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Myocardial cell heterogeneity in the human heart with respect to myosin ATPase activity

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Summary

The pH sensitivity of the Ca^{2+}-activated myosin ATPase in atrial, ventricular and conduction tissue of human hearts has been established. Heterogeneity with respect to ATPase activity is shown not only to exist between the atrial, the ventricular myocardium and the conduction system but also *within* both the ordinary atrial and ventricular myocardium and *within* the conduction system. These observations are related to the polymorphism of the myosin molecule and suggest that fibre types with different contractile properties co-exist in the human heart.

Introduction

Recent biochemical and immunohistochemical studies have conclusively shown that atrial and ventricular myosin in mammalian hearts have a different composition (Sartore *et al.*, 1978, 1981; Dalla Libera *et al.*, 1979; Syrovy *et al.*, 1979; Wikman-Coeffelt & Srivastava, 1979; Schiaffino *et al.*, 1980; Whalen *et al.*, 1981). Furthermore, the activity of the Ca^{2+}-activated ATPase of atrial myosin is higher than that of ventricular myosin (Long *et al.*, 1977; Flink *et al.*, 1978; Yazaki *et al.*, 1979). The correlation between ATPase activity and myocardial function has recently been reviewed (Scheuer & Bhan, 1979). Myosin Ca^{2+}-activated ATPase activity has been related to velocity of muscle shortening in skeletal (Barany, 1967) and cardiac (Carey *et al.*, 1979) muscle and the shortening velocity has been shown to be faster in atrial than in ventricular fibres (Urthaler *et al.*, 1975, 1978).

With histochemical techniques it is possible to reflect myosin ATPase activity. Histochemical typing of slow and fast skeletal muscle fibres is largely based on the different activity and pH sensitivity of the slow and fast myosin ATPases (Brooke & Kaiser, 1970; Guth & Samaha, 1970). The ATPase reaction at pH 9.4 is most widely employed (cf. Dubowitz & Brooke, 1973) and has been validated by comparing the histochemical properties of the enzyme with the biochemical properties of the purified actomyosin (Guth & Samaha, 1969; Mabuchi & Sreter, 1980) and by the use of specific

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anti-slow and anti-fast myosin antibodies (Lutz *et al.*, 1978, 1979; Gauthier & Lowey, 1979; Billeter *et al.*, 1980).

The standard myosin ATPase reaction at pH 9.4 has also been used on cardiac muscle. Morales & Fine (1965) observed that the human atrial and nodal tissues exhibited higher activity than the ventricular fibres. Elias *et al.* (1980), on the other hand, noted no difference between ventricular and conducting fibres. However, they incubated their sections at pH 6.7 according to a method described by Meijer (1970). Morkin *et al.* (1977) showed an increased myosin ATPase activity upon thyroxin treatment in rat. These authors also performed control experiments to establish that the ATPase activity was myofibrillar and not mitochondrial in origin. Thornell *et al.* (1978) showed with the standard ATPase reaction that the activity in Purkinje fibres was higher than in the ordinary ventricular myocardium in the bovine heart. Further, it was shown that the ATPase activity of Purkinje fibres was more acid stable than that of the ordinary myocardial cells. The difference in ATPase activity and acid stability was correlated to differences in the composition of the myosin light chains of Purkinje fibres and ordinary myocardium (Thornell *et al.*, 1978). This difference between Purkinje fibre and ordinary myocardial myosin has recently been confirmed on the basis of more elaborate biochemical techniques as two-dimensional gel electrophoresis and peptide mapping (Whalen *et al.*, 1980, 1982).

The aim of the present study was to examine a possible variability of human cardiac muscle cells with respect to Ca^{2+}-activated ATPase activity. Further, the optimal pH to obtain maximal differences in enzyme activity between atrial and ventricular myocardium has been established.

Heterogeneity with respect to ATPase activity is shown to exist not only between the conduction system and the atrial and the ventricular myocardium but also *within* both the ordinary atrial and ventricular myocardium and *within* the conduction system.

Materials and methods

Selective areas of human hearts of previously healthy subjects, aged 20–30 years and who died sudden violent deaths, were removed within 48 h after death. Previous studies have shown that the myosin ATPase activity is not reduced at that time after death (Swynghedauw *et al.*, 1971; Klotz *et al.*, 1975). Tissue blocks containing the upper part of the interventricular septum and the lower part of the interatrial septum and blocks composed of atrial tissue, ventricular tissue and moderator bands were rapidly frozen by immersion in isopentane, pre-cooled with liquid nitrogen. Serial sections about 10 μm thick were cut in a Ditte cryostat.

Series of sections were stained for myofibrillar ATPase (myosin adenosine triphosphatase, Ca^{2+}-activated, EC 3.6.1.3) according to Brooke & Kaiser (1970) with an initial pre-incubation in a sodium barbital acetate buffer at pH 4.0, 4.2, 4.4, 4.6, 4.8 or in a 100 mM 2-amino-2-methyl-1-propanol buffer (containing 18 mM $CaCl_2$) at pH 10.2, 10.4, 10.6, 10.8 or 11.0 for 5 min at room temperature. After evaluation of these series of sections further sections were incubated at selected pH levels (4.2, 4.3, 4.4 and 10.6, 10.7 and 10.8). Some sections were fixed in 1% formaldehyde in a sodium cacodylate buffer for 30 or 60 s or were pre-washed in Triton X-100 for 5 min before staining for ATPase at pH 9.4. Other sections were incubated with

antibodies against the intermediate filament protein skeletin (Eriksson *et al.*, 1979). The sections were examined under a Leitz Dialux 20 Photomicroscope.

Results

ATPASE ACTIVITY IN ORDINARY ATRIAL AND VENTRICULAR MYOCARDIUM

In sections stained for the routine myofibrillar ATPase no marked differences in activity were seen between atrial and ventricular cells. (Fig. 1A).

Effects of formaldehyde fixation

After a short formaldehyde fixation, the ventricles showed no ATPase activity while atrial cells still exhibited activity (Fig. 1B). In high magnification the atrial cells showed a heterogeneous activity (Fig. 4).

Effects of acid pre-incubation

A marked inhibition of the ATPase activity in both atria and ventricles was seen at pH 4.0 (Fig. 1C). At pH 4.2 and 4.3 there was an apparent difference in activity between atrial and ventricular tissue (Fig. 1D). The inhibition of activity being most evident in the atria (Fig. 1D). In high magnification it was moreover obvious that the atrial cells showed a heterogeneous activity (Figs. 2D, 4D). At pH 4.4, 4.6 and 4.8, and up to neutral pH on the other hand, no difference in activity in and between atria and ventricles was seen (not illustrated). Vessels and capillaries were easily distinguished and showed high activity in sections incubated at pH 4.3 and below (Figs. 1–4).

Effects of alkaline pre-incubation

A marked inhibition of the ATPase activity in the ventricular myocardium was seen above pH 10.6 (Fig. 1E) while the atrial ATPase activity was inhibited only above

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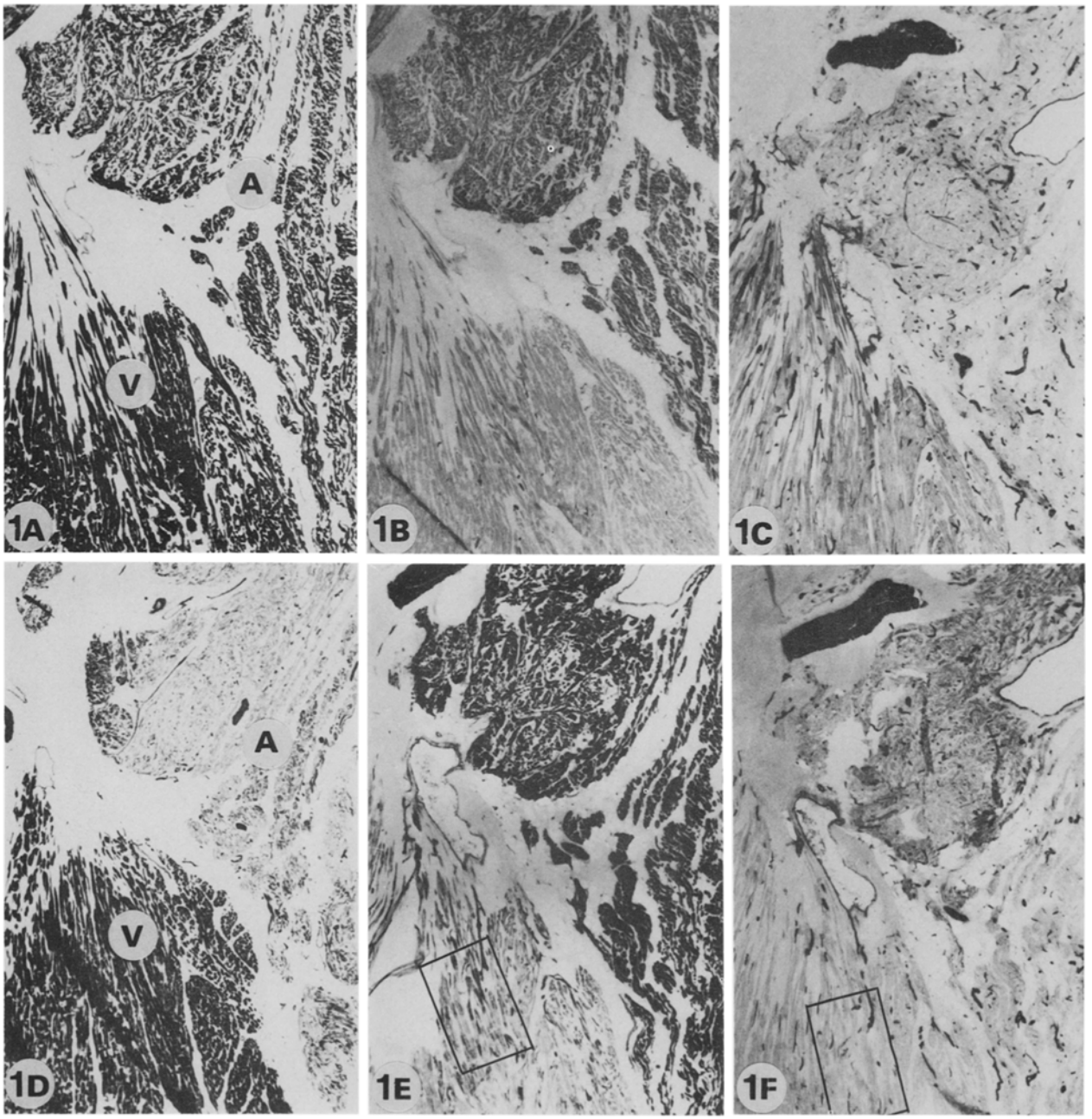
Fig. 1. Cryostat serial sections of upper part of the ventricular septum (*V*) and the lower part of the atrial septum (*A*). (A) Section stained with the standard ATPase procedure at pH 9.4. (B) Section fixed with formaldehyde 30 s prior to staining with the standard ATPase procedure at pH 9.4. (C–F) Sections pre-incubated at acid or alkaline pH before staining for ATPase at pH 9.4: (C) pH 4.0; (D) pH 4.2; (E) pH 10.6; and (F) pH 10.8. × 25.

Fig. 2. (A) Higher magnification of indicated area in Fig. 1F. The myocardial ATPase is totally inhibited at pH 10.8. Vessels show strong activity (arrows). (B) High magnification of indicated area in Fig. 1E. Myocardial heterogeneity with respect to ATPase activity is seen in the ventricular myocardium. Some areas with high ATPase activity correspond to vessels (arrows). (C) High magnification of ventricular myocardial cells incubated at pH 10.6. Note that the ATPase activity is confined to the myofibrils and that a distinct transverse band-pattern can be seen. Some cells show strong activity, others weak activity. Note the distinct shift in activity between individual cells at intercalated discs (arrows). (D) Atrial myofibres in high magnification. Section pre-incubated at pH 4.2. Some cells show strong activity, others weak activity. Note the distinct shift in activity between individual cells at intercalated discs (arrows). The ATPase activity is confined to the myofibrils. (A), (B), × 100; (C), × 700; (D), × 670.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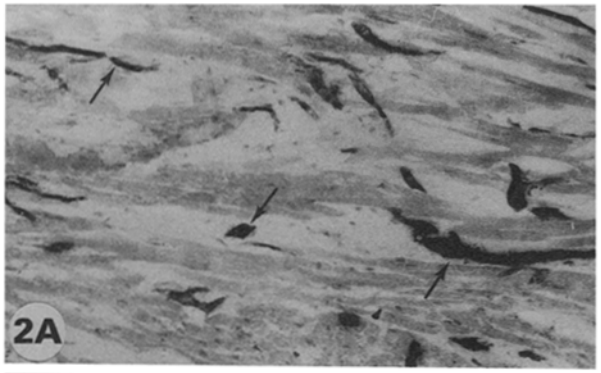
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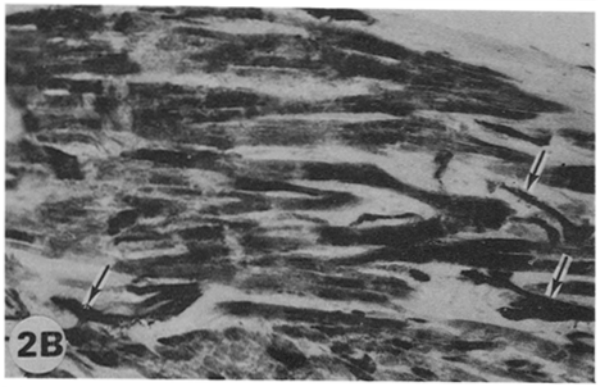
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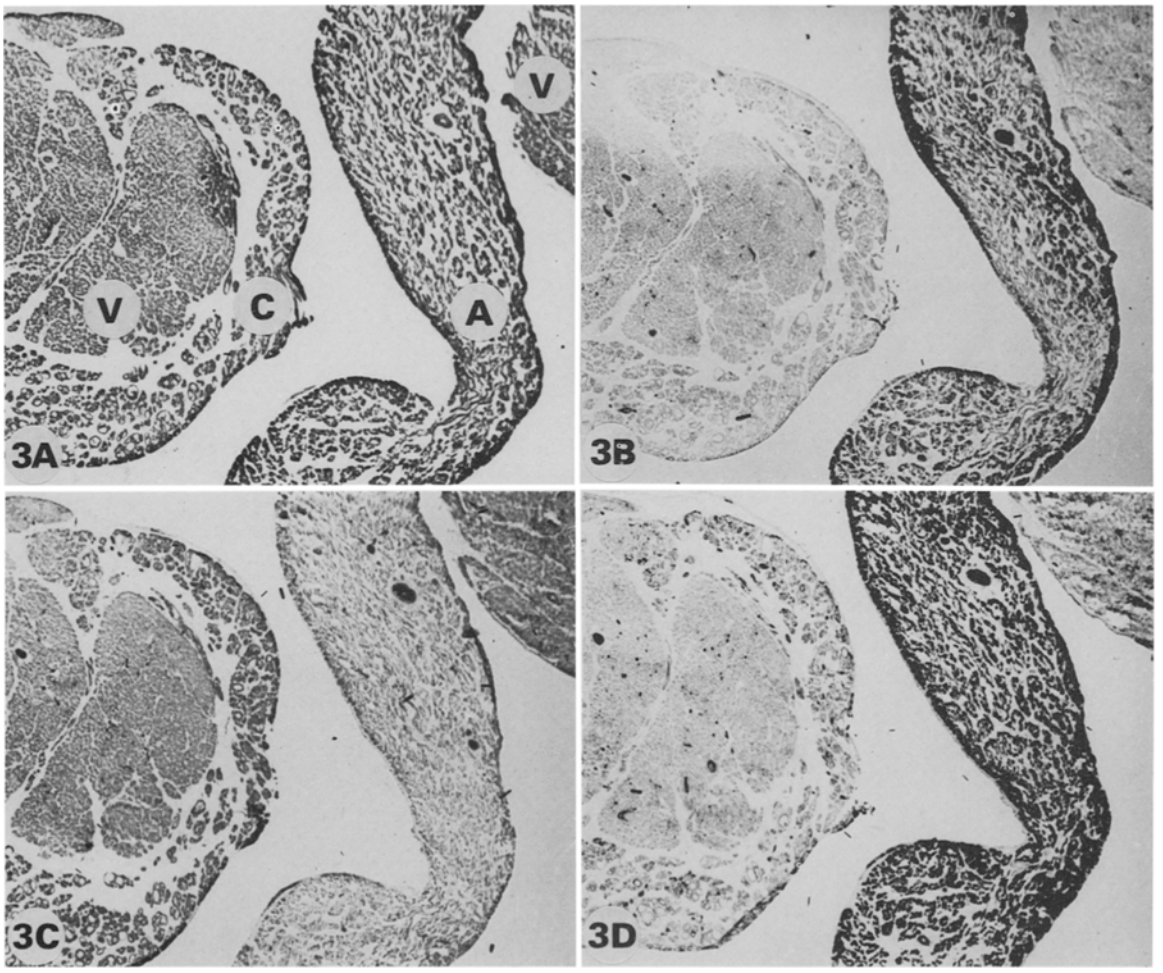


Fig. 3. Cryostat serial sections of blocks composed of the moderator band with ordinary ventricular myocytes (*V*) and conduction cells (*C*), atrial (*A*) and ventricular (*V*) tissue. (A) Standard ATPase procedure, pH 9.4. (B) Formaldehyde fixation prior to ATPase procedure, pH 9.4. (C) Acid pre-incubation, pH 4.2. (D) Alkaline pre-incubation, pH 10.6. Note the heterogeneity in ATPase activity for the conduction, ordinary ventricular and atrial cells in (B), (C) and (D). Blood vessels show high activity in all sections. × 25.

pH 10.8 (Fig. 1F). Vessels and capillaries on the other hand showed high activity at that pH (Figs. 1F, 2A). In sections incubated at pH 10.6 it was seen in high magnification that some individual ventricular myocardial fibres in the upper part of the ventricular septum still exhibited high activity (Fig. 2B, C).

In all sections in which some activity was seen it was evident that the staining product was confined to the myofibrils. A distinct cross-striation pattern was clearly seen in high magnifications (Fig. 2C, D). A marked intermyofibrillar staining was never seen.

ATPASE ACTIVITY IN THE CONDUCTION CELLS OF THE MODERATOR BAND

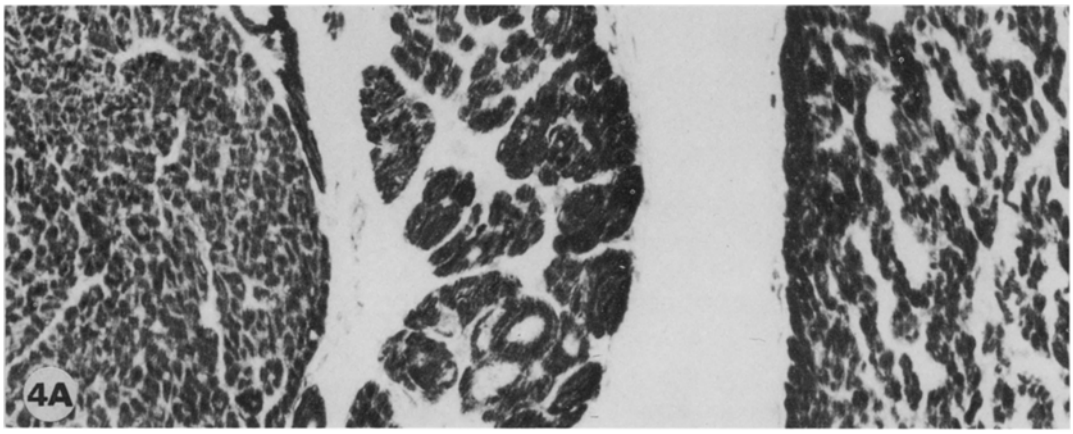
The conduction cells, distinguished by their morphology and their higher reactivity with antibodies against skeletin than ordinary myocardial cells (Eriksson *et al.*, 1979; Thornell & Eriksson, 1981), showed the same activity as the ordinary ventricular myocardial cells in sections stained for standard ATPase (Figs. 3A, 4A). After a short formaldehyde fixation most conduction cells showed inhibited activity as did the ordinary ventricular cells (Figs. 3B, 4B). At pH 4.2, fibre heterogeneity with respect to ATPase activity was apparent. Some conduction cells showed higher activity than did both the ordinary ventricular and the atrial myocytes (Figs. 3C, 4C). After pre-incubation at pH 10.6, some Purkinje fibres showed inhibited ATPase activity as did ordinary ventricular myocytes, others showed an activity identical to that of most atrial myocytes (Figs. 3D, 4D).

Discussion

This study shows that the adult human heart is composed of muscle cells with heterogeneous ATPase activity as seen in sections after acid and alkaline pre-incubation or formaldehyde fixation. Heterogeneity is shown to exist not only between the ordinary atrial and ventricular myocardium but also to exist within the atrial and within the ventricular population of the ordinary muscle cells. Further, it is also evident that the ATPase in the conduction cells in the moderator band shows a different sensitivity to acid and alkaline pre-incubation than do the ATPases of the ordinary atrial and ventricular myocytes.

Several different ATPases are present in myocardial cells. The present method used for demonstrating ATPase activity is generally accepted to reveal the Ca^{2+}-activated myosin ATPase. Arguments for possible contribution of other ATPases as the SR-ATPase and especially the mitochondrial ATPase have been put forward (Guth, 1973; Samaha & Yunis, 1973). A possible contribution of these ATPases in the present study cannot be ruled out. However, the ATPase activity was confined to the myofibrils and no activity was seen in regions rich in mitochondria nor was the staining pattern effected by a pre-wash in Triton X-100 which solubilizes membranes (McFarland & Inesi, 1971; Schiaffino & Pierobon-Bormioli, 1973). Thus the heterogeneous ATPase activity shown here is likely to reflect differences in the myosin composition of the cardiac muscle cells. Our results are – with respect to differences in ATPase activity between atrial and ventricular myocardium – in agreement with previous results on the biochemical myosin ATPase activity. Yazaki *et al.* (1979) have reported that the ATPase activity of ventricular myosin is more labile in alkaline environment than that of atrial myosin. A good correlation between the histochemical pH profile of myosin ATPase staining and the biochemical pH profile has also been shown for skeletal and cardiac muscles in birds (Talesara & Goldspink, 1978).

Of special interest in the present study is the finding that the human heart shows heterogeneity in composition for all three types of myocytes: the atrial, the ventricular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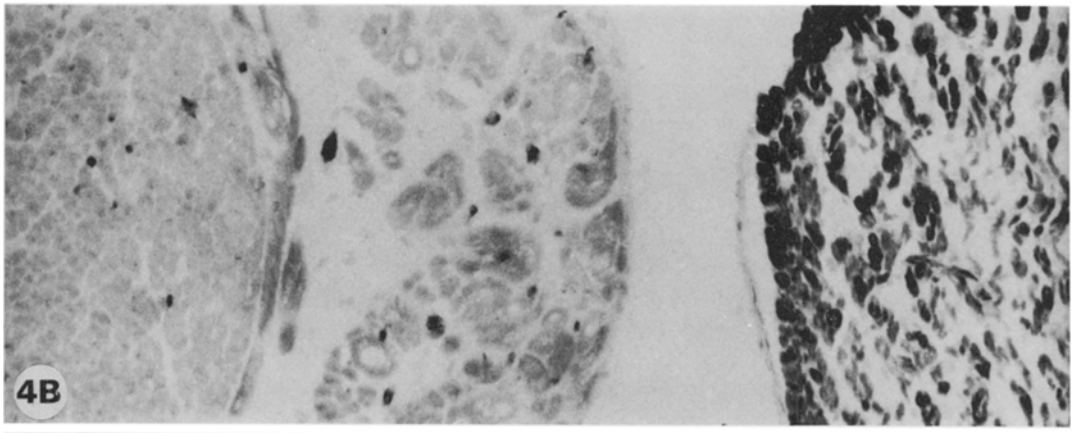
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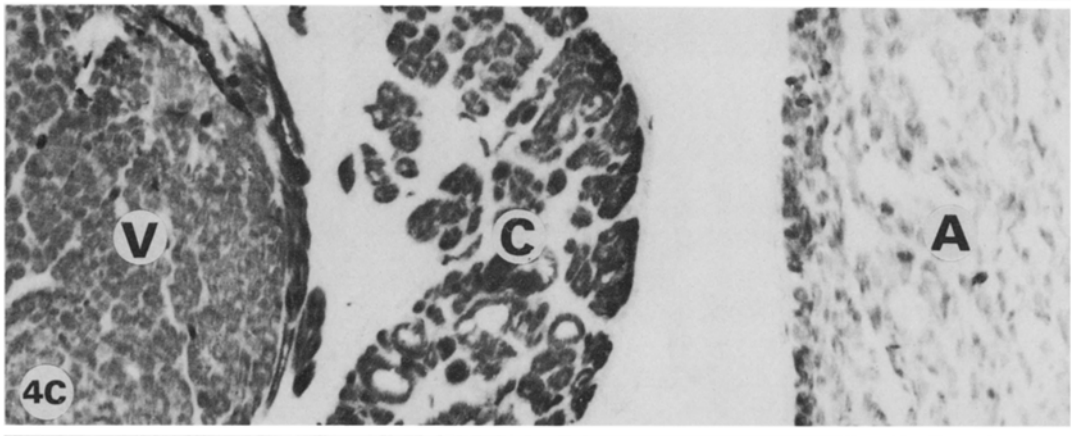
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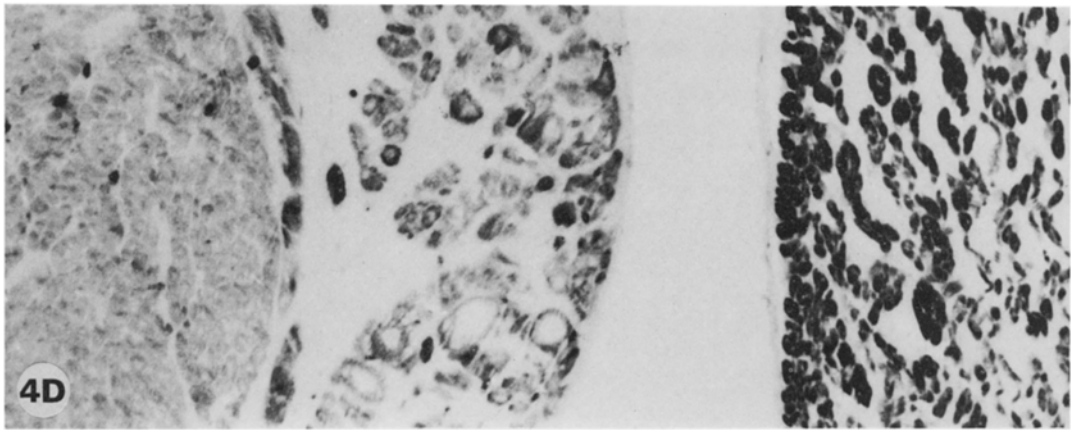
4D

and the conduction tissue. Price *et al.* (1980a,b) have reported that the myosin light chain subunits in the atria and ventricles of adult human hearts were different when characterized by isoelectric focusing and two-dimensional polyacrylamide gel electrophoresis. No differences were observed, however, between light chain subunits in the right and the left ventricles (Price *et al.*, 1980a). Myosin in the atria and particularly the left atrium, on the other hand, contained components similar to the ventricular light chain components (Price *et al.*, 1980b). The findings of both atrial and ventrical components in atria might be in accordance with the heterogeneity within atrial tissue reported herein. However, further studies are needed to determine the precise nature of the ventricular light chain components in the atria and their influence on the ATPase activity. The absence of heterogeneity of myosin light chains in the ventricles in the studies of Price *et al.* might reflect a low percentage of heterogeneous cells in the ventricles or to be a question of how the material was sampled. The main difference between the myocytes of different ATPase activity and pH sensitivity is most likely to be due to differences in their myosin heavy chains.

From recent biochemical and immunohistochemical studies on hearts of several species, it seems likely that heterogeneity of cardiac muscle fibres with respect to the myosin composition is a general property of the mammalian heart (Sartore *et al.*, 1981). Direct evidence for myosin polymorphism in cardiac myofibres was reported by Hoh *et al.* (1978) in an electrophoretic study on intact myosin from rat and by Sartore *et al.* (1978) in an immunomicroscopic study by use of antimyosin antibodies. Sartore *et al.* (1981) have recently shown, by use of specific antiserum against bovine atrial myosin in combination with the indirect and direct immunofluorescence technique, a marked myosin heterogeneity in both the working ventricular myocardium and in the Purkinje fibre system of the bovine and of the rabbit heart. Sartore *et al.* (1981) also reported a great variability in staining between species and that the staining reactivity was related to the developmental and the functional state of the heart, clearly indicating that fibre types with different contractile properties may indeed co-exist in the ventricular myocardium.

No immunohistochemical studies on myosin heterogeneity in the human heart have so far been published. However, muscle cell heterogeneity in the human heart can also be demonstrated with specific antimyosin antibodies (Schiaffino & Gorza, personal communication). Collaborative work to correlate ATPase activity and antimyosin staining is now in progress.

Fig. 4. Higher magnification of parts of the sections shown in Fig. 3. (A) Standard ATPase procedure, pH 9.4. (B) Formaldehyde fixation prior to ATPase procedure pH 9.4. (C) Acid pre-incubation, pH 4.2. (D) Alkaline pre-incubation, pH 10.6. Ordinary ventricular (*V*), conduction (C) and atrial (*A*) cells respectively. Note the heterogeneity of ATPase staining within the conduction cells and within the atrial cells. × 130.

Acknowledgements

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