM-2-specific antibody reacting with all myocytes, whereas the anti–SM-1-specific antibody reacted with none. This is consistent with the elimination of the SM-1 isoform in hypertrophied muscles.

RAPID and substantial enlargement of chicken skeletal muscle can be produced by the addition of a load to the humerus of the wing. The mechanism responsible for this growth is believed to be related to the stretching and subsequent tension developed in the wing muscles (Feng et al., 1962; Sola et al., 1973; Ashmore, 1982; McDonagh and Davies, 1984). Both fast twitch muscles such as the patagialis (Holly et al., 1980; Barnett et al., 1980) and slow tonic muscles such as the anterior latissimus dorsi (ALD)1 muscle (Sola et al., 1973; Laurent et al., 1978; Sparrow, 1982; Gollnik et al., 1983) can be induced to enlarge to a mass between 70 and 100% greater than contralateral control muscles within 1 wk after the onset of hypertrophy.

As far as the cellular features of this growth response are concerned, it is clear that a hypertrophy of existing fibers occurs (Barnett et al., 1980; Ashmore, 1982; Gollnik et al., 1983). However, it is less clear whether hyperplasia also occurs (Sola et al., 1973; Barnett et al., 1980; Gollnik et al., 1983). Biochemical properties have also been examined in hypertrophied muscle and, as in other models of muscle growth, the RNA concentration (Laurent et al., 1978; Barnett et al., 1980) and the fractional rate of protein synthesis (Laurent et al., 1978) have been found to increase. In addition, the activity of several enzymes is changed in hypertrophied muscle. For example, assays for succinic dehydrogenase and citrate synthetase indicate substantial increases in the oxidative potential of stretched fast twitch muscles (Gutmann et al., 1970; Holly et al., 1980). In contrast, little if any change in mitochondrial enzymes are noted in stretched slow tonic ALD muscle (Holly et al., 1980), although the pattern of fibers stained for alkali-stable ATPase does change profoundly (Sola et al., 1973; Holly et al., 1980). In control ALD muscles, two populations of muscle fibers are discernible that have a light or dark staining intensity when stained for succinic dehydrogenase. Hypertrophy results in the decline and eventual disappearance of darkly stained fibers.

Although histochemical assays indicate two types of ATPase activity in individual ALD muscle fibers, the cellular distribution of isomyosins in these muscles has not been investigated. It is known, however, that the chicken ALD muscle contains two major myosin bands separated by pyrophosphate gel electrophoresis of the proteins in the native state (d’Albis and Gratzer, 1973; Hoh et al., 1976). These are designated SM-1 and SM-2 depending on their speed of migration, with the SM-1 band migrating faster. Peptide mapping of the SM-1 and SM-2 isomyosins isolated from whole tissue or synthesized in a reticulate lysate system indicate that the differences between these two bands are due to differences in myosin heavy chains rather than light chains (Matsuda et al., 1982) and that the two heavy chains are encoded by separate mRNAs (Matsuda et al., 1983). Anti–myosin monoclo-

1. Abbreviation used in this paper: ALD, anterior latissimus dorsi.
onal antibodies have been characterized (Bormioli et al., 1980; Shafiq et al., 1984) that react uniformly with all ALD muscle fibers. Whether these react specifically with the myosin heavy chain associated with either SM-1 or SM-2, or react with an epitope in common to both isoforms is unknown.

It is of great interest that the expression of the two myosin heavy chain genes changes during the normal development and growth of ALD muscles. Electrophoretic analysis of myosin in the native state demonstrated that the SM-1 isoform declines with age from ≈80% of the total myosin in 14-d-old embryos to <20% in adult chickens (Hoh, 1979; Matsuda et al., 1982). The data presented here show that hypertrophy enhances both the rate and extent of this process.

Also, monoclonal antibodies raised against myosin heavy chain were screened for their specificity to SM-1 and SM-2 isomyosins in the chicken ALD muscle. It is reported here that the isomyosins separated by gel electrophoresis in the native state are antigenically distinct. We have also examined whether the SM-1 and SM-2 isomyosins are distributed homogeneously within the ALD muscle or reside in discrete populations of cells. Using monoclonal antibodies in immunocytochemical staining of normal ALD muscles, we have determined that SM-1 and SM-2 isomyosins are largely distributed in discrete cell populations in normal adult chicken ALD muscles. In addition, immunocytochemical staining of hypertrophied ALD muscles reveals that the population of SM-1 containing muscle fibers is eliminated and all muscle fibers are reactive with the anti–SM-2 antibody. Preliminary reports of our observations have been published (Kamel et al., 1985; Kennedy et al., 1985).

Materials and Methods

Animal Procedures

5-wk-old or newly hatched male White Leghorn chickens were obtained from Spafas Inc., Norwich, CT. White Leghorn chickens older than 1 yr of age were purchased from a local breeder. A lead weight covered with soft gauze was wrapped around one wing of each experimental chicken just above the humero-ulnar joint. Each experimental chicken served as an internal control because only one wing was weighted. Chickens were weighed daily and the mass of the shackle was maintained at 10% of the chicken's body mass throughout the experiment. To confirm that the experimental treatment did not cause gross changes in growth, body mass was recorded as a function of time for experimental chickens and a group of age-matched control chickens without the overload. The regression slopes were compared between experimental and control groups using analysis of covariance to determine if growth rates were equal.

Control and experimental chickens were chosen at random and killed with CO2 gas on days 0, 3, 8, 14, 21, and 28 after the placement of lead weights on the wings of 5-wk-old experimental animals. The ALD was excised from both sides of each chicken, weighed to the nearest milligram, and cised from both sides of each chicken, weighed to the nearest milligram, and used to determine if growth rates were equal.

Electrophoresis of Native Myosin

Myosin was extracted from minced ALD muscle samples with 10 vol of Guba/Straub's buffer (pH 6.5) containing 1 mM EDTA (DeAlbis et al., 1979). Electrophoresis was carried out at 1–4°C according to the procedure of Hoh et al., 1978. Polyacrylamide gels were prepared with 3.88% acrylamide and 0.12% bisacrylamide in buffer containing 30 mM sodium pyrophosphate (pH 8.80), 1 mM EDTA, 2 mM cysteine, 10% glycerol, a 1:286 dilution of N,N,N',N'-tetramethyl-ethylenediamine, and 1.75 mg/ml ammonium persulfate. 6–8 μg of myosin were loaded onto each gel and electrophoresis was conducted for 24 h with a constant voltage gradient of 11 V/cm. The gels were stained with Coomassie Brilliant Blue R-250. After destaining, the relative proportion of proteins in the gels was quantitated by densitometry using a spectrophotometer (model DMS 90; Varian Associates, Inc., Palo Alto, CA) and the scan digitized using a digitizer (model 9874A; Hewlett-Packard Co., Palo Alto, CA).

Characterization of Monoclonal Antibodies

Monoclonal antibodies designated CCM-52, HPM-7, and HPM-9 were used as probes for different myosin heavy chains in control and experimental muscles. Antibody CCM-52 was prepared against myosin isolated from day 12-18 embryonic chicken heart (Clark et al., 1982). Antibodies HPM-7 and HPM-9 were prepared against myosin isolated from the human psoas muscle. All three antibodies were directed against myosin heavy chains, as detected by staining of immunotransfers after SDS gel electrophoresis of myosin or its chymotryptic fragments. The specificity of the antibodies for SM-1 or SM-2 isomyosins in the ALD muscle was determined by immunoperoxidase staining of myosin bands separated by electrophoresis on pyrophosphate gels and transferred to nitrocellulose. Immunofluorescent procedures on cryostat sections also revealed that all three antibodies reacted with cardiac myosin and none of these antibodies reacted with fast skeletal muscle myosin in the posterior latissimus dorsi muscle.

Immunotransfer Technique

Native myosins separated by polyacrylamide gel electrophoresis were transferred to nitrocellulose by the electroblotting technique described by Towbin et al. (1979). The procedure was modified by electrophoresing 10% SDS through the gels to facilitate the transfer of myosin (Theo Walliman, personal communication). After transfer, the nitrocellulose was cut into strips and incubated for 1 h with the appropriate concentration of antibody CCM-52, HPM-7, or HPM-9 in a solution containing 20 mM Tris buffer (pH 7.5), 500 mM NaCl, 0.05% Tween-20, and 1% BSA. After this, strips were washed and treated with a 1:1000 dilution of horseradish peroxidase-conjugated rabbit anti–mouse or rabbit anti–rat IgG. Finally, the nitrocellulose strips were treated with 4-chloro-1-naphthol substrate for color development of the antibody reactive peptides.

Immunofluorescence Procedure

Cross sections of chicken ALD muscles were taken from the center of the muscle and labeled by indirect immunofluorescence with monoclonal antibodies CCM-52, HPM-7, and HPM-9. Muscle samples were taken at various times after the application of the overload. The contralateral ALD muscle served as the control. At least four chickens were sacrificed at each time point studied. Muscles were rapidly frozen in a dry ice/isopentane bath in OCT-embedding medium (Miles Scientific Div., Miles Laboratories Inc., Naperville, IL). 10-μm sections were cut in a cryostat at −20°C and transferred to glass microscope slides coated with 1.0% gelatin to enhance adhesion of the sections. After immersion in acetone at −5°C for 5 min, slides were incubated with one of the three primary anti–myosin antibodies at predetermined dilutions for 1 h at room temperature. This was followed by incubation with the appropriate rhodamine-conjugated IgG (Miles Scientific Div., Miles Laboratories Inc.) as the secondary antibody for 1 h at room temperature. Rabbit anti–mouse IgG served as the secondary antibody for CCM-52 and rabbit anti–rat IgG served as the secondary antibody for both HPM-7 and HPM-9 antibodies. Between each step slides were washed with three changes of PBS for a total of 30 min. After the final wash, the sections were covered with glycerol in PBS, coverslipped, and examined with a Leitz microscope equipped for epifluorescence. Stained sections were photographed on Kodak plus-X film at an exposure of 2 min. Control procedures show that the observed fluorescence was due to specific labeling because elimination of primary, secondary, or both antibodies resulted in a loss of staining. Nonmuscle tissue showed no reactivity with any of the antibodies and thus served as an internal control for background fluorescence.

Results

Evaluation of Hypertrophy

Because 5-wk-old chickens are still growing, hypertrophy must be superimposed on normal muscle growth. To evaluate whether placing a weight on the wings of experi-
Table I. Hypertrophy and Isomyosin Transitions in Overloaded Chicken ALD Muscles

| Age  | Control Muscle Mass | % SM-1 Isomyosin |
|------|---------------------|------------------|
|      | n  | mg            |                  |                  |
| Control | Overloaded |                  |                  |
| Day 0 | 9  | 116.2 ± 11.9  | 31.9 ± 2.2       |
| Day 3 | 4  | 132.4 ± 5.0   | 38.8 ± 3.3       |
| Day 8 | 6  | 166.2 ± 9.2   | 39.9 ± 8.3       |
| Day 12| 9  | 190.9 ± 9.7   | 34.9 ± 4.5       |
| Day 21| 5  | 229.5 ± 20.9  | 25.5 ± 5.4       |
| Day 28| 4  | 316.7 ± 26.8  | 21.7 ± 6.0       |

The ALD muscles of 5-wk-old chickens (experimental Day 0) were overloaded for 3, 8, 12, 21, and 28 d. The overloaded muscles and contralateral control muscles were weighed and samples prepared for myosin extraction and pyrophosphate gel electrophoresis. The relative proportion of SM-1 isomyosin was determined from the digitized area of densitometry tracings of pyrophosphate gels. Average values for muscle weight in milligrams and SM-1 proportion by percent of the total protein for n samples are given ± SE. Overloaded muscles had masses significantly different from control values (P < 0.05).

The hypertrophy in overloaded muscles was evaluated by comparing the wet masses of the muscles on the overloaded side to those of the contralateral control ALD muscles. The mass of the experimental ALD muscles was significantly greater than that of control ALD muscles throughout the experiment (Table I). As early as 3 d after the application of the overload, the mass of the ALD muscle on the experimental side was 1.7 times greater than that of the contralateral control ALD muscle (227.9 mg vs. 132.4 mg). After 28 d the overloaded ALD muscles weighed an average of 861.4 mg, while the ALD muscles on the contralateral control side weighed an average of only 316.7 mg.

Analysis of Native Myosins

The effect of hypertrophy on the isomyosin composition of the ALD muscle was evaluated using native gel electrophoresis. Two myosin bands are seen in control muscles corresponding to SM-1 and SM-2 (Fig. 1). The relative proportion of myosin in control muscles present in the faster migrating SM-1 band decreases dramatically during the first few weeks after hatching from a value of 67.5% in 6-d-old chickens to 31.9% at 5 wk; and to 21.7% at 9 wk (Fig. 2). The transition from SM-1 to SM-2 isomyosin does not continue after 9 wk of age because the amount of SM-1 isomyosin in the control ALD muscles at this point (21.7%) is not significantly different from that in chickens older than 1 yr of age (19.8%).

The replacement of SM-1 myosin by SM-2 myosin is greatly enhanced by hypertrophy (Figs. 1 and 2). After 28 d of overload, SM-1 isomyosin in the experimental ALD muscles represented only 1.2% of the total protein, whereas the contralateral control ALD muscles contained 21.7% SM-1 isomyosin. The overloading leads to a near complete disappearance of SM-1 in chickens at 6 wk of age, which is far in excess of that seen during normal development and growth.
Antigenicity of SM-1 and SM-2 Isomyosins

Given the fact that SM-1 and SM-2 isomyosins contain distinct myosin heavy chains, we used monoclonal antibodies to search for epitopes on the proteins that contribute to these differences. To screen our antibodies we used immunoperoxidase staining of myosin bands resolved by electrophoresis of myosin in the native state and transferred to nitrocellulose sheets. The results for three antibodies (HPM-7, HPM-9, and CCM-52) are reported here. Antibody HPM-7 reacted with both SM-1 and SM-2 isomyosin, whereas antibody HPM-9 reacted with only the SM-2 isomyosin and antibody CCM-52 with only the SM-1 isomyosin (Fig. 3 A).

Additional proof of the specificity of our antibodies within the ALD muscle was obtained by staining myosin obtained from overloaded ALD muscles. We have shown above that after 28 d of overloading, all or nearly all of the SM-1 myosin isoform is eliminated in the hypertrophied ALD muscles. Likewise, the reactivity of antibody CCM-52 on transfers of these gels is lost, while antibodies HPM-7 and HPM-9 retain their reactivity with the remaining SM-2 band (Fig. 3 B). Previous reports (Clark et al., 1982) indicated that antibody CCM-52 had little reactivity with adult ALD muscles. Given the present results, this is not surprising since the chickens used in these earlier experiments were older than 1 yr of age and of a strain (White Rock) that expresses little SM-1 isomyosin. Based on this evidence, antibody CCM-52 will be designated the anti-SM-1 antibody and antibody HPM-9 will be designated the anti-SM-2 antibody in these studies.

Cellular Distribution of Isomyosins

Further experiments were conducted to determine the cellular location of SM-1 and SM-2 myosin heavy chains within the chicken ALD muscle. There are two possibilities for the distribution of these myosins: (a) an individual muscle fiber could contain both myosin heavy chain types or, (b) an individual muscle fiber could contain exclusively one type of myosin or the other. Indirect immunofluorescent staining of serial cryostat sections from 5 wk-old chicken ALD muscles with antibodies HPM-7, HPM-9, and CCM-52 was used to answer this question. Antibody HPM-7 reacted equally well with every fiber in the muscle, while the reactivities of the anti-SM-1 and anti-SM-2 antibodies were almost exclusively...
distributed (Fig. 4). These results suggest that the myosin heavy chains associated with SM-1 and SM-2 proteins have discreet cellular localizations. However, on rare occasions fibers were detected which stained strongly with both anti-SM-1 and anti-SM-2 antibodies (Fig. 4), indicating that both SM-1 and SM-2 are present. Therefore, a small percentage of the total population of muscle fibers contain both myosin isoforms. The relative proportion of each isoform in a cell could not be determined due to the nonquantitative nature of the immunofluorescent technique.

We have reported that hypertrophy induces a rapid replacement of the SM-1 isomyosin by the SM-2 form. Immunofluorescent staining of these muscles demonstrates that in muscle overloaded for 28 d, all the myofibers that react with the anti-SM-1 antibody are replaced by fibers staining exclusively with the anti-SM-2 antibody (Fig. 5). These results are consistent with hypertrophy-associated replacement of SM-1 isomyosin by the SM-2 variant in ALD muscle fibers. Similarly, when the number of anti-SM-1- and anti-SM-2-reactive myocytes in normal ALD muscles are quantitated at different times during development, a decline in the number of anti-SM-1-reactive cells and an increase in the number of anti-SM-2-reactive cells is seen (Table II). 100% of the myocytes in 7-d-old chickens react with the anti-SM-1 antibody, whereas only 21.1% react in chickens older than 1 yr of age. The number of myocytes reacting with the anti-SM-2 antibody increases from 56.0% at 7 d to 87.4% in chickens 1 yr old. It is doubtful that the decrease in the number of SM-1-containing cells that occurs during development or the elimination of this population during hypertrophy is due to a degenerative loss of muscle fibers that originally express SM-1. It is more likely that the isoform transitions that occur are due to a switch in the synthesis and/or degradation of SM-1 and SM-2 proteins in individual myocytes. 10 d after the application of the overload, all cells stain with the anti-SM-2 antibody, whereas a faint reactivity remains on some cells with the anti-SM-1 antibody. This indicates that coexpression is a feature of the early stages of isoform replacement (Fig. 6), which is consistent with a switch in the expression from SM-1 to SM-2 isomyosin in individual ALD muscle cells. Although both normal development and hypertrophy result in an isomyosin transition from SM-1 to SM-2, the rate and extent of the transition appears to be exaggerated by hypertrophy.

### Discussion

The ALD muscle on the overloaded side of experimental chickens hypertrophied to a mass > 2.5 times that of the contralateral control muscles. The features of the model responsible for the growth are unclear. Initially, the ALD muscle is passively stretched as the chicken's wing droops to the floor of the cage. After a few days, the chickens are able to readjust their posture to a near normal condition with their wings held closer to their bodies. Thus, there seems to be a component of both passive stretch and work involved in this

| Table II. Cellular Isomyosin Transition |
|----------------------------------------|
| Isomyosin distribution                |
| Immunocytochemistry | Electrophoresis | |
| Age          | % SM-1 | % SM-2 | % SM-1 | % SM-2 |
| 7 d          | 100.0 | 56.0 ± 1.8 | 63.2 ± 3.5 | 36.8 ± 2.6 |
| 6 wk         | 37.3 ± 3.5 | 74.1 ± 3.4 | 31.8 ± 6.6 | 68.2 ± 6.7 |
| 1 yr         | 21.1 ± 3.5 | 87.4 ± 3.3 | 14.8 ± 3.4 | 85.2 ± 3.3 |

Normal ALD muscles from 7-d, 6-wk, or 1-yr-old chickens were excised and adjacent strips were prepared as samples for immunofluorescent analysis or pyrophosphate gel electrophoresis of native myosin extracts. Cryostat sections were stained with either the anti-SM-1 or anti-SM-2 antibodies and the number of fibers staining with each antibody was counted and recorded as the percent of the total number of fibers counted. At each age, the average percentage of four samples is presented ± SE. At least 150 muscle fibers were counted from each sample. SM-1 and SM-2 isomyosin were quantitated from pyrophosphate gels as described in Materials and Methods and are presented for comparison.
hypertrophy model. A hypertrophic response similar to the one reported here has been achieved using a surgically implanted spring apparatus to simulate a model of passive stretch only (Holly et al., 1980; Barnett et al., 1980). Also, in vitro studies (Vandenburgh and Kaufman, 1979) show that responses characteristic of growth can be achieved when muscle cell cultures are grown on a stretchable membrane. It therefore seems likely that passive stretch is sufficient to produce hypertrophy in chicken skeletal muscle and is probably the major stimulus here.

The present experiments demonstrate that the two types of myosin present in the chicken ALD muscle are immunologically distinct. The SM-1 myosin predominates in the neonatal chicken and is expressed in all muscle fibers at this time. Approximately 60% of the muscle fibers also contain the SM-2 isoform, so that there is a relatively large coexpression of the two myosin variants in individual muscle cells. As chickens continue to develop there is a transition in the relative proportion of SM-1 and SM-2 isomyosins with SM-2 eventually predominant. Even in older chickens, however, there is a small population of cells that coexpress both slow myosin isoforms. Growth induced by stretch enhances this process and results in an ALD muscle with fibers exclusively containing SM-2 isozyme.

Clearly a number of developmentally regulated gene switches occur in myosin types in chickens. Very early on there appears to be an expression of a "primordial" type of myosin in all embryonic-striated muscle (Sweeney et al., 1984). In fast twitch muscles of chicken such as the pectoralis, embryonic, neonatal, and adult forms of myosin heavy chain have been identified (Lowey et al., 1983; Benfield et al., 1983; Bandman et al., 1982; Bader et al., 1982). The situation is less clear in the slow tonic ALD muscle. Evidence for an embryonic form of slow myosin that is distinct from either adult fast or slow myosin heavy chains has been reported (Benfield et al., 1983; Matsuda et al., 1983). It is unclear whether this embryonic form of myosin heavy chain is identical to (Matsuda et al., 1983) or different from (Benfield et al., 1983) the fast muscle embryonic myosin heavy chain. The SM-1 and SM-2 myosin isoforms are both present in embryonic and adult ALD muscles. Therefore, it is not possible to identify one isoform or the other as embryonic, neonatal, or adult. However, it is clear that there is a developmentally regulated gene switch: the expression of the SM-1 gene is repressed while the expression of the SM-2 gene is induced. The present experiments show that this gene switch can be influenced by increasing the load on a muscle.

A similar situation is present in the mammalian ventricle where two myosin heavy chain genes (alpha and beta) are expressed at different levels during development (Everett et al., 1983; Lompre et al., 1981). A variety of interventions, of which thyroid hormone is the most potent (Hoh, 1978), can be superimposed over the genetic program to alter the level of expression of these two genes. So far, the only manipulation which is known to alter the preprogramming of the developmentally regulated gene switch in ALD muscle SM-1 and SM-2 isozymes is the induction of hypertrophy by overloading. We have demonstrated that overloading induces hypertrophy and enhances the repression of the SM-1 gene. It is probable that unlike the situation in the mammalian ventricle, the isoenzyme switching in the chicken ALD muscle is not hormonally mediated, but is somehow related to the increased load imposed on the muscle. Both the response to overloading presented here and the natural progression of development result in muscle growth. However, our results indicate that there is not a straightforward relationship between growth of the ALD muscle and repression of the expression of the SM-1 gene, for in the normal ALD muscle while the muscle weight increases between 5 wk and 1 yr, the proportion of cells that express the SM-1 gene changes very little. In contrast, in the overloaded ALD muscle a similar weight increase is accompanied by a nearly complete disappearance of muscle fibers that express the SM-1 gene. It is possible that the suppression of the expression of the SM-1 gene is induced by a stimulus additional to the one that promotes growth. Our results indicate that this stimulus depends on the load the muscle must carry. During development this load is represented by the weight of the wing, which increases with the age of the bird. In our experiments this natural increase in weight is augmented by the added load. Hence, in this situation all muscle fibers repress expression of the SM-1 gene so that the muscle can cope with the additional load. The mechanism by which the overload causes the suppression of the expression of a particular myosin isoform and/or induces the expression of another is not understood, although the changes may be related to a different activity pattern in overloaded muscles. The present model provides a simple situation that allows the study of this problem.

Funding for this research was provided in part by grants from the Muscular Dystrophy Association of America, U.S. Public Health Service grants.

Figure 6. Replacement of SM-1 isomyosin by SM-2 isomyosin in hypertrophied ALD muscle. Hypertrophied ALD muscles were obtained 10 d after the application of an overload. Serial cryostat sections were stained with monoclonal antibodies HPM-7 (A), CCM-52 (B), or HPM-9 (C). All muscle cells stain with antibodies HPM-7 and HPM-9, whereas a faint reactivity remains on some myocytes stained with antibody CCM-52. Bar, 200 μm.
HHL-16637 and HL-20592 from the National Heart, Lung, and Blood Institute, and by the Medical Research Council of Great Britain.

Received for publication 8 February 1986, and in revised form 27 May 1986.

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Kennedy et al. Myosin Expression in Hypertrophied Muscle