Expression of Smooth Muscle and Nonmuscle Myosin Heavy Chains in Cultured Vascular Smooth Muscle Cells*

(Received for publication, May 5, 1986)

Arthur S. Rovner, Richard A. Murphy, and Gary K. Owens‡

From the Department of Physiology, School of Medicine, University of Virginia, Charlottesville, Virginia 22908

We explored the hypothesis that discrepancies in the literature concerning the nature of myosin expression in cultured smooth muscle cells are due to the appearance of a new form of myosin heavy chain (MHC) in vitro. Previously, we used a very porous sodium dodecyl sulfate gel electrophoresis system to detect two MHCs in intact smooth muscles (SM1 and SM2) which differ by less than 2% in molecular weight (Rovner, A. S., Thompson, M. M., and Murphy, R. A. (1986) Am. J. Physiol. 250, C861–C870). Myosin-containing homogenates of rat aorta cells in primary culture were electrophoresed on this gel system, and Western blots were performed using smooth muscle-specific and non-muscle-specific myosin antibodies. Subconfluent, rapidly proliferating cultures contained a form of heavy chain not found in rat aorta cells in vivo (NM) with electrophoretic mobility and antigenicity identical to the single unique heavy chain seen in nonmuscle cells. Moreover, these cultures expressed almost none of the smooth muscle heavy chains. In contrast, postconfluent growth-arrested cultures expressed increased levels of the two smooth muscle heavy chains, along with large amounts of NM. Analysis of cultures pulsed with [35S]methionine indicated that subconfluent cells were synthesizing almost exclusively NM, whereas postconfluent cells synthesized SM1 and SM2 as well as larger amounts of NM. Similar patterns of MHC content and synthesis were found in subconfluent and postconfluent passaged cells. These results show that cultured vascular smooth muscle cells undergo differential expression of smooth muscle- and nonmuscle-specific MHC forms with changes in their growth state, which appear to parallel changes in expression of the smooth muscle and nonmuscle forms of actin (Owens, G. K., Loeb, A., Gordon, D., and Thompson, M. M. (1986) J. Cell Biol. 102, 343–352). The reappearance of the smooth muscle MHCs in postconfluent cells suggests that density-related growth arrest promotes cytodifferentiation, but the continued expression of the non-muscle MHC form in these smooth muscle cells indicates that other factors are required to induce the fully differentiated state while in culture.

Chamley et al. (1977) developed a technique for smooth muscle cell (SMC') culture based on an enzymatic dissociation of intact tissues. These investigators found that vascular smooth muscle cells underwent a process of "phenotypic modulation" in culture (Chamley-Campbell et al., 1979) which involved their apparent conversion to a cell type characterized by proliferation, synthesis, and secretion rather than contraction. During this process, the cells acquired large amounts of rough endoplasmic reticulum and Golgi apparatus, whereas there was a loss of cytoplasmic thick filaments and the progressive diminution of myosin immunofluorescence staining by a smooth muscle-specific antibody produced against chicken gizzard myosin (Chamley and Campbell, 1974; Chamley et al., 1977; Gröschel-Stewart et al., 1975). Sometimes, primary cultures which had been plated at an initial high density (>5 x 10⁶ cells/ml) showed a return of smooth muscle myosin immunofluorescence after the attainment of confluency (Chamley-Campbell and Campbell, 1981), although in passaged cultures, there was a persistence of the "synthetic state" and a continued lack of smooth muscle myosin antigenicity (Chamley and Campbell, 1974; Chamley et al., 1977). These types of observations led Chamley-Campbell et al. (1979) to suggest that cell culture caused a permanent loss of many of the characteristics typical of contractile smooth muscle cells, including the possession of myosin.

Several recent studies suggested that the concept of "synthetic" versus "contractile" phenotype is overly simplistic in describing the state of differentiation of SMCs in culture. Vascular SMCs display contractions after several days in culture in response to the application of agonists such as angiotensin II (Gunter et al., 1982). Established smooth muscle cultures continue to express high affinity receptors to contractile agonists (Gunter et al., 1982; Colucci et al., 1984). After growth arrest by heparin, changes in ionic conductance in response to the application of vasoactive amines are evident (Martin and Gordon, 1983). Myosin light chain phosphorylation is seen in postconfluent cultures of rat mesenteric artery SMCs in response to the binding of angiotensin II (Anderson et al., 1981). It has also been shown that the amount of smooth muscle-specific a-actin, as a proportion of total actin, is greatly decreased during the proliferative phase of cultured cell growth (Fatigati and Murphy, 1984). However, smooth muscle actin increases dramatically upon growth arrest by the withdrawal of mitogens or by contact inhibition at high cell density.

*This research was supported by a National Institutes of Health Predoctoral Training Grant 5 T32 HL07284-09 (to A. S. R.) and National Institutes of Health Grants 2 PO1 HL19242-09 and 5 PO1 HL19242-10. Portions of this work have previously been presented at the 1985 Federation of American Societies for Experimental Biology Summer Conference on Smooth Muscle Physiology, June 24–28, 1985, Saxton's River, VT, and at the 1986 Federation of American Societies for Experimental Biology meeting, St. Louis, MO, April 13–18, 1986. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡To whom reprint requests should be addressed: Dept. of Physiology, School of Medicine, Box 449, University of Virginia, Charlottesville, VA 22908.

1 The abbreviations used are: SMC, smooth muscle cell; SDS, sodium dodecyl sulfate.
densities (Owens et al., 1986; Strauch and Rubenstein, 1984). These observations illustrate that SMCs may exhibit a continuum of phenotypes with varying relative degrees of contractile and synthetic characteristics while in culture. However, the issue of whether myosin expression is altered by the conditions of culture remains unclarified.

Larson et al. (1984b) showed that both subconfluent and postconfluent cultures of rat mesenteric artery cells were immunofluorescently stained by polyclonal antibodies to human uterine (ASMM) and platelet (APM) myosins. These investigators also showed by quantitative SDS-polyacrylamide gel densitometry that the total amount of myosin present at these stages of culture remained fairly constant. A small diminution in ASMM-specific staining was noted at confluency, whereas APM staining remained constant (Larson et al., 1984b). It is possible that the discrepant observations of Chamley-Campbell et al. (1979) and Larson et al. (1984b) were due to the expression of smooth muscle myosin heavy chain variants in culture which were not resolved by the higher percentage SDS-acylamide gels employed by the latter group.

We tested the smooth muscle myosin-specific antibody of Gröschel-Stewart et al. (1976) and the anti-human platelet myosin antibody of Fujiwara and Pollard (1976) which had been used in the previous investigations and performed Western blots on extracts of cultured rat aorta smooth muscle cells which had been electrophoresed on SDS-3-4% acrylamide gradient gels. Our first objective in this study was to test for the appearance of myosin heavy chain isoforms with unique antigenicity and/or mobility under the conditions of cell culture. Our second objective was to document possible shifts in the pattern of myosin heavy chain expression associated with changes in the growth state of smooth muscle cell cultures.

MATERIALS AND METHODS

Cultured Vascular Smooth Muscle Cells—Smooth muscle cells were obtained by enzyme digestion from the thoracic aortas of young adult Sprague-Dawley rats (Hilltop Lab Animals, Scottsdale, PA) and cultured as previously described (Owens et al., 1986). Freshly obtained cells were counted in triplicate in a hemocytometer and were then seeded onto plastic culture dishes at a density of 10^4 cells/cm^2 in medium 199-10% fetal calf serum (HyClone Laboratories), penicillin, streptomycin, and 10 mM HEPES (pH 7.4). Plates were incubated at 37°C with a humidified atmosphere of 5% CO_2, 95% air.

Harvest of Cells and Preparation of Denaturing Gel Samples—Smooth muscle cell cultures were examined at subconfluent and postconfluent growth stages. Under these conditions, the subconfluent cultures are growing logarithmically, whereas postconfluent cultures are quiescent (Owens et al., 1986). Cells were removed from the culture dishes by trypsinization, diluted with phosphate-buffered saline to inactivate the enzyme, and centrifuged at 120 x g for 10 min. The cell pellet was quick-frozen by immersion of the centrifuge tube into an acetone-dry ice slurry and stored at -80°C until further processing.

Cultured cells were prepared for denaturing gel analysis in one of two ways. 1) Cell pellets were suspended at 5000 cells/ml in SDS sample buffer containing 50 mM Trizma (Tris base) hydrochloride (pH 6.8 at 22°C), 1% (v/v) SDS, 25 mM dithiothreitol, 10% (v/v) glycerol, and 0.001% (v/v) bromphenol blue tracking dye. They were triturated within the centrifuge tube using a pipette and then sonicated (Heat Systems-Ultrasonics, Farmingdale, NY, Model 370). The disrupted cell suspension was boiled (4 min) and stored as frozen aliquots at -80°C. 2) Cell pellets were sonicated as above at 5-15 x 10^9 cells/ml in phosphate-buffered saline containing protease inhibitors: leupeptin (4 mg/ml), aprotinin (4 mg/ml), chymostatin (2 mg/ml), soybean trypsin inhibitor (20 mg/ml), phenylmethylsulfonyl fluoride (1 mM), and 1 mM EDTA. The majority of this homogenate was mixed with an equal volume of twice-concentrated SDS sample buffer and boiled. The remainder was assayed for DNA content (Lubarch and Paigen, 1980) or protein (Lowry et al., 1951). This method allowed us to normalize the volume of sample loaded from cells at different stages of culture to a constant amount of DNA or protein.

Denaturing gel samples from intact cardiac and smooth muscle were prepared by mixing pyrophosphate extracts from these tissues with an equal volume of twice-concentrated denaturing sample buffer and boiling as above. These extracts were prepared as described by Hoh et al. (1978) except that the extracting buffer was 80 mM in tetrasodium pyrophosphate and contained the proteolytic enzyme inhibitor mixture described above.

Discontinuous SDS-Polyacrylamide Gels—The denaturing SDS gel system of Laemmli (1970) was used for all analyses of intact myosin heavy chains. A very porous separating gel of 3-4% acrylamide gradient with low myosin loadings (<4 µg/ lane) allowed discrimination of heavy chain variants differing slightly in molecular weight (Rovner et al., 1986).

Immunoblot Analysis—Proteins in SDS-polyacrylamide gels were transferred onto nitrocellulose paper and assayed immunologically using the general protocol established by Towbin et al. (1979). Transfers were conducted using a Hoefer Transphor apparatus at currents between 0.5 and 1.2 A for 45-90 min. The nitrocellulose was washed with 0.1% Amido Black in 50% methanol plus 10% acetic acid to verify protein transfer from the gel, and blocking was carried out in 1% BSA/PBS (pH 7.4) containing 0.01% Tween 20 and 0.01% bovine serum albumin. Protein blots were assayed using two myosin-specific polyclonal antibodies. The rabbit anti-chicken gizzard myosin antibody (ACG) was prepared by Dr. Ute Gröschel-Stewart (Technische Hochschule Darmstadt), whereas a rabbit anti-human platelet myosin antibody (APM) (Fujiwara and Pollard, 1976) was obtained from Dr. David Latson (Boston University). A goat anti-rabbit second antibody conjugated to horseradish peroxidase was used as the marker for the antigenically reacted proteins. Preimmune rabbit serum was used as the negative control for these assays.

RESULTS

Immunological Analysis of Myosin Heavy Chains in Primary Cultures—The myosin heavy chain complement of rat aortic smooth muscle cells at the subconfluent and postconfluent growth stages of primary culture was examined using Western blots of SDS-3-4% acrylamide gradient gels (Fig. 1). These samples were compared with denatured homogenates of intact smooth muscle tissues and with striated muscle myosin heavy chains.

General protein staining with Amido Black revealed that homogenates of swine stomach and intact rat aortic media each contained two putative myosin heavy chains with mobilities somewhat less than the heavy chains found in rabbit fast-twitch skeletal muscle and rat left ventricle (Fig. 1A, lanes 1-4). These two heavy chains, designated SM1 and SM2 in order of increasing mobility, have been found in several different mammalian smooth muscle tissues and differ in molecular weight by approximately 4000 (Rovner et al., 1986). Whereas the skeletal and cardiac heavy chains failed to react with either the smooth muscle-specific or nonmuscle-specific myosin antibodies (Figs. 1, B and C, lanes 1 and 2), the smooth muscle heavy chains were equally labeled in these tissues by the anti-chicken gizzard myosin antibody (Fig. 1B, lanes 3 and 4). There was no cross-reactivity of the swine smooth...
Muscle heavy chains with the anti-platelet myosin antibody, whereas the rat aortic heavy chains were very faintly labeled (Fig. 1C, lanes 3 and 4). The platelet sample seemed to contain a much greater proportion of high molecular weight proteins relative to the total amount of protein loaded than did the rat aortic extract, which may have been due to inconsistencies in the protein assay used or in the electrophoretic transfer to nitrocellulose. Platelet myosin heavy chain migrated as a single band with mobility slightly greater than that of SM2. This heavy chain was not recognized by the smooth muscle-specific myosin antibody (Fig. 1B, lane 5) but was labeled by the nonmuscle myosin antibody (Fig. 1C, lane 5).

Rat aorta cells in the subconfluent stage of primary culture contained small amounts of the SM1 heavy chain as assayed by general protein staining, and this heavy chain was clearly labeled by the ACG antibody (Fig. 1, A and B, lane 6). However, these cells also contained large amounts of a heavy chain which had a mobility slightly greater than that of the SM2 variant seen in intact smooth muscle tissues, identical to the mobility of human platelet heavy chain (Fig. 1A, compare lane 5 with lanes 6 and 7). This high mobility heavy chain was unlabeled by the smooth muscle myosin-specific antibody (ACG) in either cultured cells or platelets, but was strongly recognized by the anti-platelet myosin antibody (APM) Fig. 1, B and C, lanes 5–7). Because of its electrophoretic and antigenic similarity to platelet myosin, the high mobility heavy chain in cultured smooth muscle cells was designated NM. Swiss 3T3 cells (American Type Culture) and cultured endothelial cells from rat heart (gift of P. DiCorletto, Cleveland Clinic) also contained this single heavy chain band which was stained with the anti-platelet myosin antibody (data not shown). Growth-arrested cells from postconfluent cultures showed all three heavy chains on general protein staining (Fig. 1A, lane 7). The two lower mobility, smooth muscle bands were recognized only by ACG (Fig. 1B, lane 7), whereas APM preferentially labeled the highest mobility, NM form (Fig. 1C, lane 7).

Immunological Analysis of Myosin Heavy Chains in Passaged Cultures—Samples from subpassaged smooth muscle cells were examined to determine 1) whether the smooth muscle forms are permanently lost in subpassaged cells as suggested by Chamley-Campbell et al. (1979) and 2) whether the differences in expression of smooth muscle and nonmuscle myosin heavy chains between subconfluent (proliferating) and postconfluent (quiescent) cells are unique to primary culture. At the subconfluent, log stage of growth, these passaged cultures showed only the NM form when tested against both the gizzard and platelet myosin-specific antibodies (Fig. 2, lanes 3). Postconfluent cultures contained large amounts of NM and smaller quantities of the SM1 heavy chain, whereas SM2 was barely detectable (Fig. 2, lanes 4).

Myosin Heavy Chain Synthesis and Content in Cultured Vascular Smooth Muscle Cells—Smooth muscle cell cultures were pulsed 4 or 12 h with 80 or 40 μCi/ml [35S]methionine, and protein-stained SDS gels containing these samples were compared to autoradiographs in order to obtain data relating myosin heavy chain synthesis and content. The results of these comparisons are shown in Fig. 3. Subconfluent primary cultures in the log phase of growth contained only the NM heavy chain whereas, consistent with earlier results on Western blots, quiescent postconfluent cultures contained small but fairly comparable amounts of SM1 and SM2 and much larger amounts of NM (Fig. 3A, left panel). The corresponding autoradiograph from these gel lanes indicated that myosin heavy chain synthesis in the subconfluent cultures involved almost exclusively the NM form, whereas postconfluent cultures synthesized both the smooth muscle heavy chains and the nonmuscle form in ratios similar to those seen on Coomassie staining (Fig. 3A, right panel). Proliferating postconfluent cells examined 4 days after plating (instead of the 6 days of growth allowed in the samples illustrated) contained small amounts of the smooth muscle heavy chains as well as NM, but synthesized almost exclusively the nonmuscle heavy chain form (data not shown). These data indicated that pro-
Myosin Heavy Chain Expression in Cultured Smooth Muscle Cells

FIG. 2. Nitrocellulose transblots of aorta and platelet homogenates as well as homogenates of passaged rat aorta cells electrophoresed on porous SDS-3–4% acrylamide gradient gels. Lane 1, pyrophosphate extract of rat aortic media, 33.0 μg of total protein; lane 2, human platelet homogenate, 8.25 μg of total protein; lane 3, subconfluent rat aorta cells in second passage, 297 × 10^3 cells; lane 4, postconfluent rat aorta cells in first passage, 220 × 10^3 cells. A, Amido Black protein stain; B, immunoperoxidase staining with anti-chicken gizzard myosin antibody; C, immunoperoxidase staining with the anti-human platelet myosin antibody.

FIG. 3. Qualitative analysis of myosin heavy chain content and synthesis in cultured rat aorta smooth muscle cells by SDS gel electrophoresis and subsequent autoradiography. A, protein-stained gel and corresponding autoradiograph of primary cultured rat aorta cells. The first lane in the left panel was stained with Coomassie Brilliant Blue, whereas the right lane was silver-stained. DNA loaded: left lane, 2.43 pg; right lane, 0.76 pg. B, Coomassie-stained gel and corresponding autoradiograph of first passage rat aorta cells. DNA loaded: left lane, 2.0 μg; right lane, 3.0 μg.

A. Protein Stain
B. Autoradiograph

DISCUSSION

Using a smooth muscle-specific antibody, Chamley-Campbell and co-workers (Chamley et al., 1977; Gröschel-Stewart et al., 1975; Chamley-Campbell et al., 1979) reported that myosin is lost in cultured smooth muscle cells as part of the process of phenotypic modulation, although they found that it is variably re-expressed in primary cultures depending on initial plating densities and growth conditions. In contrast, Larson et al. (1982, 1984b) reported that myosin is maintained at almost constant levels in subconfluent and postconfluent cultures, as assessed by immunofluorescence and quantitative SDS gel densitometry. We have found that myosin persists in cultured cells in significant amounts throughout proliferation, as was demonstrated by Larson et al. (1984b). However, subconfluent cultures of smooth muscle cells express a myosin heavy chain variant which is distinguishable...
from the smooth muscle forms seen in vivo (Rovner et al., 1986) on the basis of electrophoretic mobility and reactivity with a smooth muscle myosin-specific antibody (Groschel-Stewart et al., 1976). Because it co-migrates with the myosin heavy chain from human blood platelets and other nonmuscle cells and is strongly recognized by the anti-platelet myosin antibody, we have designated this heavy chain NM. The APM polyclonal antibody appears to cross-react very slightly with the smooth muscle cells of intact rat aorta, consistent with the findings of previous immunocytochemical studies (Larson et al., 1984a). However, along with our electrophoretic and immunological findings, previous studies showing that the peptide maps from smooth muscle and nonmuscle heavy chains differ (Burridge, 1974; Burridge and Bray, 1975) argue strongly that the two forms are distinct in their structural and physicochemical properties.

Our data also demonstrate that there is differential expression of the smooth muscle and nonmuscle (NM) myosin variants at different stages of culture. Synthesis of the smooth muscle forms is greatly attenuated in logarithmically growing cultures, but increases with growth arrest, leading to the appearance of significant amounts of SM1 and SM2 in postconfluent cultures. The nonmuscle heavy chain, in contrast, is continuously synthesized during all phases of cell growth and is the predominant heavy chain present at subconfluent and postconfluent time points. These observations may explain why Larson et al. (1982, 1984a) saw continued APM staining in all phases of primary culture even though staining with Groschel-Stewart's ACG antibody (Groschel-Stewart et al., 1976) was lost during the subconfluent phase of growth in the studies of Chamley et al. (1977).

As in primary cultures, NM was seen at all phases of growth in passaged cultures. At the subconfluent time point, there was no staining by the smooth muscle-specific heavy chain antibody, but growth-arrested cultures re-expressed SM1 and SM2. A phenomenon we found to be reproducible in several different SMC lines was the increase in the ratio of the SM1 heavy chain to the SM2 heavy chain in first passage quiescent cultures compared to primary cultures. However, in the absence of strict quantitative data concerning the rates of synthesis or turnover of the two heavy chain proteins, or on the levels of message RNAs coding for these peptides, we do not feel it possible to speculate as to the control mechanism responsible for this change in heavy chain stoichiometry with passage.

Our finding of continued smooth muscle heavy chain expression in passaged cultures refutes the suggestion by Chamley et al. (1977) Chamley-Campbell et al. (1979) that established cultures have been permanently modulated to a synthetic phenotype in which myosin is lost from the cells. Furthermore, we found that total loss of the smooth muscle forms of myosin was not a prerequisite for onset of proliferation of our cultures, as reported by Chamley et al. (1977) and Chamley-Campbell et al. (1981). There is the possibility that the biochemical approach used in our study provided a more sensitive assay for smooth muscle myosin forms than immunocytochemical techniques, thus explaining the different findings with the same antibody. Alternatively, the difference in our results and those of Chamley et al. (1977) may relate to the use of a different cell type in the present study (rat versus monkey or rabbit aorta) or to differences in the methods of cell culture (enzymes used in initial cell isolation and passage, composition of medium, technique).

The appearance of the NM heavy chain could be due to its de novo expression in cells which previously expressed only the smooth muscle-specific heavy chains. Alternatively, the conditions of culture could favor the proliferation of a subpopulation of cells which express only the nonmuscle heavy chain. Our data favors the former possibility for the following reasons. First, critical morphological and immunocytochemical examination of our cultures using a smooth muscle-specific isoactin monoclonal antibody (Owens et al., 1986) failed to reveal any nonmuscle contamination, ruling out the possibility that NM appears due to selection of contaminating cell types. Second, analysis of the intact rat aorta by SDS gels and autoradiography failed to reveal any NM heavy chain content or synthesis, even at very high loadings. This argues against the idea that culture caused the preferential selection of a pre-existing population of SMCs which expressed exclusively NM. Third, our Western blot analyses indicated that proliferating cultures which contained almost exclusively NM myosin later expressed significant quantities of SM1 and SM2 in the postconfluent state. Our limited supply of the two polyclonal antibodies did not allow us to identify the heavy chain content of individual quiescent or dividing cells identified by labeling with [3H]thymidine. However, in our previous studies (Owens et al., 1986), dual labeling of cells with the smooth muscle actin-specific monoclonal antibody and [3H] thymidine indicated that smooth muscle isoactins are differentially expressed in given cells depending upon their growth stage and that all cells respond similarly. Collectively, these observations suggest that the changes seen in heavy chain synthesis and content were due to changing patterns of expression within a fairly homogeneous population of smooth muscle cells.

A source of artifact in our studies could have been the use of sample loadings for different stages of culture which contained disparate amounts of myosin heavy chain. This possibility was tested by examining loading curves of different samples over a wide range of protein, DNA, or number of cells. Samples from cultures in sub- or postconfluent growth contained comparable amounts of total heavy chain on porous gels when loaded with equal amounts of protein or DNA. Furthermore, densitometric evaluation of 8-12% acrylamide gradient gels, utilized to cause migration of the different heavy chains as a single band, indicated that the amount of myosin as a proportion of the total cellular protein or DNA did not change significantly with growth stage of SMCs in culture. This relative constancy of total myosin heavy chain content is consistent with the earlier findings by Larson et al. (1984b) and suggests that our electrophoretic results were not affected by differences in the amount of myosin loaded on gels.

The results of the present study have shown that the pattern of expression of the nonmuscle- and smooth muscle-specific forms of myosin heavy chain at different stages of culture closely parallels that seen for actin (Owens et al., 1986). The nonmuscle forms of both actin and myosin are prevalent during the log phase of culture. However, in quiescent postconfluent cultures, there appears to be a coordinate induction of the smooth muscle-specific forms of these contractile proteins. Previous studies examining the expression of smooth muscle α-actin in rat aorta cultures grown in a defined serum-free medium indicated that growth arrest alone appears to trigger the synthesis of smooth muscle-specific actin forms (Owens et al., 1986). However, the present results show that the level of the smooth muscle-specific forms of myosin heavy chain expressed in postconfluent cultures does not reach that seen in vivo, as was found for the different actins. Although we feel that description of these cells as being in a synthetic state would be inappropriate, it is nonetheless clear that the cell phenotype brought about by growth arrest is modulated from that seen in vivo. This indicates that...
other factors yet to be identified are necessary for SMCs in cultures to reach their mature, fully differentiated state.

Acknowledgments—We wish to thank Dr. Julie Chamley-Campbell for providing the anti-chicken gizzard myosin antibody and Dr. David Larson for the anti-platelet myosin antibody. We also thank Marcie Corjay and Diane Singer for technical assistance and Barbara Green for administrative support.

REFERENCES