Metabolism of myosin heavy chain in steady-state chick skeletal muscle cultures

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Synthesis, accumulation and breakdown of the 200000-mol.wt. heavy subunit of myosin were analysed over an 11 day period in muscle cell cultures isolated from the leg muscle of 12-day chick embryos. Muscle cells accumulated myosin heavy chain rapidly from days 2 to 5 and maintained a maximum, constant myosin-heavy-chain concentration between days 7 and 11. Myosin-heavy-chain content and breakdown rate were compared in steady-state muscle cultures grown either in the presence of an optimum batch of horse serum (control) or in the presence of horse serum that had been pre-selected for its ability to inhibit several-fold the rate of synthesis of myosin heavy chain (inhibitory). The quantity of myosin heavy chain in the inhibited cultures was decreased in direct proportion to the decrease in the rate of synthesis of myosin heavy chain; however, the half-lives of myosin heavy chain (control, 17.7h; inhibitory, 17.0h) were virtually identical. In contrast, the absolute rate of breakdown of myosin heavy chain, expressed as molecules/min per nucleus, was approx. 5-fold lower in the inhibited cultures (4.3×10^3 molecules/min per nucleus) than in the control cultures (21.7×10^3 molecules/min per nucleus). Thus, inhibition of myosin-heavy-chain synthesis in this case was accompanied by diminished myosin-heavy-chain concentration and absolute breakdown rate at the altered steady state, but relative myosin-heavy-chain breakdown rates were unchanged.

Breakdown of the myofibrillar protein fraction in skeletal muscle is sensitive to nutritional stress and rehabilitation, muscle hypertrophy, denervation, neuromuscular diseases, hormone administration, altered intracellular Ca^{2+} concentrations and proteinase inhibitors (Fulks *et al.*, 1975; Goldberg, 1969a,b; Kameyama & Etlinger, 1979; Laurent *et al.*, 1978a,b,c; Maruyama *et al.*, 1978; Millward *et al.*, 1974, 1975a,b; Riebow & Young, 1980; Rourke, 1975a,b; Segal *et al.*, 1974). However, the intracellular signalling mechanisms that co-ordinate synthesis and breakdown of specific polypeptides are unknown. The clarity of this regulatory system could perhaps be enhanced by defining the metabolic pattern of one specific myofibrillar component, rather than by measuring average metabolism of a heterogeneous mixture of the major myofibrillar proteins.

The 200000-mol.wt. subunit of myosin can be quantitatively purified with ease from muscle cell cultures. Because myosin is the major myofibrillar constituent, and because alterations in myosin-heavy-chain metabolism may accurately reflect

metabolism of the entire myofibrillar unit, it should therefore be informative to define specifically the metabolic properties of myosin heavy chain in cultured muscle cells. Thus, the purposes of the present study were to: (i) define the period in cell culture when embryonic chick muscle cells reach a steady-state condition in which there is no net change with time in the quantity of myosin heavy chain; (ii) compare the decay rate of myosin heavy chain as measured by pulse-chase experiments with the decay rate mathematically derived from data on myosin-heavy-chain concentration and synthesis rate and (iii) assess the effects on myosin-heavy-chain breakdown of serum that promotes sub-optimal myosin-heavy-chain synthesis rates.

Experimental

Muscle cell cultures

Leg muscle from 12-day chick embryos was removed and placed in 15 ml of complete culture medium. This medium consisted of 85% (v/v) Eagle's minimum essential medium (Grand Island

Biological Co.), 10% (v/v) pre-selected horse serum, 5% (v/v) chick embryo extract, 50 units of penicillin/ml, 50 µg of streptomycin/ml, and 2.5 µg of Fungizone/ml. The embryonic muscle tissue was dissociated with a vortex mixer at maximum speed for 20 s as described by Young *et al.* (1975). Cells were counted with the aid of a haemocytometer and were plated at a density of 5×10^6 cells/10 cm Falcon tissue culture dish. Dishes had previously been coated with approx. 100 µg of collagen. Cells were incubated in the complete medium described above in an atmosphere of air and CO₂ (19:1, v/v), and complete medium was replenished daily. Since myoblast fusion is essentially complete after 3 days in culture under the conditions employed here, 10^{-7} M-fluorodeoxyuridine was added on day 3 to inhibit fibroblast proliferation and thereby enhance the proportion of multinucleated myotubes.

Muscle cultures were stained with Giemsa stain as described by Young *et al.* (1975), and at least 1000 nuclei were counted in a minimum of ten randomly chosen fields. The number of nuclei within multinucleated myotubes and the percentage of nuclei within multinucleated myotubes were calculated and used as indices of differentiation of muscle cultures. Since muscle cells are multinucleated, and since the number of nuclei in each myotube varies widely, it was not possible to express data on a cellular basis. Instead, myosin-heavy-chain synthesis rates and content were divided by the number of myotube nuclei in the culture dish to normalize on the basis of each nuclear unit and to, therefore, permit direct comparisons among experiments.

Myosin-heavy-chain content and synthesis rate

The synthesis rate and the quantity of myosin heavy chain were assessed from the same culture samples. Cells in 10 cm dishes were pulse-labelled for 4 h with 10 µCi of L-[4,5-³H]leucine/ml (New England Nuclear, Boston, MA, U.S.A.) in complete medium. The cell culture medium used for all pulse-labelling and pulse-chase experiments contained leucine at a final concentration of approx. 0.5 mM. At the end of the labelling period, the dishes were rinsed twice with cold 0.25 M-NaCl/0.01 M-MgCl₂/0.01 M-Tris/HCl, pH 7.2, and the cells were scraped from the surface into 0.5 ml of the above buffer. Cells were then homogenized with 20 strokes of a 7 ml Dounce homogenizer (Wheaton Scientific, Millville, NJ, U.S.A.; tightly fitting A pestle), and the cell homogenate was centrifuged at 1600 g for 20 min at 2°C. The NaCl concentration of the supernatant was then lowered to 0.025 M by the addition of cold water, and samples were placed at 2°C for approx. 12 h to permit myofibrillar protein aggregation. Myosin-containing material was collected by centrifugation at 6000 g for 20 min. The supernatant from this centrifugation was saved for

determination of intracellular [³H]leucine specific radioactivity as described by Airhart *et al.* (1979). Myosin-containing samples were dissolved for electrophoresis in a solution containing 1.0% (w/v) sodium dodecyl sulphate/0.5% (v/v) 2-mercaptoethanol/20% (v/v) glycerol/0.01% (w/v) Pyronin Y/0.05 M-Tris/HCl, pH 7.1. Gels contained a final concentration of 10% (w/v) acrylamide/5% (v/v) glycerol/0.1% (w/v) sodium dodecyl sulphate/0.04% (w/v) NNN'N'-tetramethylethylenediamine/0.04% (w/v) ammonium persulphate/0.12% (w/v) NN'-diallylditartardiamide/0.04 M-Tris/glycine buffer, pH 8.8. After electrophoresis, gels were stained at room temperature for 24 h in methanol/acetic acid/water (50:7:43, by vol.) containing 0.03% Coomassie Brilliant Blue R-250. Gels were then destained for 24 h in water/methanol/acetic acid (35:2:3, by vol.). Residual stain was finally removed electrophoretically by two 15 min destaining steps in an Ames model 5525 Quick Gel Destainer (Canalco, Rockville, MD, U.S.A.).

The myosin-heavy-chain band was sliced from the gels as a series of 0.8 mm slices, and slices were dried overnight at 50°C in a liquid-scintillation vial. Each slice was dissolved for 2 h at 25°C in 0.4 ml of a freshly prepared solution of 2% (w/v) HIO₄/4% (w/v) lactic acid, pH 3.0 (adjusted with NH₄OH). Samples were transferred to a cuvette, and the absorbance was measured at 560 nm, the absorbance maximum of Coomassie Brilliant Blue R-250 under these conditions. Standard curves constructed with purified myosin heavy chain were used to calculate the quantity of myosin heavy chain in each gel. Under the conditions described above, the absorption coefficient for myosin heavy chain was 40.2 ± 2.6 µg of myosin heavy chain/*A*₅₆₀ unit (Young *et al.*, 1980).

After absorbance measurement, each sample was returned to the liquid-scintillation vial, the cuvette was rinsed once with water to remove residual sample, and this rinse was combined with the sample in the liquid-scintillation vial. Aquasol (New England Nuclear, Boston, MA, U.S.A.) scintillation fluid was added to each vial and radioactivity was measured in a Beckman 3133P liquid-scintillation spectrometer; c.p.m. were converted to d.p.m. by using automatic external standardization.

Half-life of myosin heavy chain by pulse-chase experiments

Mature muscle cultures were incubated in the presence of complete culture medium containing 1 µCi of [³H]leucine/ml for 16–20 h to pre-label protein. Cultures were then incubated in complete medium (which contains 0.5 mM-leucine) without [³H]leucine during the chase period. At the end of the labelling period, and at approx. 12 h intervals thereafter for 60 h, duplicate 10 cm plates were

rinsed once each with an iso-osmotic saline solution and isolation buffer (0.25 M-NaCl/0.02 M-Tris/HCl, pH 7.2) to remove residual radioactivity. Cells were then scraped, homogenized and prepared for electrophoretic analysis of radioactivity in myosin heavy chain as described in the preceding section. To standardize and summarize data from nine experiments, the radioactivity found in myosin heavy chain at each of the times indicated was calculated as a percentage of the initial value, and the logarithm of these values was plotted as a function of time after initiation of the chase period. The amount of time necessary for the radioactivity to decrease to 50% of its initial value was used as the half-life of myosin heavy chain.

Half-life of myosin heavy chain by mathematical derivation from myosin-heavy-chain synthesis and concentration

An alternative method for evaluating the half-life of biological molecules can be derived from a mathematical description of concentration and synthetic rate (cf. Harris *et al.*, 1975). If these two parameters can be quantified, breakdown rates can be calculated without concern for potential problems of amino acid re-utilization or metabolism. For example, the equation describing changes in myosin-heavy-chain content as a function of time in muscle cultures can be expressed as:

$$dc/dt = T - CD \quad (1)$$

where dc/dt is the rate of change in the average

number of molecules of myosin heavy chain/nucleus, T is the myosin-heavy-chain translation rate in molecules/min per nucleus, C is the concentration of myosin heavy chain in molecules/nucleus and D is the first-order degradation constant for myosin heavy chain. Since dc/dt is by definition equal to zero in the steady-state condition, $T = CD$. Moreover, D is related to the half-life of myosin heavy chain (in min) by the relationship $D = \ln 2/t_{1/2}$. Consequently, the half-life of myosin heavy chain (in h) can be calculated from the myosin-heavy-chain concentration and translation rate by the following equation:

$$t_{1/2} = \frac{C \times \ln 2}{T \times 60} \quad (2)$$

Results

Myosin accumulation in muscle cultures

Cultured muscle cells initiate the fusion process after approx. 1 day, and the number of nuclei within myotubes increases rapidly during the ensuing 48 h. The maximum percentage of fused nuclei was generally between 75 and 80 (Table 1). Shortly after initiation of myoblast fusion, the gene programmes for all muscle-specific functions are activated (Young & Allen, 1979). The quantity of myosin heavy chain steadily increases from barely detectable levels in 1 day mononucleated cultures to a maximum of approx. 3.3×10^7 molecules of myosin heavy chain/nucleus in fully developed 7 day

Table 1. *Effect of muscle development on myoblast fusion and on synthesis and accumulation of myosin heavy chain in muscle cell cultures*

All values represent the mean $\pm$ one S.D. of ten cell culture dishes at each age. The number of myosin-heavy-chain molecules was calculated from the absorbance of Coomassie Brilliant Blue R250 in myosin-heavy-chain bands on the same gels used for estimates of synthesis rates. These calculations utilize a mol.wt. of 2.0×10^5 for myosin heavy chain, an absorption coefficient for Coomassie R250 binding to myosin heavy chain of $40.2 \mu\text{g}/A_{560}$ and the number of myotube nuclei in replicate culture dishes determined from microscopic evaluation of Giemsa-stained cultures. The potential contribution of constitutive myosin heavy chain by the 15–25% of contaminating fibroblasts is considered negligible in these calculations for two reasons. First, fibroblast myosin is partially soluble under the ionic conditions employed to precipitate quantitatively myofibrillar myosin heavy chain, and less than 30% of fibroblast myosin heavy chain would be recovered under these conditions (R. B. Young & O. C. Dombroske, unpublished work). Secondly, fibroblasts contain only 0.8 pg of constitutive myosin heavy chain/nucleus, approx. 15-fold less than the quantity of myofibrillar myosin heavy chain in muscle cells (Young *et al.*, 1979). Thus less than 1% of the observed myosin heavy chain could have originated from fibroblasts. Synthesis rates were calculated from 4 h pulse-labelling experiments with [^3H]leucine. These calculations employ a mol.wt. of 2.0×10^5 for myosin heavy chain, 175 leucine residues/myosin heavy chain, a [^3H]leucine specific radioactivity of 1.51×10^4 d.p.m./nmol, and the number of myotube nuclei in replicate culture dishes as determined by Giemsa staining.

Culture age (days)	Fusion (%)	$10^{-6} \times$ Myotube nuclei/culture	$10^{-6} \times$ No. of myosin heavy chain molecules/nucleus	$10^{-3} \times$ Synthesis rate (molecules synthesized/ min per nucleus)
2	40.2 $\pm$ 6.0	8.14 $\pm$ 1.42	0.3 $\pm$ 0.1	1.0 $\pm$ 0.4
3	60.0 $\pm$ 4.4	10.15 $\pm$ 1.18	3.9 $\pm$ 1.1	6.9 $\pm$ 1.2
5	76.7 $\pm$ 3.0	10.10 $\pm$ 0.95	21.6 $\pm$ 2.4	23.2 $\pm$ 1.8
7	72.5 $\pm$ 2.6	9.13 $\pm$ 0.75	34.1 $\pm$ 2.9	23.1 $\pm$ 2.7
9	72.7 $\pm$ 9.2	9.10 $\pm$ 0.80	32.5 $\pm$ 2.3	20.9 $\pm$ 4.1
11	66.5 $\pm$ 9.6	8.33 $\pm$ 0.63	33.4 $\pm$ 2.9	21.0 $\pm$ 2.6

contracting cultures (Table 1). Both the number of myotube nuclei per culture and the total quantity of myosin heavy chain per culture tend to decline slightly after maturity is attained; however, the quantity of myosin heavy chain maintained by each nuclear unit remains essentially constant at 3.3×10^7 molecules/nucleus (Table 1). Thus, muscle cell cultures between 7 and 11 days of age are at steady state with respect to myosin-heavy-chain content.

Comparison of myosin-heavy-chain half-life by the pulse-chase technique and by calculation from synthesis and accumulation rates

The first-order decay of radioactive amino acids from pre-labelled proteins is frequently employed to quantify protein breakdown, despite the inability to measure the contribution of amino acid re-utilization to the decay rate. Analysis of myosin heavy chain in mature muscle cultures by this approach revealed a half-life of 25.8 h (Fig. 1). Although this value is in reasonable agreement with myofibrillar protein decay rates measured in rats (Alemany, 1975), it is considerably shorter than the value of 6 days previously reported for myosin heavy chain in muscle cultures (Rubinstein *et al.*, 1976).

Fig. 1. First-order disappearance rate of $[^3\text{H}]$ leucine from myosin heavy chain after an overnight labelling period in the presence of $1 \mu\text{Ci}$ of $[^3\text{H}]$ leucine/ml. Each point represents the mean $\pm$ one S.E.M. of nine experiments in which measurements were made in duplicate. To correct for differences in $[^3\text{H}]$ leucine content in individual experiments, the radioactivity at zero-chase time was assigned a value of 100%, and subsequent values were calculated relative to this original one. The line best fitting these decay data was calculated by linear regression. Approx. 25.8 h were needed for radioactivity in myosin heavy chain to decline to 50% of its initial value.

When values of 33.3×10^6 molecules of myosin heavy chain/nucleus and 21.7×10^3 molecules/min per nucleus (Table 1, mean of days 7, 9 and 11) are substituted into eqn. (2) (see the Experimental section), a half-life of 17.7 h is calculated for myosin heavy chain. Because fewer assumptions are involved in calculating $t_{1/2}$ from synthesis rate and content, this method was employed in subsequent comparative experiments in steady-state muscle cultures.

Analysis of myosin-heavy-chain breakdown under conditions of sub-optimal myosin-heavy-chain synthesis rate

The ultimate purpose of our research is to define the mechanism that co-ordinates the proper balance between myosin synthesis and degradation in muscle cells. Accordingly, we wondered whether there is a constant, basal breakdown rate of myosin heavy chain, with changes in content only accomplished by alterations in synthesis rate. Or, more likely, is myosin heavy chain content altered by a combination of changes in synthesis and breakdown. As one approach to this problem, several independent batches of horse serum were evaluated in an effort to identify one that would promote typical morphological differentiation of muscle cultures, but would not permit maximum myosin-heavy-chain synthesis. Results of this screening process (Table 2) show variable results, but sample no. 2 (hereafter referred to as the inhibitory serum) was found to satisfy adequately the requirements. Sample no. 1 is the batch of serum employed to obtain all the data in Table 1 and in Fig. 1; this serum is called the control serum in the remainder of the text.

The effects of inhibitory serum on myoblast fusion and on myosin-heavy-chain synthesis rate and content are shown in Table 3. The percentage fusion and the number of nuclei within myotubes are similar to the control serum (Table 1). Moreover, the general patterns of myosin-heavy-chain accretion and synthesis are qualitatively similar to those found with control serum, in that myosin-heavy-chain content exhibits only minor changes between days 7 and 11, and the synthesis rate is relatively constant between days 5 and 11 (Table 1). In contrast with the similar qualitative patterns of development, marked quantitative differences are observed between the cultures grown in control and inhibitory serum. The myosin-heavy-chain content is approx. 33.3×10^6 molecules/nucleus in control cultures and 6.37×10^6 molecules/nucleus in inhibited cultures (means of data for days 7–11 in Tables 1 and 3, respectively). The myosin-heavy-chain synthesis rate is approx. 21.7×10^3 molecules/min per nucleus in control cultures, but only 4.34×10^3 molecules/min per nucleus in inhibited cultures (means of data for days 7–11 in Tables 1 and 3, respectively). Thus,

Table 2. Screening of several lots of horse serum for those that differentially affect the myosin-heavy-chain synthesis rate.

Cells were evaluated after 7 days in culture. Batch 1 was employed as the control serum in this study, since it promoted massive fusion and the maximum rate of myosin-heavy-chain synthesis. Batch 2 of inhibitory serum was selected for further examination because of its ability to support massive myoblast fusion, but its inability to stimulate myosin-heavy-chain synthesis to a maximum rate.

Sample no.	Fusion (%)	$10^{-6} \times$ Myotube nuclei/culture	$10^{-5} \times$ Synthesis rate (d.p.m./min per nucleus)
1	72.5	8.72	13.4
2	69.0	8.15	1.9
3	71.0	4.45	11.7
4	70.4	6.30	8.2
5	38.3	2.68	3.7

Table 3. Effect of horse serum selected for its promotion of sub-optimal rates of myosin-heavy-chain synthesis on myoblast fusion, myosin heavy chain synthesis and accumulation in muscle cell cultures

All values represent the mean $\pm$ one S.D. of nine to twelve cell culture dishes at each age. The number of myosin heavy chain molecules/nucleus and the synthesis rate were calculated as described in the legend to Table 1.

Culture age (days)	Fusion (%)	$10^{-6} \times$ Myotube nuclei/culture	$10^{-6} \times$ No. of myosin heavy chain molecules/nucleus	$10^{-3} \times$ Synthesis rate (molecules synthesized/min per nucleus)
2	51.7 $\pm$ 0.9	10.69 $\pm$ 1.14	0.48 $\pm$ 0.10	1.14 $\pm$ 0.03
3	60.0 $\pm$ 1.5	12.01 $\pm$ 1.87	1.03 $\pm$ 0.16	3.71 $\pm$ 0.67
5	70.7 $\pm$ 3.8	11.83 $\pm$ 0.62	4.67 $\pm$ 0.43	4.29 $\pm$ 0.16
7	67.5 $\pm$ 2.1	9.27 $\pm$ 1.07	6.59 $\pm$ 1.39	4.26 $\pm$ 0.40
9	66.0 $\pm$ 6.5	9.94 $\pm$ 1.07	6.79 $\pm$ 0.87	4.48 $\pm$ 0.36
11	61.0 $\pm$ 1.5	8.60 $\pm$ 0.80	5.73 $\pm$ 0.17	4.29 $\pm$ 0.57

inhibition of myosin-heavy-chain synthesis resulted in a comparably diminished content.

The myosin-heavy-chain half-life, calculated by using eqn. (2) from the data in Tables 1 and 3 (days 7–11), was 17.0h in the presence of inhibitory serum, compared with 17.7h in the control serum. Thus, inhibition of synthesis resulted in a diminished myosin-heavy-chain content, with no change in half-life. However, half-lives are not absolute measures of protein degradation. Because myosin-heavy-chain synthesis rates were measured in steady-state cultures where the accumulation rate is equal to zero (days 7–11 in Tables 1 and 3), synthesis rates are, therefore, exactly equal to breakdown rates. Thus, absolute breakdown rates are approx. 21.7×10^3 and 4.34×10^3 molecules/min per nucleus in the presence of control and inhibitory serum, respectively. In an absolute sense, therefore, the breakdown rate, as well as the synthesis rate, is markedly lower in cells exposed to inhibitory serum.

Discussion

The half-life of myosin heavy chain in control cultures was measured in preliminary studies by two methods: pulse-chase and mathematical derivation from synthesis rate and content. The observed half-life of 25.8h (Fig. 1) was much shorter than the half-life of 6 days previously reported by Rubinstein

et al. (1976). However, half-lives of approx. 1 day are not unreasonably short in view of other studies *in vivo* and *in vitro* (Alemany, 1975; Young *et al.*, 1978). At this time, we do not know why large variations in myosin-heavy-chain half-life have been observed in muscle cultures, but it apparently depends on the specific culture conditions as well as the genetic composition of the embryos used for the experiments.

Half-lives calculated by using eqn. (2) were consistently shorter than half-lives observed in pulse-chase experiments. At least two unevaluated assumptions are inherent in the pulse-chase method: (i) that there is no re-utilization of degraded amino acids for synthesis of new proteins and (ii) that there is negligible metabolism of the labelled amino acid into other amino acids that are subsequently incorporated into protein. Both of these uncertainties would tend to increase observed half-lives. That at least one of these processes is operating in muscle cells is shown by our consistent observation that calculated half-lives are shorter than observed half-lives. Skeletal muscle tissue rapidly metabolizes leucine *in vivo* (Odessey & Goldberg, 1972, 1979; Chang & Goldberg, 1978*a,b*; Goldberg & Chang, 1978); however, leucine oxidation is negligible under cell culture conditions virtually identical with those employed here (Schneible *et al.*, 1978). Thus, [3 H]leucine re-utilization most likely accounts for the discrepancy.

Measurement of protein breakdown by eqn. (2) requires quantification of the specific radioactivity of precursor pool [^3H]leucine during pulse-labelling experiments. In the present study, the specific radioactivity of the total intracellular [^3H]leucine pool was employed for calculation of myosin-heavy-chain translation rates. The specific radioactivities of labelled amino acids in the extracellular and tRNA pools are equal when amino acid concentrations are as high as those in our cell culture medium (McKee *et al.*, 1978). However, we cannot eliminate the possibility that errors in breakdown rate are introduced by the assumption that tRNA and intracellular specific radioactivities are equal.

Neither the intracellular mechanism co-ordinating the balance between myosin-heavy-chain synthesis rate and breakdown rate nor the actual proteinases that degrade myosin heavy chain are known. During the planning stages of the present research, either of two results was expected when the myosin-heavy-chain synthesis rate was partially inhibited: (i) that the myosin-heavy-chain content would be identical with that of controls and the half-life would be longer, or (ii) that the myosin-heavy-chain content would be diminished proportionally to changes in synthesis rate and the half-life would be unchanged. The second, observed, result is that the myosin-heavy-chain content of muscle cells is diminished in the altered steady-state, but relative breakdown rates (i.e., half-life) are unchanged. It is also interesting that the absolute breakdown rates (molecules/min per nucleus) would have been identical in both of the above cases (i.e., absolute synthesis must equal degradation at steady-state, and myosin-heavy-chain synthesis was intentionally suppressed by the inhibitory serum).

A note of caution is therefore extended to interpretations of protein breakdown data, particularly under conditions of cell culture. In the present experiments, for example, it is clear that proteolysis of myosin heavy chain in the presence of inhibitory serum is diminished 5-fold. It is not known whether the lower absolute breakdown rate is due to the presence of a proportionally diminished concentration of specific proteinases, or, alternatively, whether these inhibited cultures contain equivalent proteinase concentrations but their activity has somehow been inhibited. Further analysis of these possibilities should lead to a more definite comprehension of myofibrillar protein breakdown. In our view, proteolytic breakdown of myosin heavy chain in muscle cultures can be more meaningfully assessed by the absolute approach, rather than by measurement of half-life.

The inhibitory serum employed for the present experiments was selected for its ability to inhibit myosin-heavy-chain synthesis, and the possibility cannot be excluded that this serum, in fact, altered

protein degradation and that myosin-heavy-chain synthesis was indirectly altered by a proportional amount. While it is clear that synthesis and breakdown of myosin heavy chain respond similarly, it is not known if one controls the other or if both processes respond in tandem to identical intracellular signals. If the latter case is true, it is not apparent how these processes differentially respond so that the content of myosin heavy chain can be increased or decreased, depending on the specific needs of the cell.

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