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Histochemistry of Ventricular Heavy-Chain Myosins in Cardiomyopathic Syrian Hamsters Treated with D-600[1] (42719)

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Abstract. Monoclonal antibodies (MAb) have been used to study the distribution of ventricular heavy-chain (HC) myosins in cardiomyopathic UM-X7.1 Syrian hamsters. The Ab were identified as α and β anti-HC myosins because of their ability to cross-react with ventricular V_1 and V_3 myosins, respectively. Cryostat frozen sections from the midventricle region of normal and myopathic hearts were processed for demonstration of these isomyosins by indirect immunofluorescence. In myopathic hearts, there was a shift of predominant α myosin toward the β isoform with the time course of the hamster cardiomyopathy. A D-600 treatment while preventing cardiac necrotic lesions had little or no effect on the β isomyosin conversion. It is inferred that the isomyosin shift during the progression of the hamster cardiomyopathy is unrelated to the necrotizing process and merely reflects the hypokynetism of the cardiomyocytes. © 1988 Society for Experimental Biology and Medicine.

The Syrian hamster hereditary cardiomyopathy is characterized by multi focal ventricular lesions which develop after 30 days of age. The necrotizing process is most prominent between 60 and 90 days and subsides after 120 days. Healing occurs by connective tissue replacement but the residual intact myofibers progressively lose their compliance, and over 50% of the myopathic animals (UM-X7.1 line) die at 250 days of age in a state of cardiac failure (1–3). Little is known on the etiology of this hereditary cardiomyopathy. A defective plasma membrane ion movement with resulting intracellular Ca^{2+} overload has been suggested as the earliest pathologic event but biochemical just as structural changes develop only after 30 days of age (4–10). The prevention of cardiac necrotic lesions by Verapamil or by its methoxy derivative D-600 (2, 11–13) substantiates the pathogenic role of Ca^{2+}, but is at variance with the protection exerted by isoproterenol, a Ca^{2+} entry promoter (14).

In recent years, much attention has been focused on abnormalities in cardiac contractile proteins occurring in primary or in secondary dysfunctional cardiomyopathies (15–17). A progressive shift toward V_3 ventricular HC myosin has been observed during the time course of the hamster cardiomyopathy (18–20). This shift presumably relates to a change in th velocity of contraction of myofibers due to an altered Ca^{2+} activated myosin ATPase with resulting changes in the rate of energy transduction.

By means of immunocytochemistry, using monoclonal antibodies (MAb), we undertook investigating (i) the distribution of ventricular HC myosins in normal and myopathic hearts; (ii) the effect of a D-600 treatment upon the configuration of ventricular isomyosins.

Materials and Methods. *Animals.* Male and female Syrian hamsters, 28 to 30 days of age, from the UM-X7.1 myopathic line and sex-matched healthy controls supplied by Charles River Breeding Laboratories, Canada, were used. They were maintained under controlled housing conditions with free access to Purina Laboratory Chow and tap water. Thirty-six animals were divided as follows: Gr I, 8 untreated healthy controls; Gr II, 8 D-600-treated healthy controls; Gr III, 10 untreated myopathic hamsters; Gr IV, 10 D-600-treated myopathic hamsters. D-600 (Knoll A, G., Ludwigschafen, Germany) was given in a daily dose of 1 mg/kg in

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two separate sc injections. Untreated controls were injected with physiological saline.

At autopsy, hearts were rapidly removed and weighed after the atria were dissected. Transverse ring sections of the midportion (3 mm thick), including both ventricles, were mounted on a cryostat chuck and quenched in Freon 12 cooled with liquid nitrogen. The remaining heart segments were fixed in Lillie's formol sublimate and paraffin sections were stained with hematoxylin–phloxine–saffron (HPS) for routine histology.

The severity of cardiac lesions (coagulation necrosis and myolysis) was assessed following a double blind procedure using a previously described arbitrary scale of 0–3 (3).

Immunocytochemistry. Indirect immunofluorescence was carried out on 6-μm serial sections of the ventricular frozen tissue in 120-day-old hamsters. Sections were mounted on slides and exposed for 30 min in a humid atmosphere at 37°C to appropriate dilutions of two different MAb deriving from hybridomas prepared in mice which were immunized with myosin from human hypertrophic ventricular tissue (21, 22). These MAb have been identified as anti-α or anti-β myosin HC because their ability to cross-react with the V_1 and V_3-type myosins, respectively (21). The sections were then processed with a fluorescein-labeled rabbit anti-mouse immunoglobulin and examined under a Leitz microscope equipped with epifluorescence optics and appropriate filters. The specificity of the reactions was tested with nonimmune serum in the first step.

Assessment of immunolabeled myofibers. In order to evaluate the net proportion of immunolabeled myofibers in cross sections of the ventricular wall, a morphometric analysis was carried out using the Zeiss Zidas point-counting system. Micrographs from different areas of the ventricular wall between the endocardium and the epicardium were taken at 100× magnification. Each heart section required three to four micrographs enclosing a minimum of 200 fibers. Strongly positive fibers were valued 1, weakly positive 0.5, and pseudonegative or negative 0. Following several countings within different fields of the midwall ventricle, the readings were expressed as percentage of immunolabeled fibers.

Results. *Histopathology.* As shown previously, the necrotic changes in UM-X7.1 cardiomyopathic hamsters develop at 40 days of age and persist until 150 days (3). Focal myocardial lesions are scattered throughout the wall of both ventricles; the necrotizing process is either myolytic or coagulative with secondary mineralization and fibroplasia. At 120 days of age lesions often become confluent and most prominent in the septal area (Fig. 1A). The mean severity of necrosis ±SE was estimated at 2.17 ± 0.55 (scale 0–3) with 100% incidence (Gr III). Treatment of myopathic hamsters with D-600 (Gr IV) resulted in complete prevention of heart coagulative necrotic changes (Figs. 1C and 1D). One of the treated animals showed two isolated myolytic foci.

Distribution of ventricular myosins in normal and myopathic hearts. Figure 2 shows the staining pattern of isomyosins in heart sections from normal and myopathic 120-day-old hamsters. The staining reaction in normal heart sections incubated with anti-α myosin MAb (Fig. 2A) was strong and uniform. Over 90% of the cardiac muscle cells were brightly stained with the only exception being fibers proximate to blood vessels and a small population within the papillary muscles. When adjacent sections were exposed to anti-β myosin MAb, however, the reaction was generally dim (Fig. 2B) except for a limited number of fibers in the perivascular area and within the papillary muscle. Strongly positive fibers amounted to 10% and weakly positive to 25%; the reciprocal relationship in the isomyosin reactivity pattern was evident.

When dealing with myopathic heart sections, the percentage of α myosin positive fibers rarely exceeds 60% depending upon the extent of the necrotizing process (Fig. 2C). Obviously, there were more pseudo- or completely negative fibers than those in normal control hearts. Conversely, the proportion of β myosin reacting fibers was rather elevated in adjacent heart sections (Fig. 2D). The amount of strongly or weakly positive cardiac fibers could vary between 60 and 75% depending upon the severity of necrotic changes.

Effect of D-600 treatment upon configuration of isomyosins. As shown in Figs. 3A and 3B, a long-term treatment with D-600 had

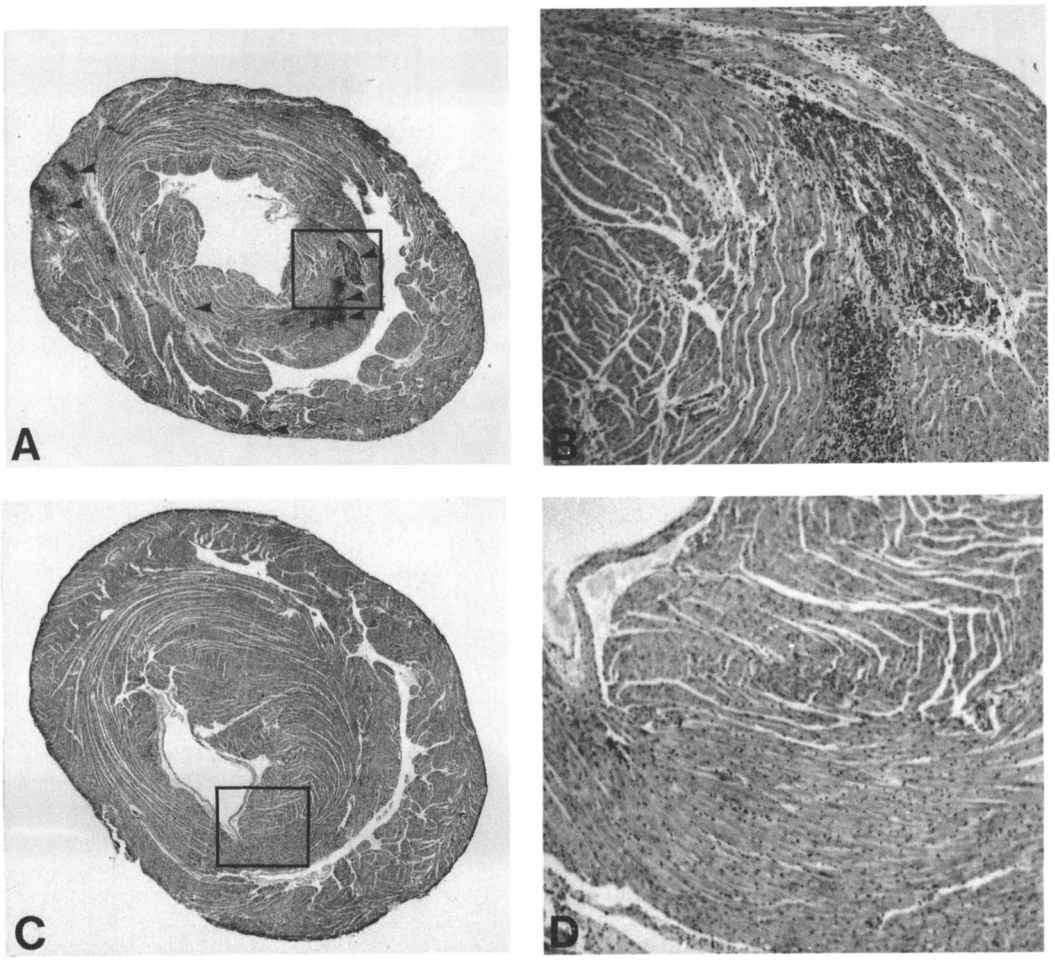

FIG. 1. (A) Microscopic appearance of heart lesions in a midventricle section of a 120-day-old cardiomyopathic hamster. Necrotic foci are scattered throughout the wall of both ventricles (HPS ×8.4). (B) Higher power micrograph illustrating the coagulative and myolytic necrotizing process (HPS ×100). (C) Heart section from a cardiomyopathic hamster treated with D-600. Note the integrity of the entire myocardium (HPS ×8.4). (D) Higher power of the septal ventricular muscle with no evident degenerative changes (HPS ×100).

little effect on the distribution of α- and β-type myosins in the normal myocardium. The reactivity of the myofibers was substantially the same as that observed in untreated hearts with 90% or more α positive fibers (Fig. 3A); reciprocally, the population of β reacting fibers was significantly lower (Fig. 3B), the strongly positive fibers being located nearby small vessels. Following a treatment with the Ca^{2+} entry blocker D-600, 80% of the myopathic heart fibers reacted with α myosin MAb much like the untreated hearts (Fig. 3C); the shift toward β myosin, however, was unchanged as evidenced by a strong reaction to β-specific MAb (Fig. 3D).

Discussion. The present histochemical observations demonstrate well that the changes in anatomical distribution of ventricular HC myosins in cardiomyopathic hamsters are quite consistent with previous electrophoretic findings (18–20). The reactivity to MAb does not necessarily reflect the absolute amount of myosin in each respective cardiocyte but merely illustrates the degeneration process as it occurs in diseased hearts. Although these studies were made on serial

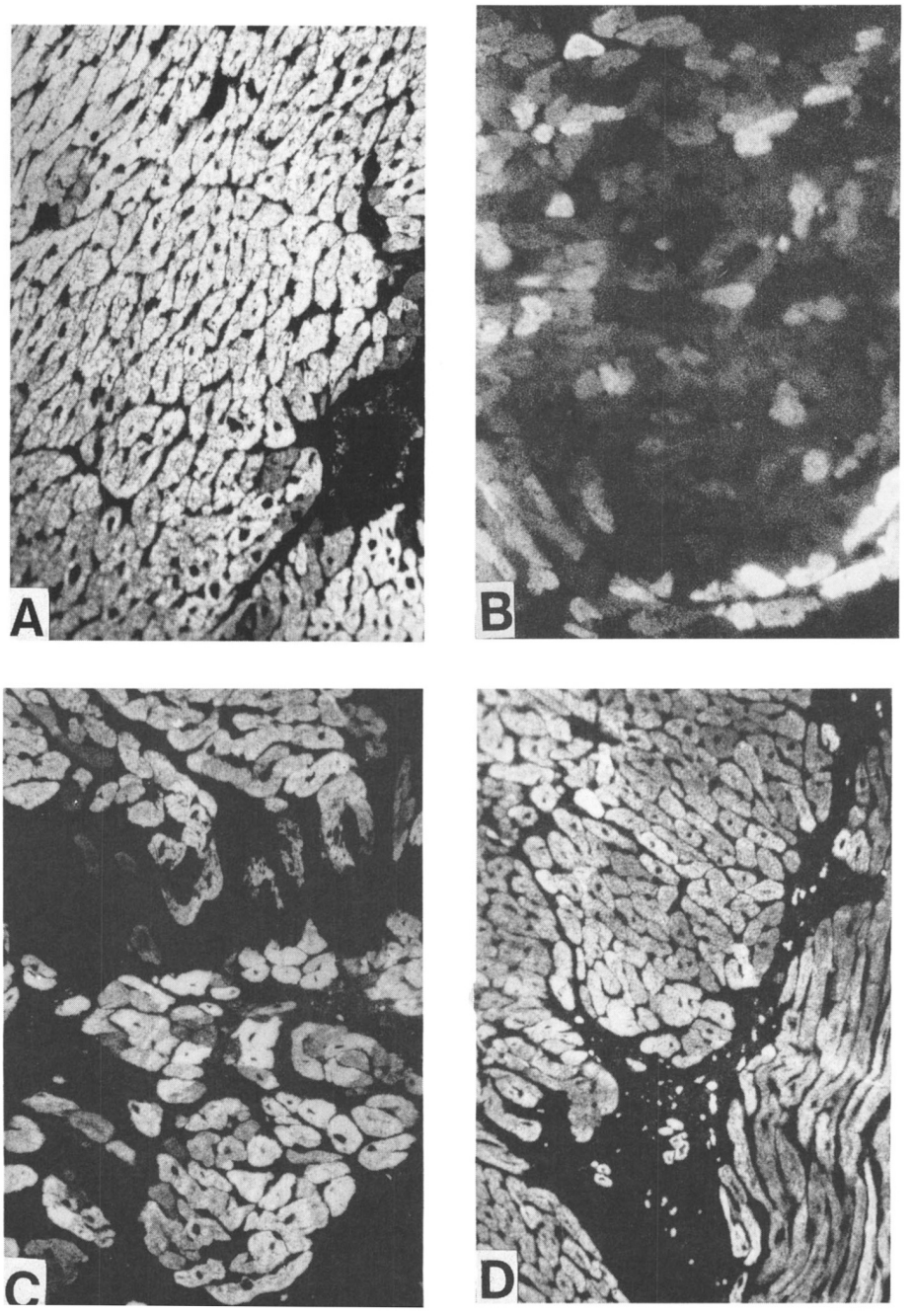

FIG. 2. Frozen sections from the midventricle processed for indirect immunofluorescence in 120-day-old normal and cardiomyopathic hamsters (×120). (A) Normal heart exposed to α MAb. The reaction is strong and uniform except for few fibers near the blood vessels. (B) Normal heart exposed to β MAb. The majority of fibers are negative or weakly positive except in perivascular areas. (C) Myopathic heart exposed to α MAb. Intact fibers display a gradation between strong and weak reaction. Necrotic areas are negative. (D) Myopathic heart exposed to β MAb. The intensity of the staining reaction is strong compared to a normal heart.

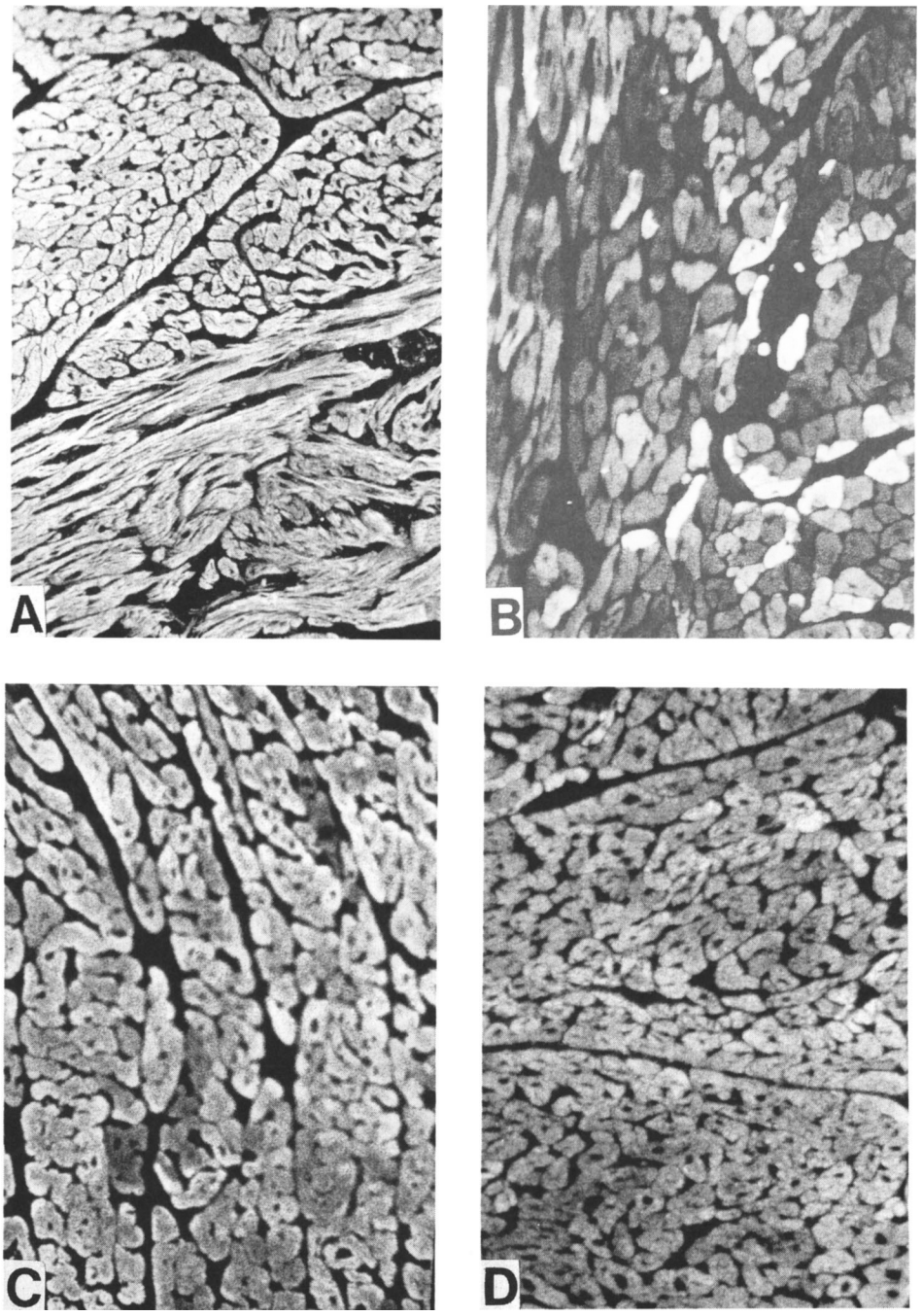

FIG. 3. Frozen sections from the midventricle processed for indirect immunofluorescence in 120-day-old normal and cardiomyopathic hamsters treated with D-600. In normal treated hearts, the reactivity of fibers to anti-α myosin (A) is strong and uniform but heterogeneous with anti-β myosin (B). In myopathic treated hearts, the morphology of fibers is well preserved; they intensely react to α myosin (C) except for a few isolated fibers. The same treated hearts, however, remain positive to anti-β myosin (D).

sections, different fields of immunolabeled fibers have been selected to obtain better focusing.

It has been suggested that the isomyosin profile in skeletal or cardiac muscle cells relates to the velocity of shortening (15, 16), the activity of myosin ATPase (24), oxygen consumption (25), neuroendocrine status (26, 27), or the relative amount of Ca^{2+} regulating membranes within a contractile cell (23). In hamster myopathic hearts, the conversion of ventricular HC myosin toward a predominant β isoform cannot be solely ascribed to the necrotizing process since the myosin shift is equally demonstrable in protected hearts following D-600 treatment. According to Malhotra *et al.* (18), several pathologic components other than a disturbed Ca^{2+} metabolism may be primarily involved in the pathogenesis of the hamster cardiomyopathy. Much of our past and recent findings agree with this understanding (27–29). Hence, the enzyme shifts in the myocardium could be associated with changes in synthesis or degradation of HC myosins as influenced by the thyroid state (30, 31). The cardiomyopathic hamster hearts on the other hand prove to be singularly resistant to catecholamine (14, 32) as well as to thyroxine treatment (33). Since each isomyosin is encoded by its own gene (34), one can easily anticipate that the regulation of gene expression in hamster hereditary cardiomyopathy is fairly complex. In the light of the present study, it all seems that the myosin shift is an adaptive mechanism unrelated to the necrotizing process.

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