Heterogeneity of Slow Skeletal Myosin Heavy Chain in Foetal Rat Skeletal Muscles

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Abstract:
A monoclonal antibody (M14) was prepared to human skeletal muscle myosin that recognises heavy chain subunit of slow skeletal and cardiac muscle type myosins. This antibody recognised all the slow skeletal muscle fibre types in adult human, rabbit and rat skeletal muscles. The distribution pattern of muscle cells stained by antibody M14 in the post-hatch chicken skeletal muscles was generally similar to SM1 myosin heavy chain. It also stained all the myocardial cells in mammalian ventricle but only a proportion of the cells in the atrium. Unlike the adult mammalian skeletal muscles, the staining pattern of 18 day foetal rat limb skeletal muscles with antibody M14 appeared markedly different and much more restricted than the distribution pattern detected by conventional slow skeletal muscle type myosin heavy chain antibodies. Antibody M14 thus demonstrated heterogeneity of slow skeletal muscle myosin heavy chain in mammalian foetal skeletal muscles. The distribution pattern of M14 antibody positive myotubes in 12 day chicken embryonic pectoral muscle was also relatively more restricted than SM1 myosin heavy chain.
Keywords: slow, myosin heavy chain, developing, adult, muscle.

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Myosin is a major contractile protein of striated muscle. It is a hexameric protein molecule composed of two heavy and four light chains. Actin binding site and ATPase activity lie in the heavy chain subunit of myosin making it an important part of the molecule. Myosin is encoded by a multigene family with its both heavy and light chain subunits existing in a number of isoforms [14]. Two myosin heavy chains, α and ß, have been described in cardiac muscle [10]. The presence of three fast muscle type myosin heavy chains IIA, IIB and IIX, has been reported in limb skeletal muscles [14, 17]. Only a single myosin heavy chain (MHC) type, however, has been described in mammalian slow skeletal muscles. Furthermore, the slow skeletal muscle type MHC in mammals is believed to be encoded by the same gene as that for cardiac muscle type (ß) MHC [11] although an additional minor slow tonic muscle-type MHC associated with intrafusal and some tonic muscle fibres in specialised muscles like extraocular and tensor tympani, has been reported [12, 15, 16].

Unlike the mammals, the cardiac ß MHC in the avian species is different from the slow skeletal muscle type myosin heavy chain [18,20] which in the chicken has been shown to be composed of two types, namely SM1 and SM2 [13]. These two slow skeletal muscle specific myosin heavy chains are recognisable by both electrophoretic and immunochemical methods and their relative proportions change during development [7, 8]. Using the slow MHC specific antibodies, the onset of muscle fibre diversity in avian species has consequently been detected much earlier [2, 3, 19, 22] than that compared with mammals. The present study was undertaken to determine if heterogeneity of slow myosin heavy chain also existed in mammalian slow twitch muscle fibres which would provide more specific markers of muscle cell differentiation during development.

Materials and Methods

Myosin was purified from human or rabbit skeletal muscles as described previously [5]. For enzyme linked immu-

noassays, some of the myosin samples e.g. porcine cardiac myosin and rabbit skeletal muscle myosin light chains were purchased from Sigma Chemical Company.

Antibody M14 was selected from hybridomas produced by fusing the spleen cells from mice immunised with human skeletal muscle myosin using the methods described previously [5]. The specificity of this antibody for myosin was tested by enzyme-linked immunoassay [9]. Its specificity was further tested by staining Western blots of different muscle myosins separated in pyrophosphate gels. The immunologic reaction of gel-separated native myosin isoforms with M14 antibody was determined as previously described [1]. The preparation and specificities of other MHC antibodies (96J, 9812 and F2) used in this study have already been described [5, 21, 22].

For immunocytochemistry, 6 μ thick serial sections were stained with different antibodies using immunoperoxidase procedure described previously [3]. Some serial sections were also stained for acid-stable myosin ATPase (pH 4.3) as described by Dubowitz and Brooke [6].

Results

A. Specificity of monoclonal antibody M14

The specificity of monoclonal antibody M14 was tested by enzyme-linked immunosorbent assays and the staining of Western blots of different myosins separated in pyrophosphate gels. The enzyme linked immunosorbent assay showed a positive reaction with myosin purified from mixed human and rabbit slow skeletal muscles but little or no reaction with myosin isolated from predominantly fast rabbit skeletal muscles like Extensor digitorum longus (EDL) or Tibialis anterior (TA) (Fig. 1). The myosin purified from rabbit or porcine hearts also reacted with this antibody (Fig. 1). No reaction, however, was observed with myosin light chains fractionated from either mixed human and rabbit skeletal or cardiac muscles. This antibody therefore appeared to be directed against the heavy chain subunit of myosin. The specificity of antibody M14 for cardiac and slow skeletal muscle type myosins was further confirmed by its staining of native proteins on pyrophosphate gels (Fig. 2). It did not react with the fast skeletal isoforms of myosin.

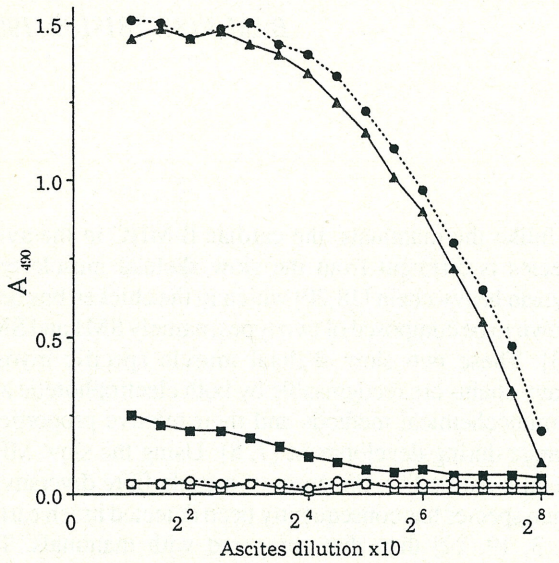

Figure 1. Reaction of antibody M14 with different myosins and myosin light chain subunits using enzyme linked immunosorbent assay. Solid circles represent reaction with human skeletal muscle myosin; hollow circles, light chains of human skeletal muscle myosin; solid rectangles, myosin from rabbit EDL muscle; hollow rectangles, light chains from rabbit cardiac muscle; solid triangles, porcine cardiac myosin.

B. Staining patterns of some adult striated muscles with antibody M14

Antibody M14 in an immunocytochemical investigation stained only a proportion of the cells in adult human skeletal muscles. The reaction of adult rat EDL muscle with this antibody was restricted to a very small proportion of muscle cells (not shown). A large majority of the fibres, however, stained with this antibody in rat soleus muscle

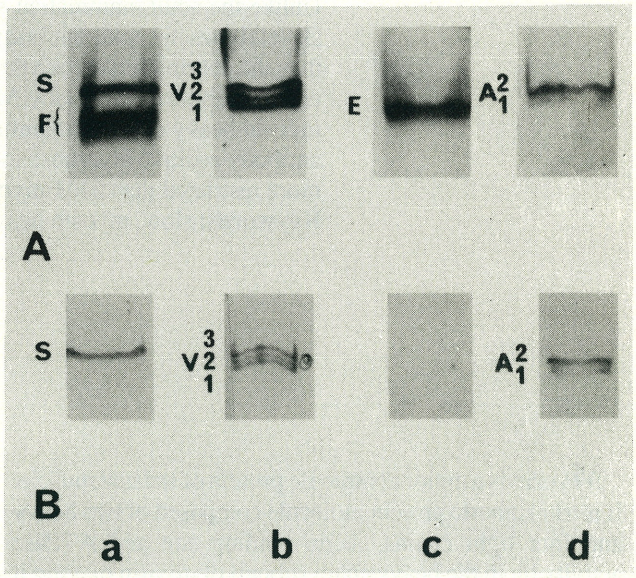

Figure 2. Specificity of antibody M14 using electrophoresis of native myosins. Myosin isoforms were separated by electrophoresis in pyrophosphate gels under nondissociating conditions. A, protein stain; B, Western blot stained with antibody M14 by immunoperoxidase procedure. Samples: a, rat diaphragm containing slow (S) and fast (F) myosins; b, rat ventricle containing ventricular V1, V2 and V3 myosins; c, rat embryonic leg muscles containing embryonic (E) myosins; d, rabbit atria containing atrial A1 and A2 myosins. Antibody M14 stained all the cardiac and slow skeletal myosins but none of the fast myosin isozymes. Due to low level expression of slow myosin detectable by antibody M14, it did not show up any staining of embryonic myosin.

(not shown). This antibody therefore appeared to recognise a myosin heavy chain associated with slow skeletal muscle fibres. The identity of this myosin heavy chain with slow MHC was further indicated by its correlation with the distribution patterns observed by two previously characterised slow MHC antibodies 96J and 9812 [5]. Its specificity for slow MHC was also apparent from its correlation with histochemical myosin ATPase reaction after acid preincubation at pH 4.3 (Fig. 3). For example, all the cells stained with antibody M14 stained dark with antibodies 96J, 9812 and histochemical myosin ATPase after acid preincubation at pH 4.3 (Fig. 3).

Although antibody M14 was raised to human skeletal muscle myosin, it also cross-reacted with some slow skeletal MHC of chicken skeletal muscles. Its staining pattern of post-hatch chicken skeletal muscles appeared generally similar to that observed with antibodies 96J (Fig 3D-F) and 9812 (not shown) specific for SM1 MHC in this species. The intermediate fibres, that showed a lower level of staining with antibodies 96J or 9812 usually did not stain significantly with antibody M14 or were comparatively lighter in their staining intensity (Figure 4).

Smooth muscle cells did not stain with antibody M14 (Fig. 5A). All the myocardial cells in the rabbit and rat ventricles, however, stained with this antibody (Fig. 5B). Unlike the ventricle, only a proportion of the cardiac muscle cells stained in the atrial compartment of the heart (Fig. 5C). The level of MHC recognised by antibody M14 in rabbit or rat atrium varied in different myocardial cells.

C. Distribution of slow skeletal muscle-type myosin heavy chain recognised by antibody M14 in some foetal rat and chicken embryonic skeletal muscles

As has been shown earlier, antibody 96J in 18 day foetal rat skeletal leg muscles stained all the primary generation myotubes present. Antibody M14, in contrast, reacted with only a subset of the primary generation myotubes at this stage of development and these cells were usually present in deeper regions of muscles which appeared to correspond

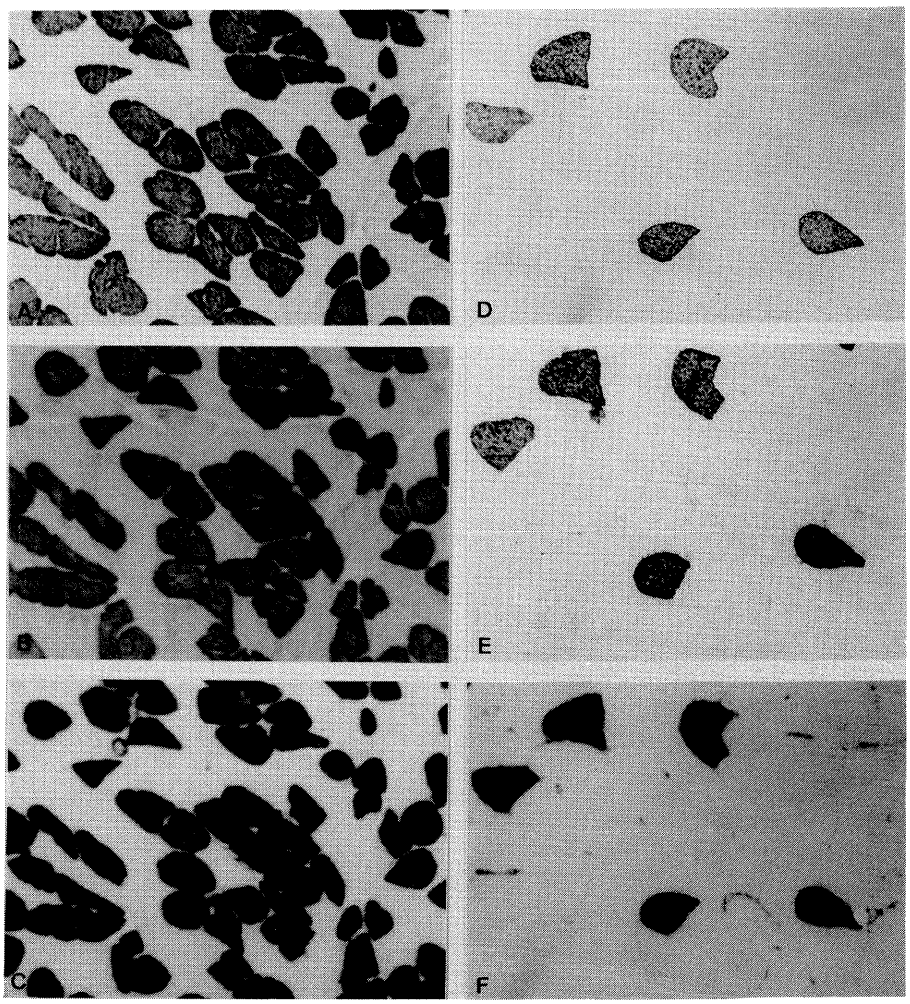

Figure 3. Serial sections of adult human biceps (A-C) and gastrocnemius muscle from a young chicken (D-F) stained with antibodies M14 (A, D) and 96J (B, E) by immunoperoxidase and by histochemical myosin ATPase after preincubation at pH 4.3 (C, F). x140.

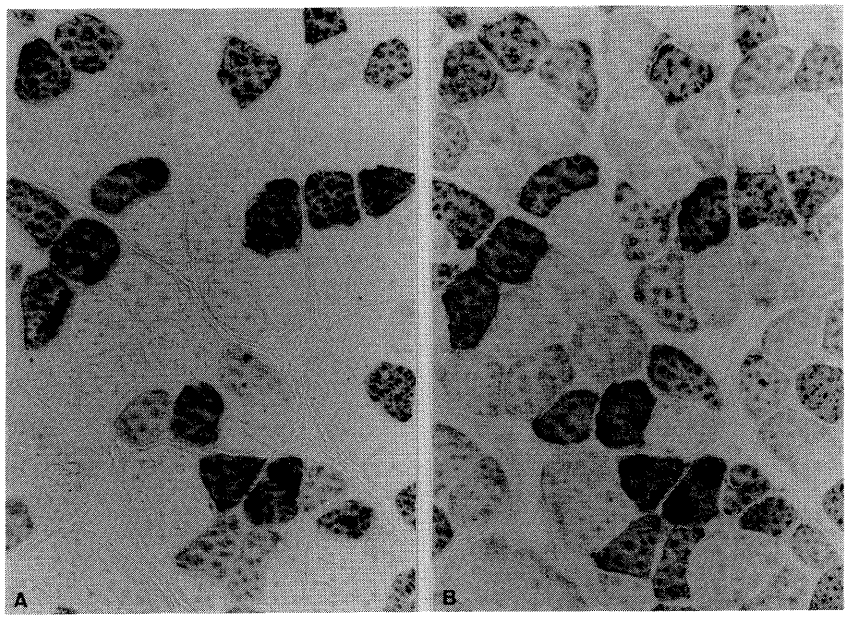

Figure 4. Serial sections of anterior latissimus dorsi muscle from a 6 week old chicken stained with antibodies M14 (A) and 96J (B) by immunoperoxidase procedure. x140.

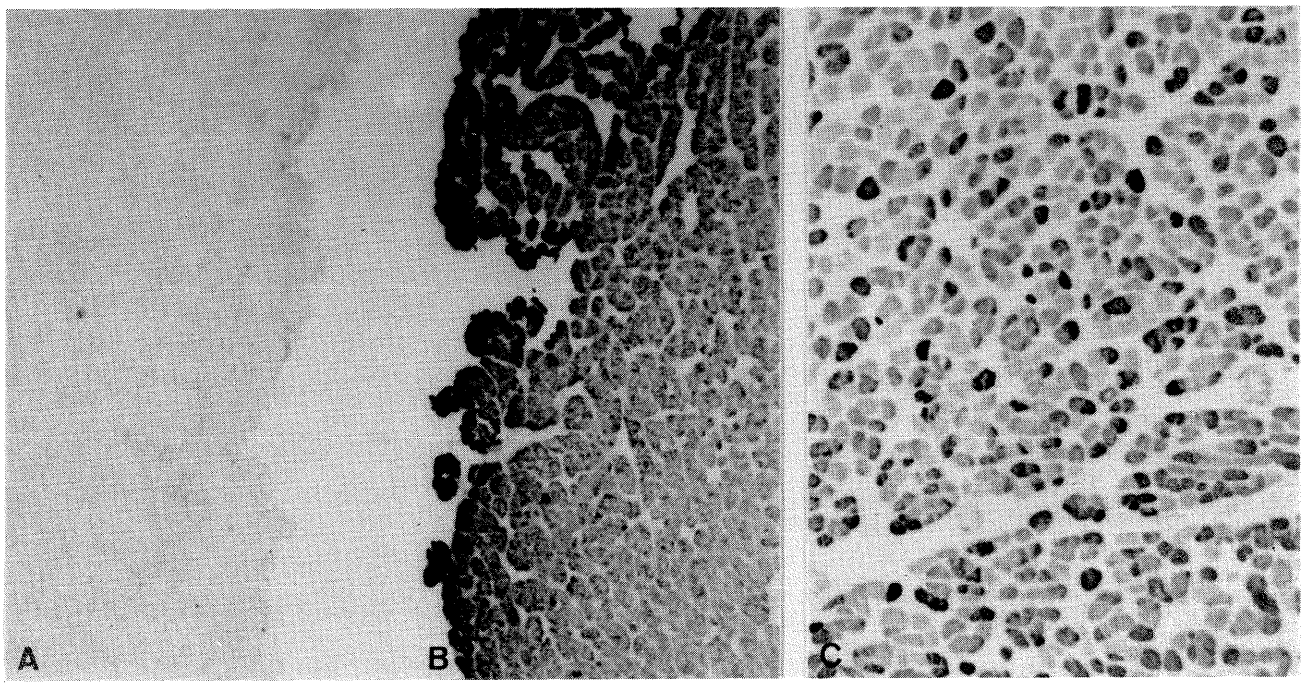

Figure 5. Sections of rabbit aorta (A), ventricle (B) and atrium (C) stained with antibody M14 by immunoperoxidase technique. x140.

to presumptive slow muscle fibres (Fig. 6). A small proportion of myotubes, particularly though not exclusively in peripheral regions of slow muscle fibre containing areas, stained weaker with antibody M14. None of the secondary generation myotubes in rat EDL or TA muscles reacted with antibody M14 at 18 days of gestation. Antibody F2, that recognises the fast isoforms of MHC [4] stained all the primary and secondary generation myotubes at this stage of development.

The distribution pattern of MHC recognised by antibody M14 was also investigated during embryonic development of some chicken skeletal muscles. As was the case in the

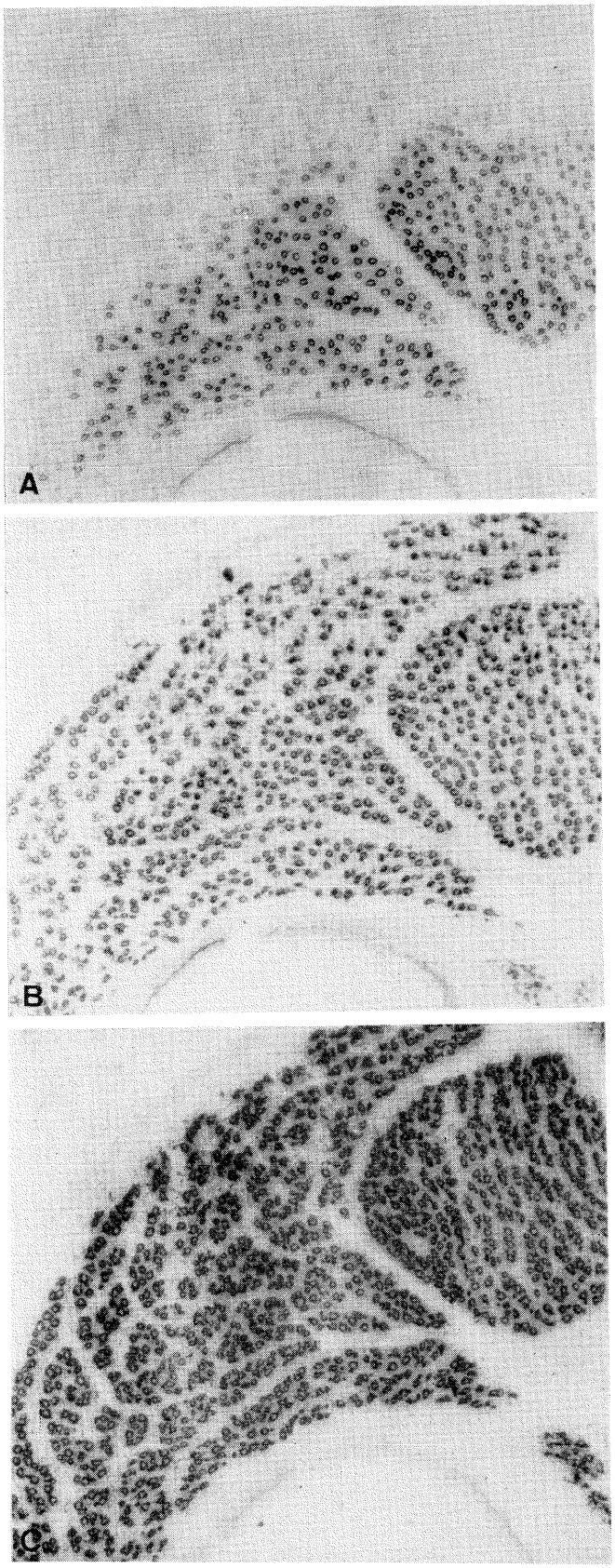

Figure 6. Serial sections through parts of extensor digitorum longus (on the right) and tibialis anterior (on the left) muscles from 18 day rat foetus stained with antibodies M14 (A), 96J (B) and F2 (C) by immunoperoxidase procedure. x64.

adult chicken skeletal muscles, the staining with antibody M14 in the 12 day chicken embryonic leg muscles appeared generally similar to SM1 MHC distribution though not exactly identical (Fig. 7). For example, a small number of myotubes in femorotibialis internus (FTI) muscle of 12 day chick embryo stained with antibody 9812 in the peripheral regions of presumptive slow muscle fibre rich areas did not react significantly or only lightly with antibody M14. The distribution of MHC recognised by antibody M14 in the pectoral muscle appeared considerably different and generally more restricted. With the exception of a few myotubes, most of the myotubes stained by antibody M14 in 12 day embryonic pectoral muscle showed more peripheral staining of the myotubes (Fig. 8). It is possible therefore that the distribution of this MHC in some myotubes is restricted to peripheral myofibrils. Antibody F2 to fast MHC [21, 22] stained all the myotubes in 12 day embryonic FTI and pectoral muscles.

Discussion

A monoclonal antibody raised to human skeletal muscle myosin is characterised in this study which recognises MHC associated with some cardiac and slow twitch fibres of adult mammalian skeletal muscles. In adult or young chicken skeletal muscles, however, it reacted with only a fraction of slow muscle fibres. The distribution pattern of the slow MHC recognised by antibody M14 in post-hatch chicken skeletal muscles corresponded to the distribution pattern of SM1 MHC recognised by two previously characterised antibodies, 9812 and 96J [2, 22]. Although the staining pattern of these muscles with antibody M14 was not exactly identical since some of the intermediate cells stained by SM1 MHC antibodies did not stain significantly or only lightly with antibody M14, it clearly did not recognise SM2 MHC. The distribution pattern of SM2 MHC in adult chicken slow skeletal muscle fibres would be generally complementary to SM1 MHC although both isoforms can be present in the same cell [8, 22]. It is therefore concluded that antibody M14 in post-hatch chicken skeletal muscles reacted with only SM1 and not SM2 MHC. It is not clear, however, whether antibody M14 recognises all the SM1 MHC population.

As expected from studies of mammalian muscles, antibody M14 also recognised MHC present in rabbit and rat ventricular muscle cells. Only a single gene so far has been described for mammalian slow skeletal/cardiac muscle ß myosin heavy chain [10]. It is not surprising therefore that antibody M14 recognised MHC present in both slow skeletal and cardiac muscles. In the atrium, however, only a fraction of the cells were stained with antibody M14. Considering the positive staining of all native cardiac myosin isozymes on Western blots, the reason for selective staining of only some atrial myocardial cells is not clear.

Unlike the adult mammalian striated muscles, the staining pattern of rat foetal skeletal muscles with antibody M14 appeared markedly different from the staining patterns observed with other slow MHC antibodies and there-

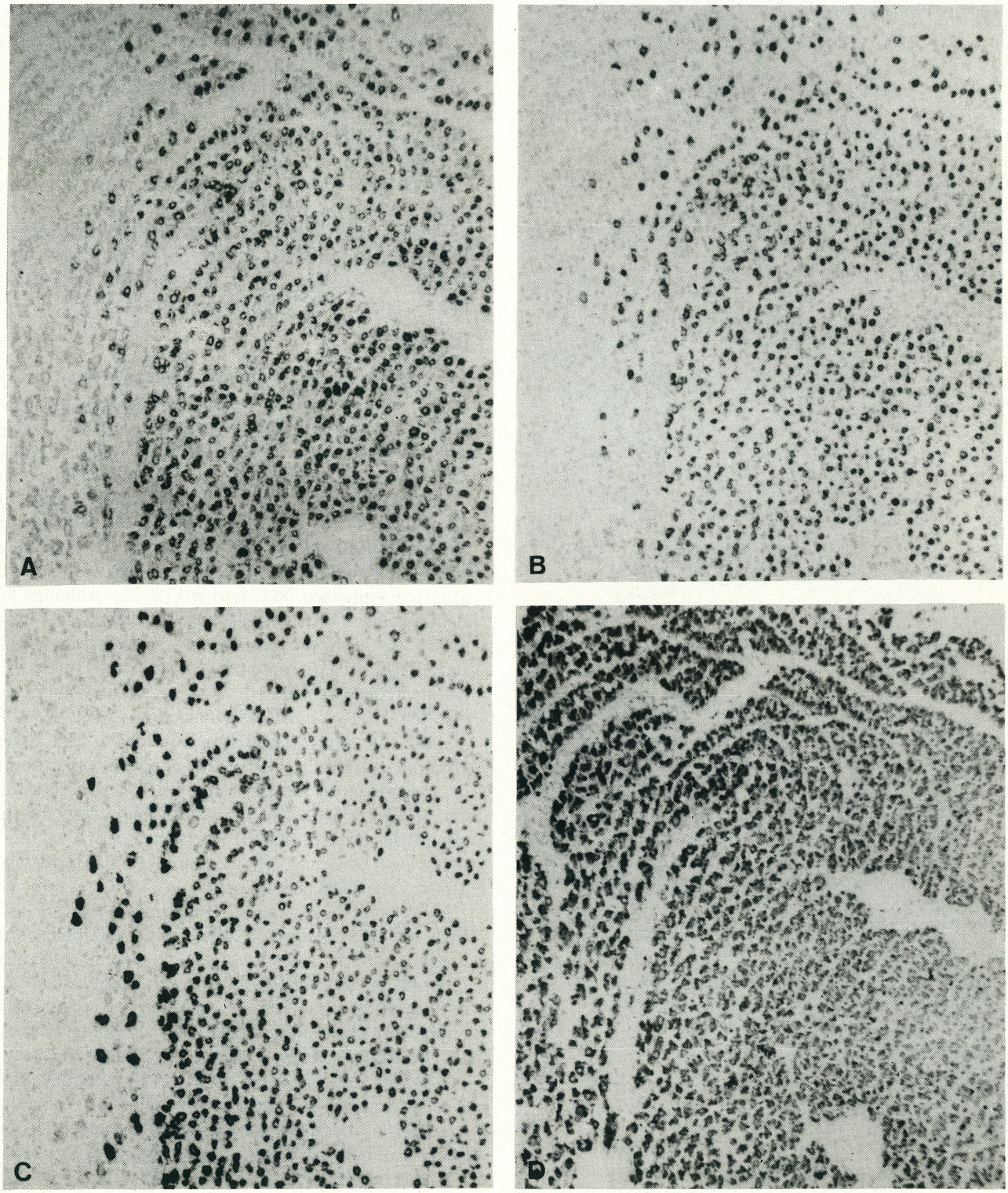

Figure 7. Serial sections of femorotibialis internus muscle from a 12 day chick embryo stained with antibodies M14 (A), 96J (B), 9812 (C) and F2 (D) by immunoperoxidase procedure. x140.

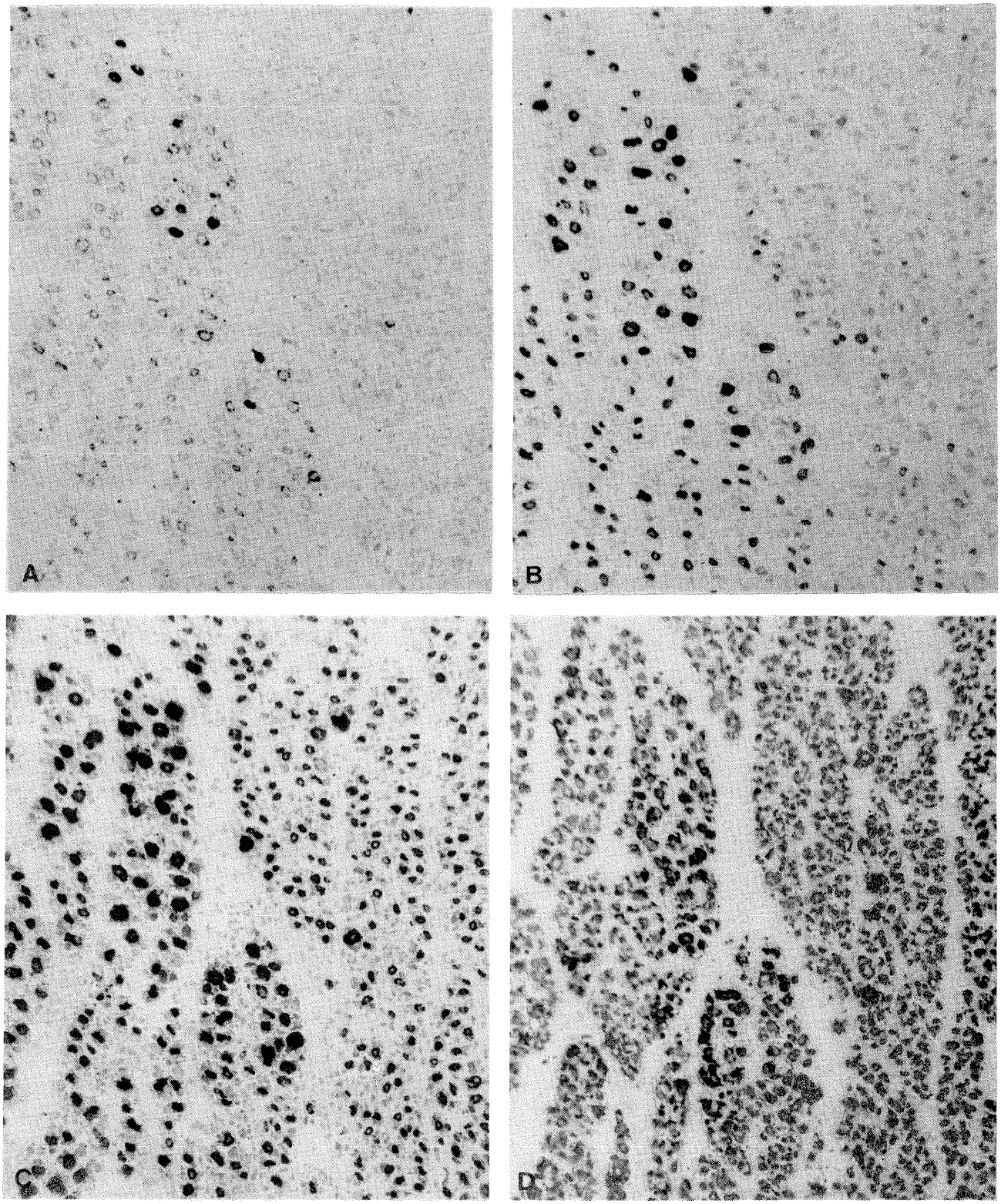

Figure 8. Serial sections through a part of pectoral muscle from a 12 day chick embryo stained with antibodies M14 (A), 96J (B), 9812 (C) and F2 (D) by immunoperoxidase procedure. x140.

fore indicated heterogeneity of slow MHC present at this stage of development. The observation that antibody M14 was more selective in its reaction with slow MHC content was also indicated by studies of embryonic chicken skeletal muscles in which it did not recognise the full complement of SM1 MHC positive myotubes in pectoral muscle. The number of myotubes stained by antibody 96J in the developing chicken embryonic skeletal muscles is much higher than that observed with antibodies specific for SM1 MHC only (e.g. 9812) because antibody 96J also recognises ventricular and a slow embryonic muscle type MHC [3, 22]. The distribution of SM1 and SM2 myosin heavy chains is usually restricted to only presumptive slow skeletal muscle cells [22]. It is possible that as is the case in chicken skeletal muscles, antibody 96J also recognises an additional cardiac muscle like or slow embryonic muscle like MHC in foetal rat skeletal muscles which is not recognised by antibody M14. Antibody M14, however, reacts with MHC present in cardiac muscle although it is not known if it recognises the whole fraction characteristic of this tissue which is recognised by antibody 96J. Antibody M14 clearly detects heterogeneity of slow MHC in rat foetal skeletal muscles which has not been described previously.

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