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SCANNING AND TRANSMISSION ELECTRON MICROSCOPY OF DISSOCIATED NORMAL AND CARDIOMYOPATHIC HAMSTER HEART CELLS*

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ABSTRACT Normal and cardiomyopathic (CM) hamster hearts were dissociated into single cells and examined by scanning and transmission electron microscopy. The size of CM and normal myocytes are comparable. In addition, both have morphologically similar intercalated discs and T-tubule openings. However, in contrast to normal, the CM myocytes contain unusually large aggregations of mitochondria. Also, cell surface pits and peripheral vesicles are more numerous in CM cells. The most striking abnormality of CM myocytes is their aberrant shapes. Many of the CM cells have branching irregular shapes or a helical morphology; the myofibrils within these cells are disarrayed or helically arranged as well[8]. Most normal cells exhibit straight cylindrical configurations with straight myofibrils. The abnormal morphology in itself would seem to provide an explanation for the decreased efficiency of heart contractions in cardiomyopathic hamsters and presumably is related to the observed heart failure in this genetically-based cardiomyopathy.

KEY WORDS genetic cardiomyopathy; hamster; dissociated heart cells; scanning and transmission electron microscopy; aberrant cardiomyocyte morphology

A genetic cardiomyopathy in Syrian hamsters (strain UM-X7.1) results in early death of the animal due to heart

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failure. The histopathology and myocardial ultrastructure of cardiomyopathic (CM) hamsters have been reported[1-3]. To date, there has been no study of dissociated single hamster heart cells. It is virtually impossible to examine the complete external structure of a myocyte in conventionally prepared intact heart tissue. However, scanning electron microscopy (SEM) of dissociated cardiomyocytes overcomes this limitation by allowing a three-dimensional imaging of the cell surface including specialized cell junctions. In conjunction with transmission electron microscopy (TEM), correlations can then be made with internal cell structures as well.

In this paper, we analyze the morphology of dissociated normal and CM hamster heart cells by SEM and TEM. Our results demonstrate that CM cardiomyocytes have several morphologically abnormal features, the most striking of which is an aberrant, helical shape.

MATERIALS AND METHODS

CELL PREPARATION Adult normal and CM hamster hearts were dissociated into single cells by retrograde aortic perfusion of the coronary vasculature with a Ca^{++} and Mg^{++}-free Krebs-Ringer solution containing 0.05% collagenase (132U/mg; Millipore Corp, USA), 0.1% hyaluronidase(710U/mg; Grand Island Biological Co, USA) and 0.5% bovine serum albumin [4]. The perfusion solution was kept at 37℃ and gassed continuously with 95% O_2+ 5% CO_2. The hearts were perfused for 45 min at a flow rate of 7 ml/min by the use of a peristaltic pump. After perfusion,the atria were discarded from the softened heart. The remaining ventricles were cut into~3mm thick slices and transferred to a 125 ml Erhlenmeyer flask containing 10-15 ml of gassed calcium-free enzyme-Krebs-Ringer solution at 37℃. After gently shaking the tissue slices for 10 min, single cells were released. The cell suspension was filtered through 2 layers of nylon mesh to remove connective tissue elements and undissociated cells, then washed with Krebs-Ringer solution and collected by low speed centrifugation. An aliquot of dissociated cells was subjected to a trypan blue exclusion test to determine viability.

ELECTRON MICROSCOPY Freshly dissociated cells were collected by low speed centrifugation and then fixed by resuspension in 2.5% glutaraldehyde, 2.0% formaldehyde and 0.1% picric acid,buffered to pH 7.3 with 0.15 M phosphate buffer at 4℃ [5]. To keep the cells dispersed in the fixing solution, they were drawn in and out of a wide-bore pipet several times for 1 h. Then the cells were rinsed in several changes of 0.15 M phosphate buffer for 30 min and postfixed for 1 h at 4℃ in 1% OsO_4 buffered to pH 7.2 with 0.15 M phosphate buffer. For scanning electron microscopy (SEM), the cells were rinsed in a saturated aqueous solution of thiocarbohydrazide (TCH) 5-10 times for a 20 min total, incubated in saturated TCH at room temperature for 10 min, rinsed in glass distilled water, osmicated again for 1 h, and rinsed with glass distilled water. The cells were dehydrated through a graded ethanol series and dried by the critical point method. The cells were mounted on aluminum stubs with silver paint, coated with gold and examined in a JEOL JSM-35 C Scanning Electron Microscope at 10 or 15 kV.

For transmission electron microscopy (TEM), after the first osmication, the cells were dehydrated in graded ethanols and propylene oxide, and embedded in Epon. Thin sections (60-80nm) were mounted on bare copper grids and doubly stained with uranyl acetate and lead citrate. The specimens were examined and

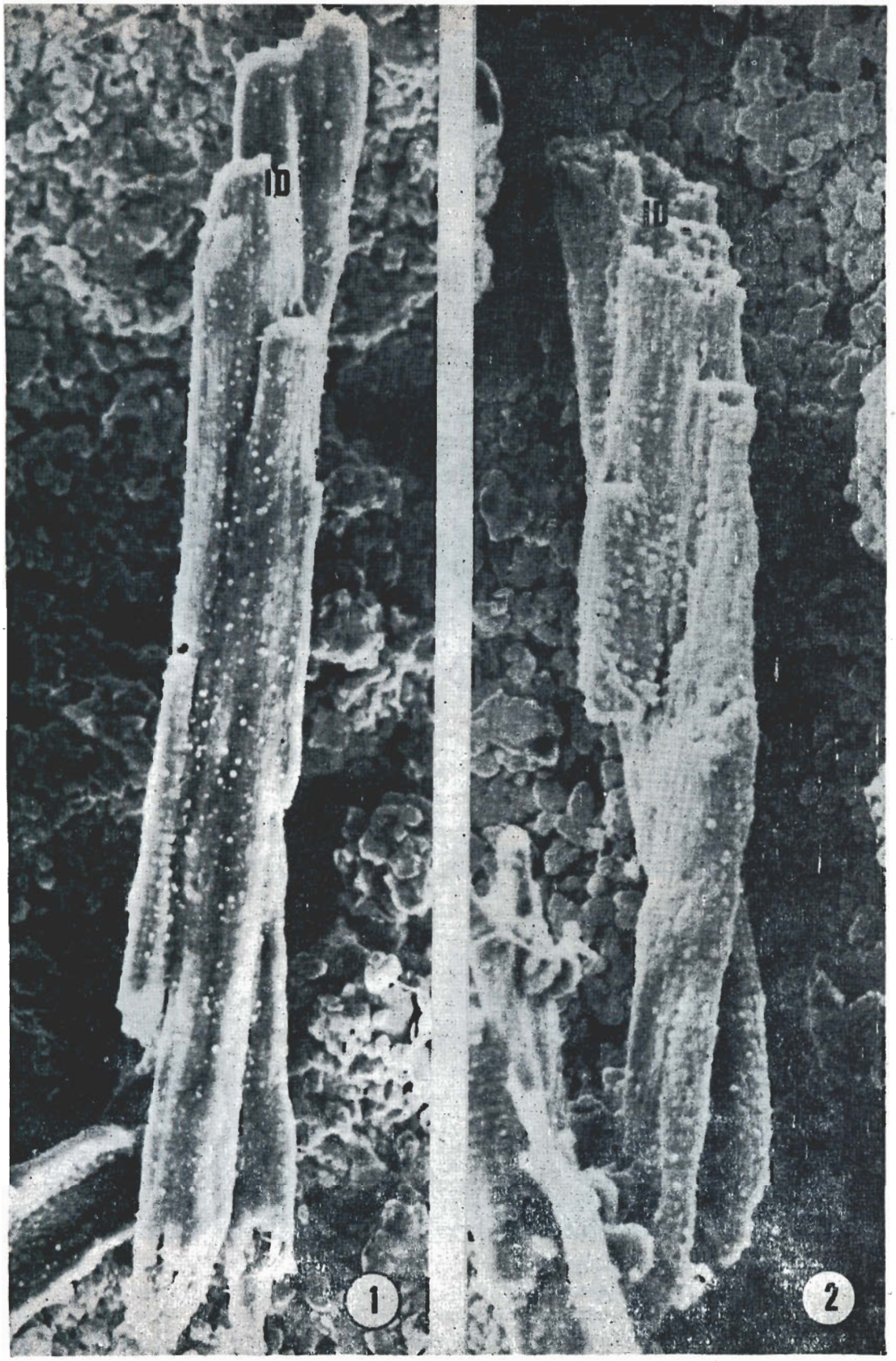
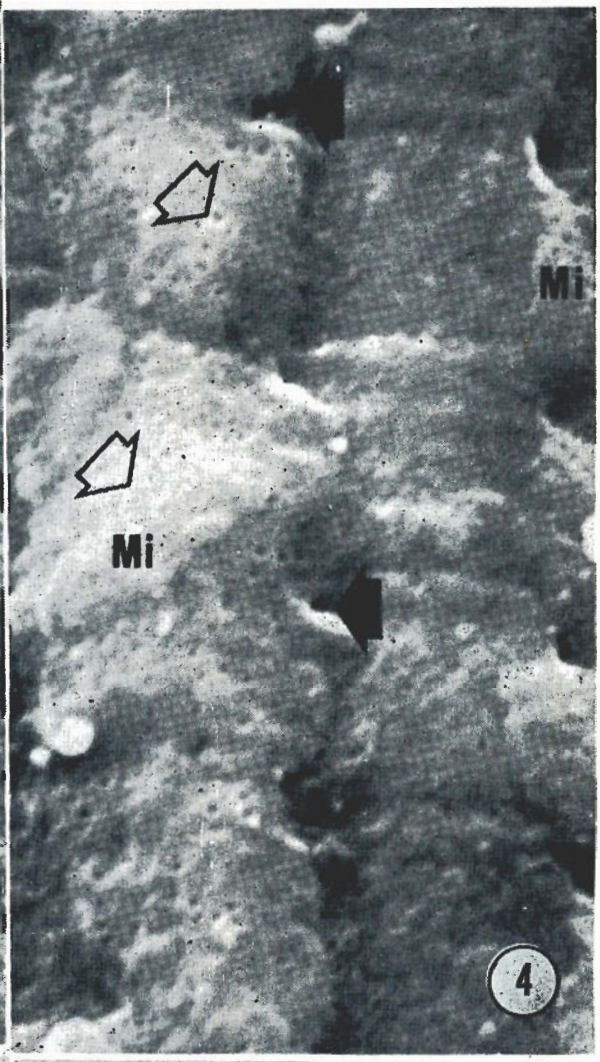


Fig 1. Scanning electron micrograph of dissociated normal hamster cardiomyocyte. The cell shows a straight, almost cylindrical morphology with straight myofibrils. Stepwise intercalated discs (ID) are apparent. ×1180.

Fig 2. Scanning electron micrograph of dissociated cardiomyopathic hamster heart myocyte. The cell has a helical shape and the myofibrils are arranged in a helical configuration. Stepwise intercalated discs (ID) appear normal in morphology. ×1150.



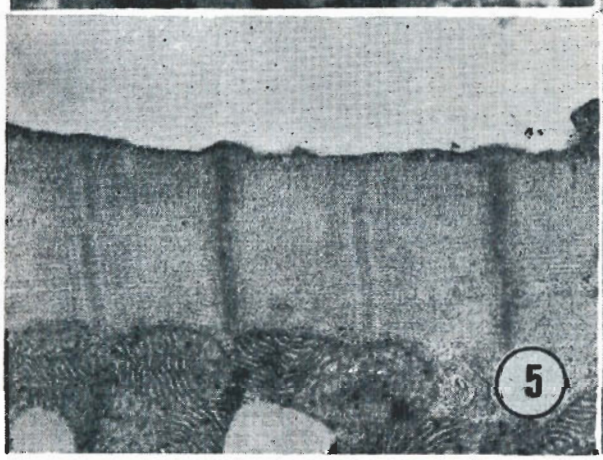


Fig 3-4. Scanning electron micrographs of the surfaces of dissociated myocytes from normal (Fig 3) and cardiomyopathic (Fig 4) hamster hearts. Both show regularly-spaced transverse tubule openings (solid arrows). The CM cell plasma membrane shows numerous surface "pits"(open arrows). The pits seem to be especially numerous over areas of peripherally-located mitochondria (Mi). ×19,000; ×24,000.

Fig 5-6. Transmission electron micrographs of the peripheral portions of dissociated myocytes derived from normal (Fig 5) and cardiomyopathic (Fig 6) hamster hearts. The main difference between normal and CM cells illustrated in these micrographs is the presence of numerous surface vesicles, "pits" (arrows) in the mutant cells (see Fig 3-4 for SEM comparison). ×30,500; ×31,600

photographed in a Hitachi HU-11DS Transmission Electron Microscope using an acceleration voltage 75 kV.

RESULTS AND DISCUSSION

Most of the freshly dissociated myocytes appear cylindrical in shape under the light microscope and were found to be viable using the vital dye (trypan blue) exclusion technique. Length and diameter measurements were performed on 80 normal and 80 CM hamster heart myocytes. The data are summarized in Table 1. No significant difference in size was found between normal and CM cardiomyocytes.

Table 1. Sizes of 80 normal and 80 CM cardiomyocytes. $\bar{x} \pm SD$. The differences are non-significant.

	Length(μm)	Diameter(μm)
Normal	105.6±19.7	20.8±5.0
Cardiomyopathic	102.9±30.8	21.0±4.0

SEM examination of dissociated normal and CM myocytes proved to be very revealing. Most normal cells exhibit straight cylindrical shapes with straight parallel myofibrils(Fig 1). In contrast, the CM cells show aberrant shapes, the most common abnormality being a helical morphology (Fig 2). Some of the CM cells also appear to be more branched than normal. Higher magnification observation in the SEM further shows that the sarcolemma of CM cardiomyocytes contains many more surface pits than normal (Figs 3–4); TEM corroborates this observation by illustrating abnormally high numbers of peripheral vesicles in CM cells (Figs 5–6) This could indicate increased pinocytotic activity in CM cells, perhaps for the movement of ions. This observation is consistent with earlier biochemical reports suggesting cell membrane alterations in CM cells [6]. The CM myocytes also have unusually large aggregations of mitochondria when compared to normal. The transverse-tubule (T-tubule) openings at the surface of normal and CM cells appear similar. Both are distributed in parallel rows at approximately 1.7 μm intervals and follow the distributions of the Z-lines of myofibrils within the cell [7].

Thus, while their size and T-tubule distribution are normal, dissociated CM cardiomyocytes exhibit several morphological features that are substantially different from normal. The most striking abnormality being a helical shape with helically-arranged myofibrils[8]. This abnormal myofibril morphology in itself would seem to provide an explanation for the decreased efficiency of heart contractions in CM hamsters and presumably is related to the observed heart failure in this genetically-based cardiomyopathy. However, the precise mechanism(s) by which CM genes act to cause these abnormalities requires further study.

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