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IMMUNOFLUORESCENT STAINING OF MYOSIN IN CULTURED CARDIOMYOPATHIC HAMSTER HEART CELLS*

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ABSTRACT Primary cultures of heart cells from normal and genetically cardiomyopathic (CM) hamsters were analyzed by immunofluorescent staining for myosin. Antimyosin specifically stains the myofibrillar A-bands. These immunofluorescent staining experiments demonstrate an abnormally disarrayed arrange-

ment of myofibrils in CM cells when compared to normal; however, the staining of individual myofibrils is similar in CM and normal. Our results suggest that the aberrant pattern of myofibrils in CM cardiomyocytes result not from secondary effects of *in vivo* development, but more likely, from primary effects of the mutation.

KEY WORDS genetic cardiomyopathy; hamster; cardiomyocytes *in vitro*; immunofluorescence; myosin; aberrant myofibrils

Strain UM-X7.1 Syrian hamsters have a genetic cardiomyopathy which results in heart failure and premature death of the animal[1]. Previously reported morphological studies demonstrate aberrant myofibril organization in heart cells of cardiomyopathic (CM) animals 30 d of age or older[2]; it has been suggested that this abnormality may result secondarily from hypoxia due to heart failure *in vivo*[3]. In the present study, to eliminate potential *in vivo* factors, we prepared primary cultures of newborn normal and CM ventricular heart cells and analyzed their myofibril formation by immunofluorescent methods. In this paper the results of immunofluorescent staining for myosin are presented

MATERIALS AND METHODS

CULTURE The hearts of three-day-old normal and cardiomyopathic (CM) hamsters were used for culture. The culture techniques employed were similar to those of Wada *et al*[4] with the following modifications. The animals were killed by cervical dislocation and the hearts were immediately extirpated using sterile techniques. A solution containing 0.08% trypsin (1:250, DIFCO) and 0.02% collagenase (132 u/mg, Millipore Co.) was used to dissociate the cells. To enrich the cultures for myocytes, the dissociated heart cell suspensions were preincubated in an Erhlenmeyer flask for 60 min at 37℃. Most of the fibroblasts attached to the bottom of the flask during this period. The remaining unattached cells (now more than 90% myocytes) were diluted to an average density of 2×10^5 dispersed cells/ml of medium. Two ml aliquots of the myocyte-enriched cell suspensions were added to each tissue culture dish with collagen-coated glass coverslips on the bottom. The culture medium consisted of Eagle's MEM containing 15% fetal calf serum, penicillin 100 u/ml, streptomycin 0.1 mg/ml and fungizone 0.25 μg/ml. The cultures were incubated at 37℃ in an atmosphere of 5% CO_2 and 95% air. The culture medium was changed every 3 d. Growth and differentiation of the cultured cells were monitored periodically using a Zeiss inverted microscope equipped with phase contrast optics.

ANTIGEN AND ANTIBODY Antibodies against highly purified myosin preparations[5,6] were raised in carefully-screened young rabbits by methods detailed in earlier papers[7,8]. The specificities of our antimyosins from both native and electrophoresed antigens were tested by the double immunodiffusion method. Each antibody preparation gave a single

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sharp precipitin line indicative of a high degree of purity (Fig 1).

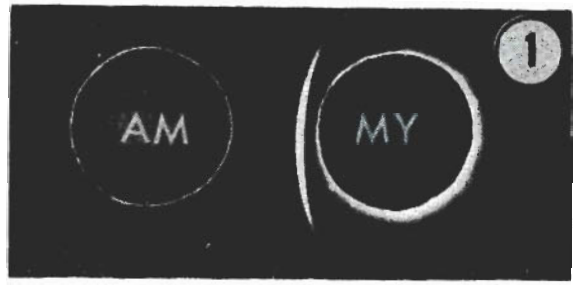
IMMUNOFLUORESCENT STAINING

After 3 d in culture, the coverslips on which the heart cells grew were rinsed in 3 changes of phosphate buffered saline (PBS) and fixed in a periodate–lysine–paraformaldehyde solution[9] for 30 min at room temperature. Then the coverslips were washed in 2 changes of PBS for 15 min, placed in 7% sucrose–PBS for 15 min, rinsed in PBS and transferred to 25% glycerol–PBS for 30 min. The cultured cells were stained by an indirect method. The primary antibody (antimyosin diluted in 25% glycerol–PBS) was applied to the fixed cells for 60 min at 37°C. After several rinses in PBS–glycerol totalling 60 min, the cells were stained with FITC–labelled goat antirabbit IgG (Miles Laboratories) diluted 1:40 with 25% glycerol: PBS for 60 min at 37°C. The controls for the antibody staining included: (a) incubation with preimmune globulins from the same rabbit that produced the specific antibodies, (b) staining with antibodies that had been absorbed with excess purified antigen, and (c) staining with the 2nd antibody only. There was very little background staining on these controls. The FITC–stained cells were viewed with a Zeiss Universal light microscope equipped with epifluorescent illumination using a xenon light source. Photographs were taken on 35 mm Kodak Plus–X film (ASA 125) at 90 s exposure times.

RESULTS AND DISCUSSION

After 3 d, primary cultures derived from the heart ventricles of both normal and CM newborn hamsters contain a mixture of cardiac myocytes and nonmuscle cells (mostly fibroblasts). This presumably resulted from the higher mitotic index of fibroblasts since most of the nonmuscle cells had been removed by our pre–incubation step as described under METHODS. In spite of this heterogeneity, the muscle and nonmuscle cells could be distinguished easily in the cultures. The muscle cells are irregular in shape and display phase–dense cytoplasm with numerous cross–striated myofibrils. They beat spontaneously at rates of 50–120 contractions/min. The fibroblasts are similar in shape to the cardiomyocytes, but have a phase–lucent cytoplasm. Furthermore, they do not contract nor do they contain cross striated myofibrils.

When the cultured cardiomyocytes are treated with FITC–labelled antibodies against myosin, intense fluorescence appears in their myofibrils; staining of fibroblasts in the same culture is very weak (only at background level). These immunofluorescent studies further reveal that antimyosin specifically stains the A–bands of myofibrils (Fig 2). The normal cardiomyocytes after 3 d in culture show intense fluorescent staining of the cross striated myofibrils. Most of the myofibrils are parallel with respect to each other and are oriented parallel to the longitudinal axis of the cell (Fig 3). By contrast, the myofibrils in CM cells are disoriented with respect to each other and many appear to form complex entangements within the cytoplasm (Fig 4). In a preliminary report, Wada *et al*[4] observed disorganized myofibrils in portions of cultured CM heart cells (strain BIO 14.6) by electron microscopy of thin sections. In the present study, immunofluorescent staining of myosin allowed us to evaluate the distributions of myofibrils in whole cells. Our studies unequivocally confirm an extensively disarrayed myofibril arrangement in cultured CM cardiomyocytes; the staining of individual myofibrils, however, appeared similar to normal.



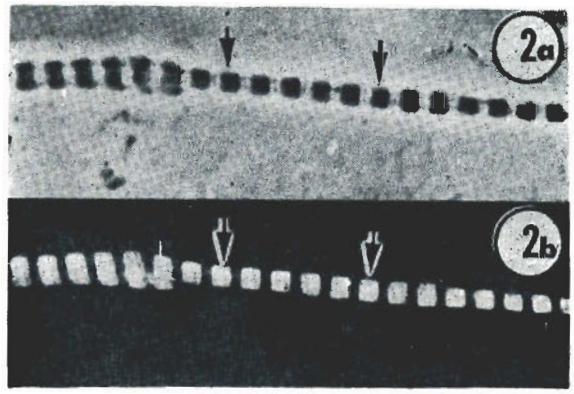
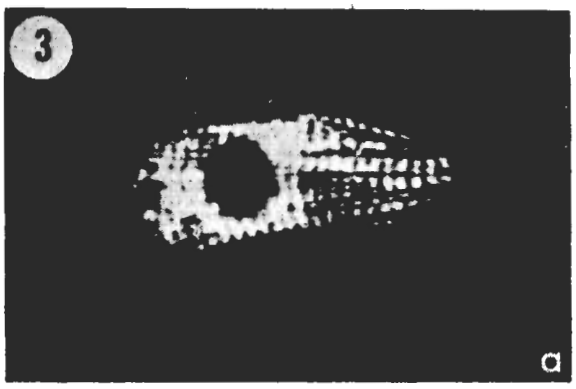


Fig 1. Double immunodiffusion plate to test antimyosin. AM, antimyosin antibodies; MY, purified skeletal muscle myosin.

Fig 2. Isolated myofibril from fresh unfixed hamster skeletal muscle stained with antimyosin. Corresponding phase contrast (a) and fluorescent (b) micrographs show that the antimyosin specifically stains the myofibril A-bands. Arrows illustrate corresponding areas.

Fig 3. Normal hamster myocytes cultured 3 d and stained for myosin by immunofluorescent methods. The A-bands of the myofibrils are stained specifically and reveal that most of the myofibrils are arranged in parallel.

Fig 4. Cardiomyopathic (CM) hamster heart myocytes cultured and stained as above. The A-bands stain specifically for myosin, however, in CM cells the myofibrils are disoriented with respect to each other.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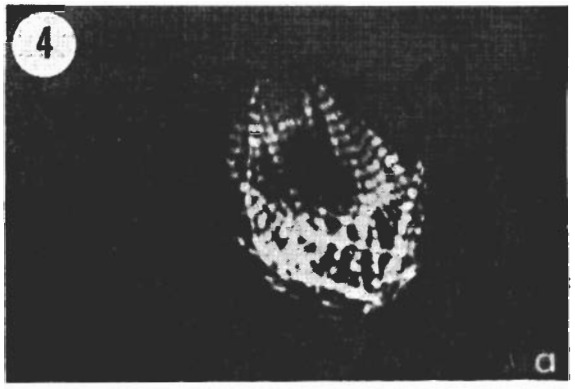
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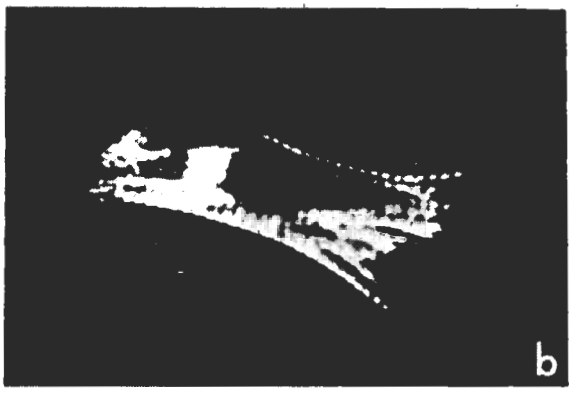
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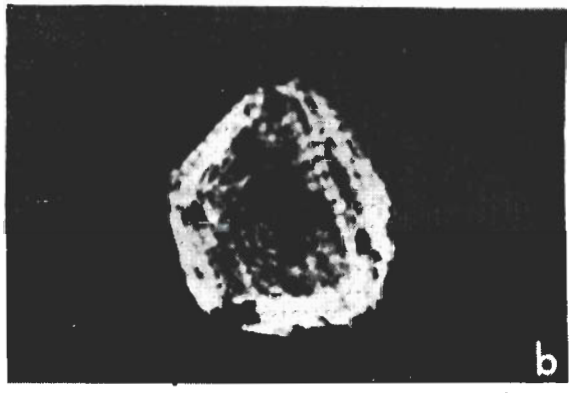
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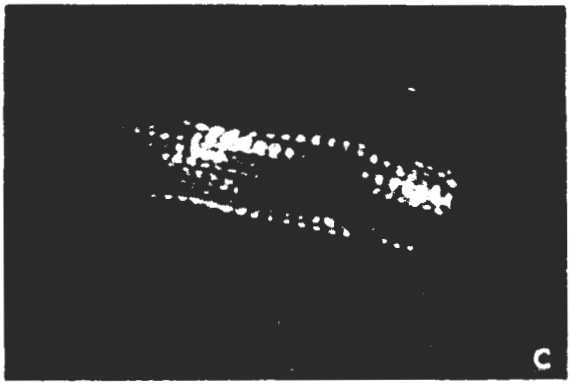
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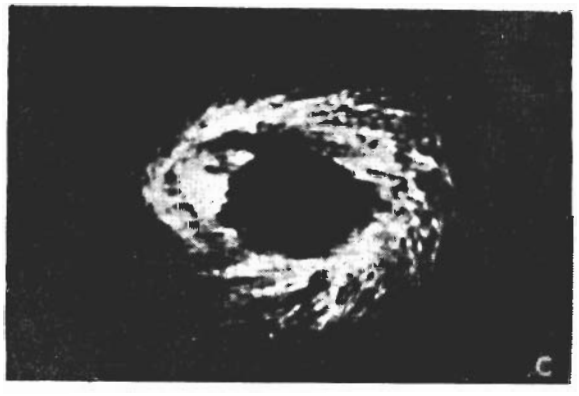
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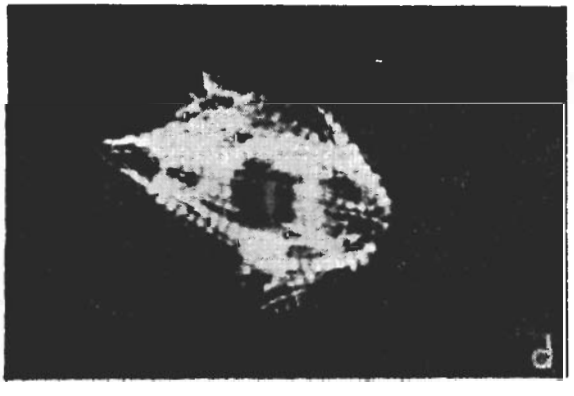
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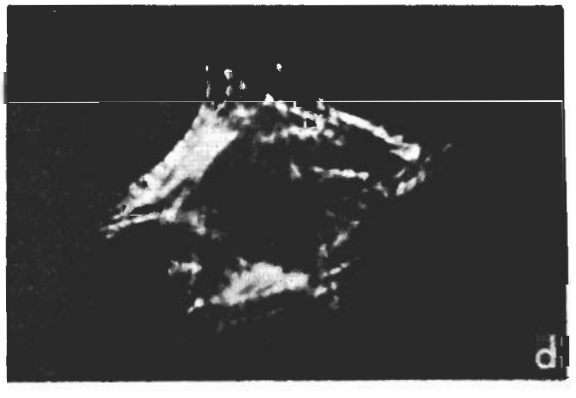
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In earlier reports[2] aberrant myofibril organization was observed in *in vivo* heart cells of cardiomypathic animals 30 d of age or older. It has been suggested that this abnormality may result from hypoxia due to heart failure *in vivo*[3]. The present study clearly demonstrates that an aberrant pattern of myofibril organization is present in cultured cells derived from very young CM animals (3 d after birth). Normal heart cells cultured under identical conditions do not show this myofibril disarray. Thus, our results strongly suggest that the myofibril disarray in CM heart cells results not from secondary effects of *in vivo* development, but more likely, from primary effects of he genetic mutation.

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