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# Demonstration of 'cardiac-specific' myosin heavy chain in masticatory muscles of human and rabbit

J. J. BREDMAN[1]*, A. WESSELS[2], W. A. WEIJS[1], J. A. M. KORFAGE[1], C. A. S. SOFFERS[2] and A. F. M. MOORMAN[2]

[1]*Department of Anatomy and Embryology, Academic Centre for Dentistry Amsterdam (ACTA) and*
[2]*Faculty of Medicine, University of Amsterdam, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands*

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## Summary

Human and rabbit masticatory muscles were analyzed immuno- and enzyme-histochemically using antibodies specific to 'cardiac' α, slow and fast myosin heavy chain isoforms. In human masseter, temporalis, and lateral pterygoid muscle 'cardiac' α myosin heavy chain is found in fibres that contain either fast, or fast and slow myosin heavy chain. In rabbit masseter, temporalis and digastric muscles, fibres are present that express 'cardiac' α myosin heavy chain either exclusively, or concomitantly with slow myosin heavy chain or fast myosin heavy chain. Our results demonstrate a much broader distribution of 'cardiac' α myosin heavy chain than hitherto recognized and these might explain in part the specific characteristics of masticatory muscles. The 'cardiac' α myosin heavy chain is only found in skeletal muscles originating from the cranial part of the embryo (including the heart muscle), suggesting that its expression might be determined by the developmental history of these muscles.

## Introduction

Myosin is an hexameric protein consisting of two heavy chains and four light chains. The myosin heavy chain (MHC) is responsible for the calcium-dependent ATPase activity that hydrolyzes ATP, thereby providing the chemical energy that is transduced into mechanical force (Huxley, 1969). The velocity of shortening of a particular fibre is directly proportional to its ATPase activity which is, in turn, strongly correlated with the MHC composition (Schwartz *et al.*, 1981; Reiser *et al.*, 1985, 1988). Using ATPase histochemistry different fibre types can be distinguished in skeletal muscle. It has been demonstrated that the distribution of these fibre types correlates with the distribution of MHC isoforms (Staron & Pette, 1987a,b).

The relatively straightforward subdivision of adult mammalian limb muscle fibres into type I (slow), IIA (fast-oxidative) and IIB (fast-glycolytic) categories cannot, however, simply be applied to the masticatory muscles. Masticatory muscles contain various myosins not normally found in other adult skeletal muscles. For example: a specific MHC (MHC-M, superfast myosin) has been found in the jaw muscles of many primates and carnivores (Rowlerson *et al.*, 1983) and combinations of MHCs, some of them being persisting embryonic or fetal isoforms (d'Albis *et al.*, 1986; Butler-Browne *et al.*, 1988), are found in human adult masseter fibres. This could explain the finding of a lack of characteristic reciprocity in ATPase reactions at acid and alkaline preincubation for many type II fibres of masseter and temporalis muscles in *Rhesus macaques* (Maxwell *et al.*, 1980) and man (Ringqvist, 1973) and a non-corresponding velocity of shortening with ATPase activity in masseter muscle of the Rhesus monkey (Faulkner, 1979).

In addition it has been shown that in adult human masseter an embryonic isoform of myosin light chain (MLC) persists (Butler-Browne *et al.*, 1988; Soussi-Yanicostas *et al.*, 1990) which is similar to the 'cardiac' specific MLC found in adult atrial myocardium (Barton *et al.*, 1985). This raises the question of whether human jaw muscles also contain the specific α MHC found in atrial myocardium (Bouvagnet *et al.*, 1984, 1987; Clark *et al.*, 1982; Wessels *et al.*, 1990b). To this end we have analyzed immuno- and enzyme-histochemically human and rabbit masticatory muscles with a panel of monoclonal antibodies, that allows the unambiguous distinction of slow (type I) MHC (identical to 'cardiac' β MHC, Yamauchi-Takihara *et al.*, 1989), fast (type IIA and/or IIB) and 'cardiac' α MHC. Our results demonstrate that 'cardiac' α MHC contributes substantially to the MHC complement of masticatory muscle.

*To whom all correspondence should be addressed.

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## Materials and methods

### *Preparation of tissue*

Samples from adult human muscles (5 specimens of masseter, 6 of temporalis, 1 of lateral pterygoid, 1 of digastric, 3 of biceps, 3 of soleus, 3 of gastrocnemius and 1 of psoas muscles) were obtained during autopsies at the Academic Medical Centre (AMC) and at the Postgraduate School of Medicine in Budapest. Embryonic heart was obtained after legal abortion and a fetal biceps femoris muscle was obtained from immature delivery at the AMC. Muscles (whole masseter, whole temporalis, whole medial pterygoid, whole digastric, part of soleus, part of anterior tibialis and a part of psoas) were dissected from three New Zealand rabbits (two males and one female) after the animals had been killed by anaesthetic overdose. For the immunohistochemical studies the tissue specimens were fixed in a mixture of methanol: acetone: acetic acid: water (35:35:5:25), dehydrated in a graded series of ethanol, cleared in chloroform, embedded in Paraplast Plus (Monoject, Ireland) and cut into 8 μm thick serial sections (Wessels *et al.*, 1988). The sections were mounted on microscope slides coated with poly-L-lysine. For Western Blot analysis, tissue specimens were frozen in liquid nitrogen and stored at −70° C. For the combined enzyme-histochemical (ATPase) study parts of muscles were frozen in liquid Freon-22 (monochlorodifluoromethane) cooled with liquid nitrogen and stored at −70° C.

### *Production of monoclonal antibodies*

Myosin was isolated according to the method of Hoh *et al.* (1976). Monoclonal antibodies (Mabs) against MHC isoforms were prepared according to the procedure of Fazekas de St. Groth and Scheidegger (1980). The production and characterization of antibodies against 'cardiac' α MHC (Mab 249–5A4) and against slow MHC (= anti type I MHC = anti β MHC) (Mab 169–ID5) has been described elsewhere (Wessels *et al.*, 1988; 1990b; de Groot *et al.*, 1989). An antibody against fast MHC (= anti type IIA, IIB and IIX myosin) (Mab 340–3B5) was raised against myosin isolated from rabbit anterior tibialis muscle. An antibody towards embryonic/fetal MHC (Mab 330–5B4) was raised against a protein extract from muscle tissue of a 15 week old human fetus.

Running ahead of the characterization of the antibodies (*vide infra*) Mab 249–5A4 is denoted as anti-α MHC, Mab 169–ID5 is denoted as anti-slow MHC, Mab 340–3B5 is denoted as anti-fast MHC and Mab 330–5B4 is denoted as anti-embryonic/fetal MHC.

### *Characterization of the antibodies*

The specificity of the antibodies towards MHC was tested by Western Blot analysis. Anti-α MHC and anti-slow MHC were previously shown to be specific for MHC (Wessels *et al.*, 1990b). The specificity of anti-fast MHC to MHC is shown in Fig. 1.

The muscle tissues were extracted essentially according to the method of Sweeney *et al.* (1989). Myosin samples were stored at −20° C in 50% glycerol (d'Albis *et al.*, 1979). After electrophoresis of the muscle extract on 10% polyacrylamide gels in the presence of SDS, the gels were blotted onto nitrocellulose sheets (BAS 85, reinforced nitrocellulose, Schleicher and Schuell, Dassel Germany), using the Biorad Minitransblot (1–2 h, 50 V). Prestained molecular weight marker (PSM) proteins (SDS–7B, Sigma, USA) were transferred simultaneously to enable identification of antibody binding bands. Nitrocellulose sheets were first stained with Amido Black to demonstrate the protein bands (3 min, 0.1% w/v amido black in 10% v/v methanol, 10% v/v acetic acid, 80% v/v distilled water, followed by rinsing in a solution of 5% v/v methanol and 7.5% v/v acetic acid in water to remove back-ground staining). After destaining the nitrocellulose sheets in TEN-ST-BSA (50 mM Tris-HCl, 5 mM EDTA, 150 mM NaCl (pH 7.4) containing 0.1% w/v SDS, 1% Triton-X-100 and 3% BSA) the sheets were rinsed in TEN-ST, cut and incubated with the respective antibodies, diluted in TEN-ST-BSA (4° C, overnight). Between each incubation step, the strips were washed in TEN-ST (Wessels *et al.*, 1990b).

### *Immunohistochemistry*

The indirect unconjugated immunoperoxidase technique (PAP-technique) according to Moorman *et al.* (1984) was applied to detect the binding of the specific monoclonal antibodies with the MHC isoforms. After deparaffination, the sections were treated with hydrogen peroxide (3% v/v in PBS) for 30 min to reduce endogenous peroxidase activity followed by pre-incubation in TENG-T (10 mM Tris, 5 mM EDTA, 150 mM NaCl, 0.25% gelatin, 0.05% Tween–20, pH 8.0) for 30 min to reduce non-specific binding. The pretreated human and rabbit sections were incubated with the various monoclonal antibodies (room temperature, overnight). When sections of human tissue were used the incubations were followed by incubation with rabbit anti-mouse immunoglobulin, goat anti-rabbit immunoglobulin and rabbit peroxidase-antiperoxidase respectively. As incubations of rabbit tissue with goat anti-rabbit immunoglobulin gave high background staining, the antibody binding was detected using goat anti-mouse, donkey anti-goat and goat peroxidase-antiperoxidase complex. Sera were diluted in phosphate buffered saline (PBS). All incubations were followed by three washes in PBS for 5 min. The immunocomplex formed was visualized by incubation of the sections with 0.5 mg $ml^{-1}$ 3,3' diaminobenzidine, 0.02% hydrogen peroxide in 30 mM imidazole, 1 mM EDTA (pH 7.0). Sections were mounted in Entellan. The sections were occasionally pretreated (before the hydrogen peroxide treatment) with pronase (0.1 mg $ml^{-1}$, 15–30 min), as described by Christensen and Strange (1987), to optimize binding of the antibodies with the antigens.

Serial frozen sections of rabbit masseter (superficial part, i.e. MSS1; see Bredman *et al.*, 1990a) and human masseter (deep part) and rabbit temporalis, digastric, soleus, anterior tibialis and psoas muscles were mounted on microscope slides coated with AAS (3-aminopropyltriethoxysilane) (Henderson, 1989), fixed overnight in methanol: acetone: acetic acid: water (35:35:5:25) at −20° C, washed in PBS for three times 5 min, pretreated with pronase (5 min) and allowed to react with the various antibodies as described above.

### *Histochemistry*

Serial sections of rabbit masseter (superficial part; MSS1) and human masseter (deep part) and rabbit temporalis, digastric, soleus, anterior tibialis and psoas muscles were mounted on glass slides coated with AAS. The sections were

incubated for $Ca^{2+}$ activated adenosine triphosphatase (ATPase) at pH 9.4 (Staron *et al.*, 1983; Bredman *et al.*, 1990a). Pre-incubations with a range of acid pH 4.2–4.6 and a range of alkali pH 10.1–10.6 were used to distinguish the several histochemical fibre types (Staron & Pette, 1986; Bredman *et al.*, 1990a). The different alkali values were taken because they allow type IIC' fibres to be distinguished.

## Results

*Characterization of the antibodies*

All antibodies used bind specifically to a prominent protein band at approximately 200 kD, characteristic for MHC (Fig. 1 and Wessels *et al.*, 1990b).

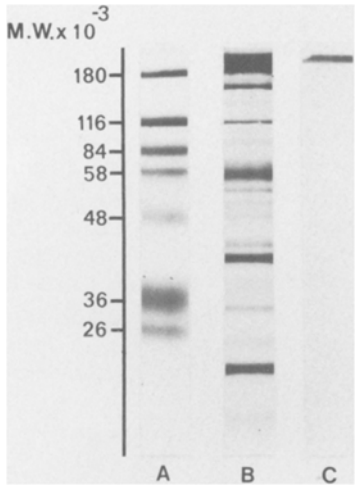


**Fig. 1.** Identification of anti-fast (Mab 340–3B5) (lane C) MHC by immunoblotting analysis of a muscle protein extract from adult human soleus after 10% SDS-polyacrylamidegel electrophoresis. To enable identification of the antibody-binding band a prestained molecular weight marker was transferred simultaneously (lane A). The protein pattern of soleus is visualized by Serva blue staining in lane B.

The specificity of the antibodies on tissue sections was tested by comparing the immunohistochemical reaction with the $Ca^{2+}$ ATPase reaction on frozen sections of different rabbit skeletal muscles. Fig. 2 shows that the anti-slow MHC stained fibres (Fig. 2C) correspond with the ATPase determined type I fibres (Fig. 2B) and that the anti-fast MHC stained fibres (Fig. 2D) correspond with the ATPase determined type IIA fibres (Fig. 2A). The anti-fast MHC stained type IIB and IIX fibres also, as demonstrated on sections of anterior tibialis and psoas muscles from rabbit, and on sections of extensor digitorum longus muscle of rat.

In human fetal heart muscle anti-α MHC reacted strongly with atrial myocardium but not with the ventricular myocardium (Fig. 3A). Anti-slow MHC (Fig. 3B) showed only reaction with the ventricular myocardium but no reaction with the atrial myocardium. The third antibody, anti-fast MHC (Fig. 3C), showed a negative reaction with both atrial and ventricular myocardium, in agreement with previous reports in the literature (De Groot *et al.*, 1989; Wessels *et al.*, 1990b). In rabbit fetal heart the three antibodies showed the same pattern as in human fetal heart.

To establish possible cross-reactivity of the panel of antibodies with embryonic/fetal myosin the antibodies were tested on 16 weeks human fetal biceps femoris muscle (Fig. 4). The anti-α MHC showed no reaction with muscle cells apart from the reactivity with intrafusal muscle spindle fibres (Wessels *et al.*, 1990a; 1990c) that served as such as a positive control (Fig. 4A). The anti-slow MHC showed a positive reaction with the primary slow myotubes (Fig. 4B) (see also Thornell *et al.*, 1984a). The anti-fast MHC showed no reaction (Fig. 4C), in accordance with the results of Hoh & Yeoh (1979), Fitzsimons & Hoh (1981), Whalen *et al.* (1982) and Pons *et al.* (1986). The anti-embryonic/fetal MHC reacted with all fibres (Fig. 4D). Therefore it is concluded that anti-α MHC does not cross-react with embryonic/fetal myosin. (This antibody was not used further in this study). Recently we carried out a study on developing masseter muscle using antibodies directed against the embryonic and neonatal isoforms of MHC. This study indicates that the actual number of distinct fibre types might even be greater (Bredman *et al.*, unpublished results).

Finally, the specificity of the panel of antibodies was tested on adult human arm (biceps), leg (soleus, gastrocnemius), trunk (psoas) and adult rabbit leg (soleus, anterior tibialis) and trunk (psoas) muscles. In all muscles the 'cardiac'-α antibody showed no reaction with the extrafusal fibres. In Fig. 5 (A,D) sections of human soleus and gastrocnemius exemplify these findings (only the intrafusal muscle spindle fibres show a positive reaction with this antibody). Some fibres reacted with anti-slow MHC (Fig. 5B,E) and the rest with anti-fast MHC (Fig. 5C,F).

All in all, our histochemical data show that the antibodies used are specific for 'cardiac' α, slow-twitch and fast-twitch MHC, respectively.

*Fibre types in masticatory muscle*

Application of the antibodies on serial sections of human jaw muscles resulted in an altogether different picture. The anti-α MHC reacted with a number of muscle fibres in the masseter (Fig. 6A), temporalis (Fig. 6D) and lateral pterygoid (not shown). The fibres were common in the three muscles (20 to 45% of the

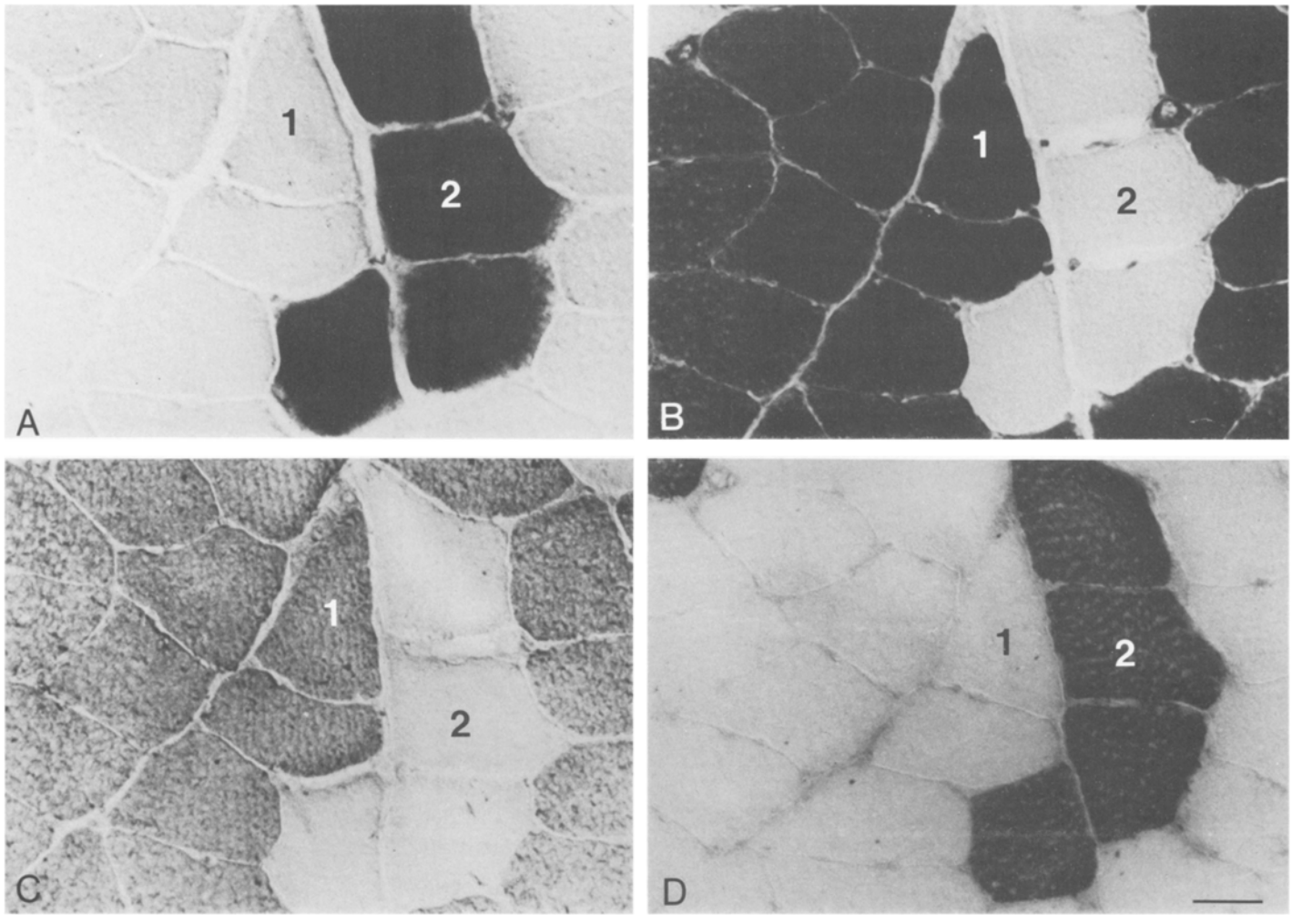


**Fig. 2.** Antibody characterization on serial transverse sections of rabbit soleus muscle showing alkali-stable ATPase activity (pH 10.6 preincubation) (A); acid-stable ATPase activity (pH 4.4 pre-incubation) (B) and the immunoreactivity with anti-slow MHC (C); anti-fast MHC (D). Type I (1) and type IIA (2) fibres are indicated. The scale bar represents 40 μm.

fibres in the masseter). The amount of positive staining fibres is region and person dependent. This is in agreement with the observations of Eriksson & Thornell (1983), who found considerable interindividual variability in human jaw muscle fibre content. In the human masseter and temporalis muscles four different fibre types were found (see Fig. 6A–F and Table I); one type containing only slow MHC, another type containing 'cardiac' α and fast MHC, a third type containing slow and fast MHC and a fourth type containing 'cardiac' α, fast and slow MHC. (No fibres contained exclusively fast MHC.) In the digastric (anterior and posterior belly) muscle no 'cardiac' α-containing fibres were found (Fig. 6G) only fibres containing slow, fast or slow and fast MHC were present (Fig. 6H, I; Table I).

In rabbit jaw muscles a similar pattern was found. Anti-α MHC stained positively in masseter (Fig. 7A), temporalis (Fig. 7D), medial pterygoid (not shown) and digastric (Fig. 7G) muscles. In the rabbit jaw muscles different fibre types were found (see Fig. 7 and Table II). In contrast to the situation in human jaw muscles the fibres either expressed slow and 'cardiac' α MHC, exclusively fast MHC (IIA and/or IIB MHC), exclusively 'cardiac' α MHC or fast and 'cardiac' α MHC (not shown in Fig. 7). A fibre type containing exclusively slow MHC was found only in digastric muscle. In Table II serial sections of immunohistochemical and ATPase reaction were compared. Using a range of alkali pH values revealed a type IIC' fibre.

The amount of anti-α MHC positive staining fibres in masseter was found to depend on the age of the

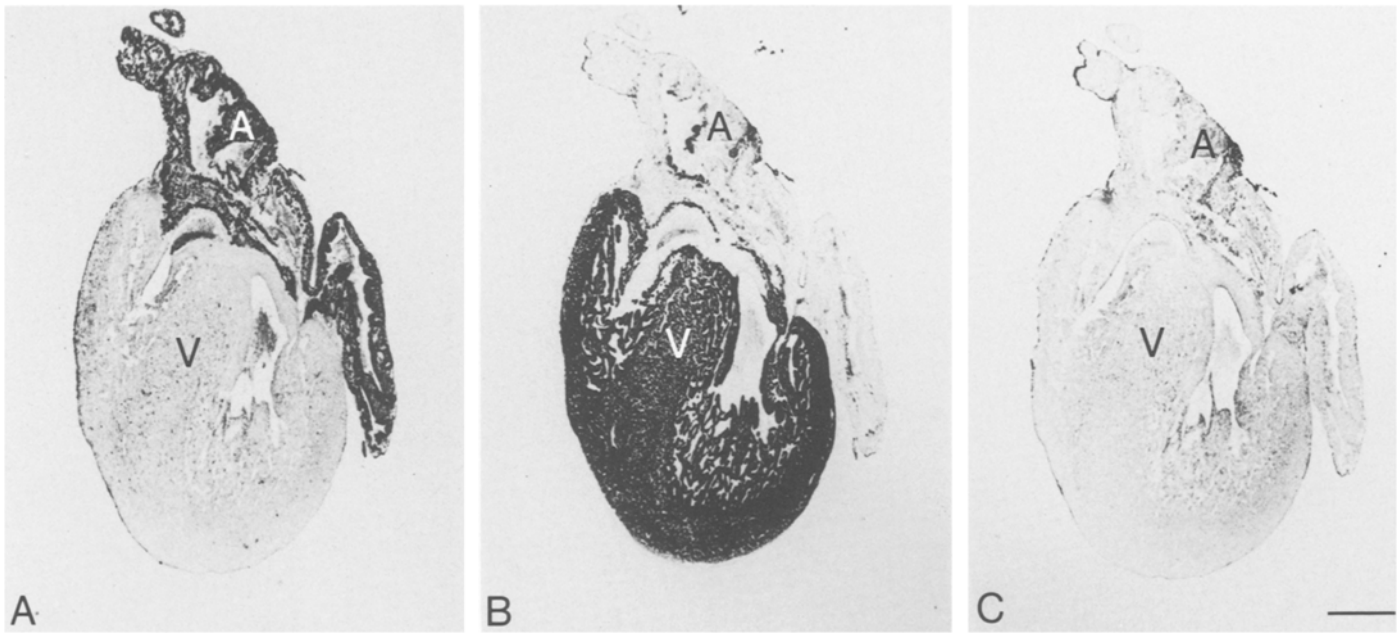


**Fig. 3.** Antibody characterization on serial sections of 6–7 weeks human embryonic heart incubated with anti-α MHC (A), anti-slow MHC (B) and anti-fast MHC (C). A = atrium, V = ventricle. The scale bar represents 0.2 mm.

**Table 1.** Fibre types in adult human jaw muscles.

| | *fibre types* | | | | |
|---|---|---|---|---|---|
| | *1* | *2* | *3* | *4* | *5* |
| Enzymehistochemistry | I | II | n.t. | IIC | n.t. |
| Immunohistochemistry | I | IIα | IIC | IICα | II |
| anti-α MHC | − | + | − | + | − |
| anti-slow MHC | + | − | + | + | − |
| anti-fast MHC | − | + | + | + | + |

The fibre types were determined by enzyme-histochemistry (ATPase reactivity) and by the MHC composition as determined by immunohistochemistry. I = slow type, II = fast type, IIC = intermediate type, (for immunohistochemistry I/II would be a more appropriate classification than IIC.). n.t.: not present in the tested part of the deep masseter muscle.

animals. In neonatal rabbits these fibres were absent; during postnatal life the amount of anti-α MHC positive staining fibres increased. These results will be dealt with in a forthcoming paper.

## Discussion

The occurrence of 'cardiac' α MHC in the jaw muscles is an interesting finding, made possible by the combined use of antibodies specific for cardiac and skeletal muscle. To our knowledge this is the first time that a 'cardiac' α MHC has been found outside heart muscle. Formally, the possibility cannot be excluded that our antibody recognizes a highly related MHC. We judge this unlikely in view of the specificity of this antibody as determined on a variety of muscles. Some of the antibodies directed against skeletal muscle used in other studies might cross-react with 'cardiac' α MHC. As this test is usually not included, the presence of this protein might therefore have remained unnoticed hitherto. The notion is underlined by our observation that a 'type I'-specific monoclonal antibody, as determined on limb muscle sections, turned out to react also with cardiac α MHC in sections of heart muscle. This antibody was raised against MHC preparation of adult rabbit psoas muscle and reacted in rabbit masseter with all histochemically determined type I, IIC and IIC' fibres, obviously not distinguishing between the presence or absence of α MHC.

The speed of contraction of a single skeletal muscle fibre is largely determined by its MHC content (Reiser *et al.*, 1985; Schiaffino *et al.*, 1988; Eddinger & Moss, 1987) but also by its MLC composition (Sweeney *et al.*, 1988). It appears that fibres containing type I MHC (slow), a myosin identical to ventricular MHC (Yamauchi-Takihara *et al.*, 1989), have the slowest speed of contraction, and those containing type IIA

**Table 2.** Fibre types in jaw and limb muscles of adult rabbit.

| | *fibre types* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *1* | *2* | *3* | *4* | *5* | *6* | *7* | *8* |
| Enzymehistochemistry | I | I | IIB | IIA | IIC | IIC | IIC′ | IIC′ |
| pH 4.3 | + | + | − | − | +− | +− | + | + |
| pH 4.5 | + | + | +− | − | +− | +− | + | + |
| pH 10.1–10.3 | − | − | + | + | + | + | + | + |
| pH10.4–10.6 | − | − | + | + | + | + | − | − |
| Immunohistochemistry | I | Iα | IIB | IIA | IIC | IICα | α | Iα |
| anti-α MHC | − | + | − | − | − | + | + | + |
| anti-slow MHC | + | + | − | − | + | − | − | + |
| anti-fast MHC | − | − | + | + | + | + | − | − |

The fibre types were determined by enzyme-histochemistry (ATPase reactivity) and by the MHC composition as determined by immunohistochemistry. I = slow type, II = fast type, IIC = intermediate type. Type IIC′ can only be distinguished at pH 10.1–10.6 and is classified by Rowlerson *et al.* (1988) as type IIC (pH 10.2) and by Bredman *et al.*, (1990a) as type I fibres (pH 10.6). Fibres 1, 3, 4 and 5 are present in leg muscles; fibres 2, 4, 6, 7 and 8 are present in masseter muscle (fibre type 3 is not found in the tested part of the masseter, but is found in the MSS4 compartment (Bredman *et al.*, 1990a) of male rabbits (Bredman *et al.*, unpublished)); fibres 3, 4, 7 and 8 are present in temporalis muscle; fibres 1, 2, 4 and 8 are present in digastric muscle and fibres 4, 6 and 8 are present in tongue muscle.

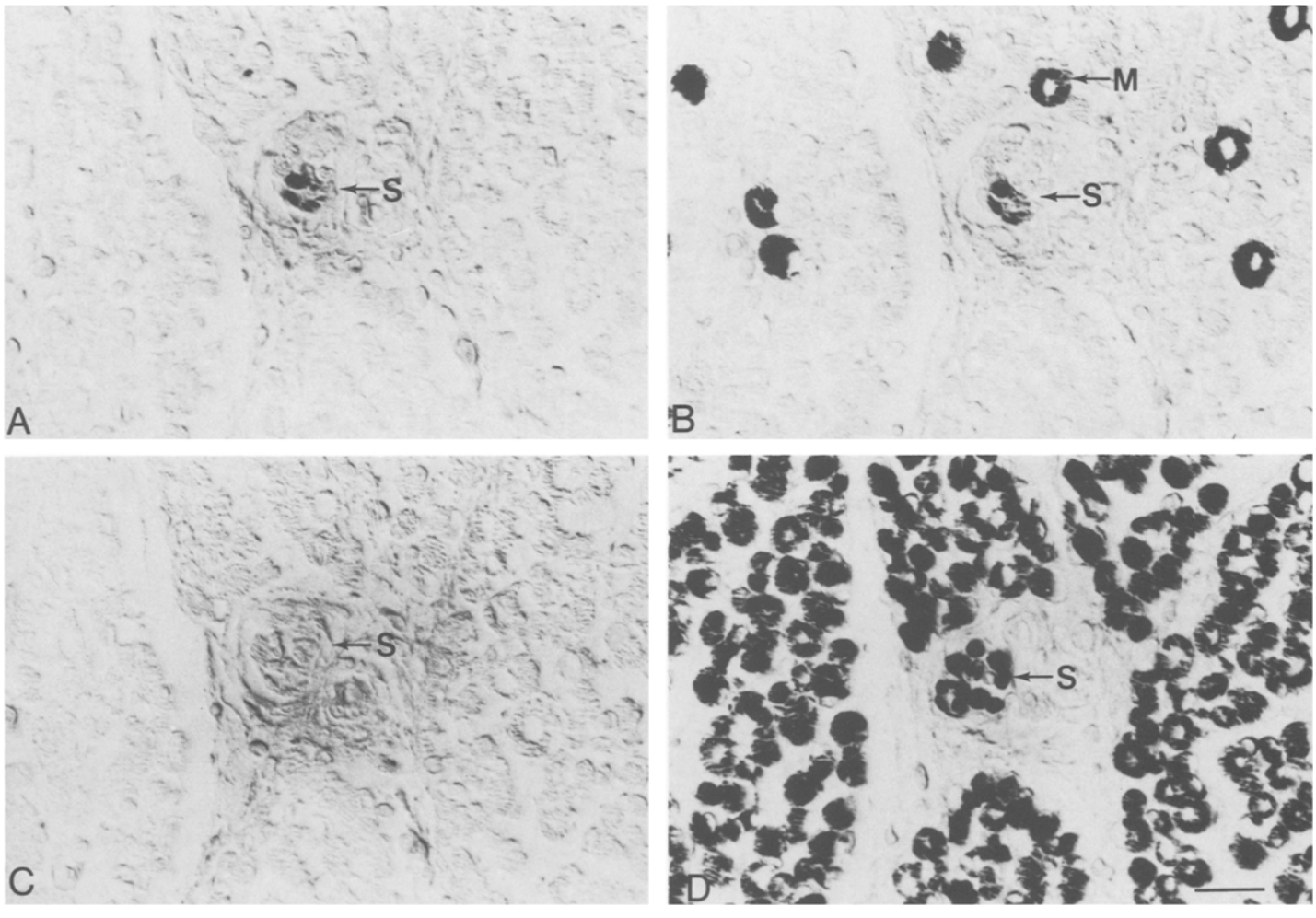


**Fig. 4.** Antibody characterization on serial sections of 15 weeks human fetal biceps femoris muscle incubated with anti-α MHC (A), anti-slow MHC (B), anti-fast MHC (C) and anti-neonatal MHC (D). Primary slow myotubes (M) and a muscle spindle (S) are indicated. The scale bar represents 20 μm.

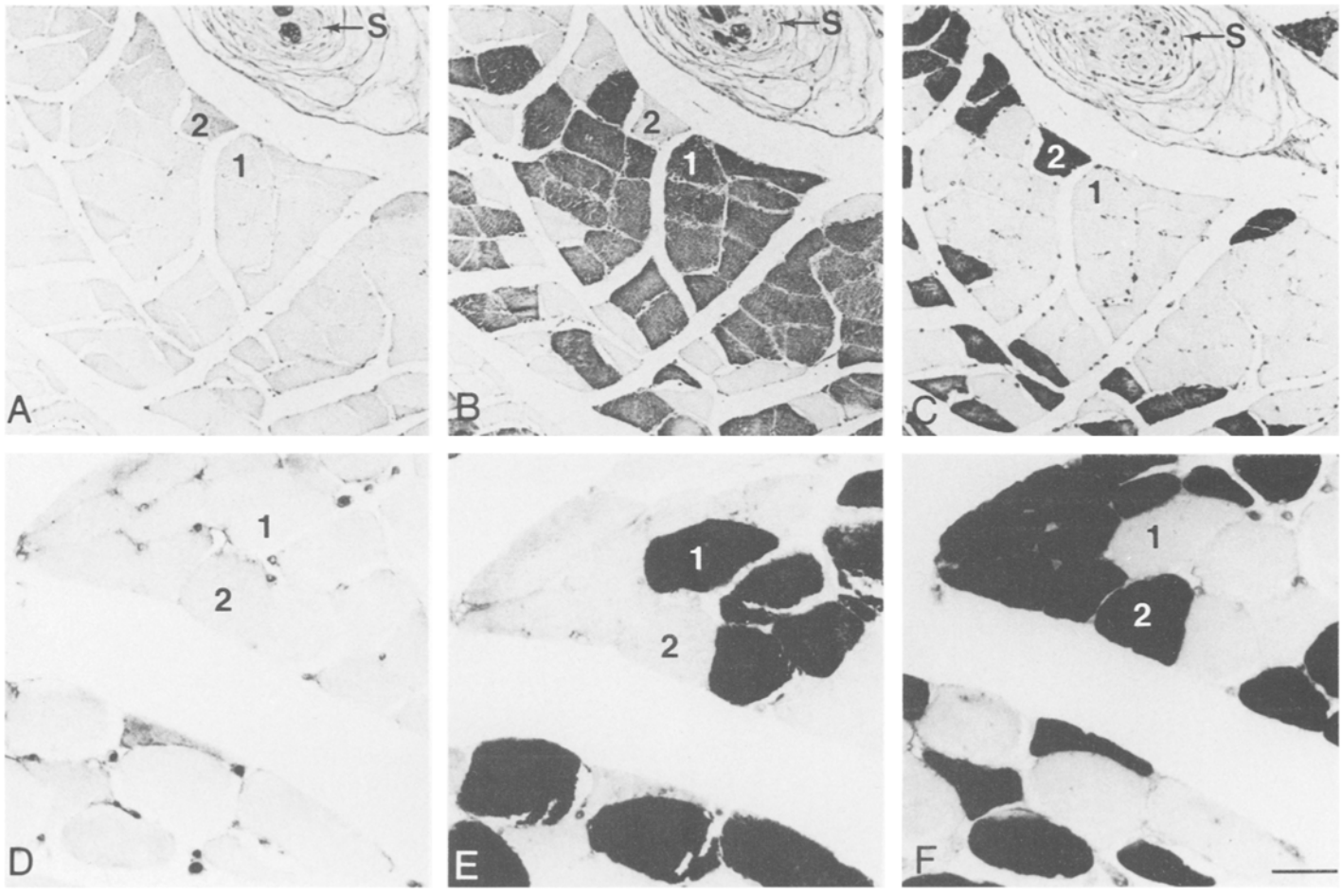


**Fig. 5.** Antibody characterization on serial sections of adult human soleus (A–C) and gastrocnemius (D–F) muscle incubated with anti-α MHC (A,D), anti-slow MHC (B,E) and anti-fast MHC (C,F). Fibres containing slow MHC (1), fibres containing fast MHC (2) and a muscle spindle (S) are indicated. The scale bar represents 40 μm.

MHC a speed of contraction four times faster. Mixtures of different MHCs in a single fibre result in intermediate contraction velocities (Reiser *et al.*, 1985).

The jaw muscles differ from most limb muscles in several aspects. First, a large portion of the muscle fibres contain more than one type of MHC (compare Danieli-Betto *et al.* (1986) for limb muscles and Thornell *et al.* (1984b) for jaw muscles). Our finding of fibres that express slow and 'cardiac' α MHC or fast and 'cardiac' α MHC adds to this notion. It may indicate that in jaw muscles a finer gradation of contraction speeds per fibre is required.

Second, in jaw muscles, more often than in limb muscles, aberrant MHC isoforms are found. In this paper we describe the occurrence of 'cardiac' α MHC in jaw muscles. Rowlerson *et al.* (1983) described a type MHC-M ('superfast') in the jaw muscles of most carnivores and primates (but not present in human). This isoform replaces MHC-IIB and it probably produces a higher speed of contraction than MHC-IIB (Rowlerson *et al.*, 1983). We did not find 'superfast' MHC in human and rabbit jaw muscles (Bredman *et al.*, unpublished). Finally, fetal/neonatal isoforms of MHC have been shown to persist in adult rodent (d'Albis *et al.*, 1986) and human (Butler-Browne *et al.*, 1988) jaw muscles. These forms are generally associated with low speeds of contraction. We also found neonatal MHC in adult human and rabbit jaw muscles (Bredman *et al.*, unpublished).

'Cardiac' α MHC is intermediate in speed of contraction between slow and fast MHC; it has ATPase activity three times higher than slow (ventricular) MHC (McNally *et al.*, 1989). Interestingly, in the jaw muscles of human 'cardiac' α MHC is present in the fibres containing either fast MHC or fast and slow MHC, but not in the fibres containing only slow MHC. In the masseter muscle of the rabbit it is predominantly present in those fibres that contain slow MHC. It can be questioned whether in large species (like human) the fast jaw muscle fibres are slowed down

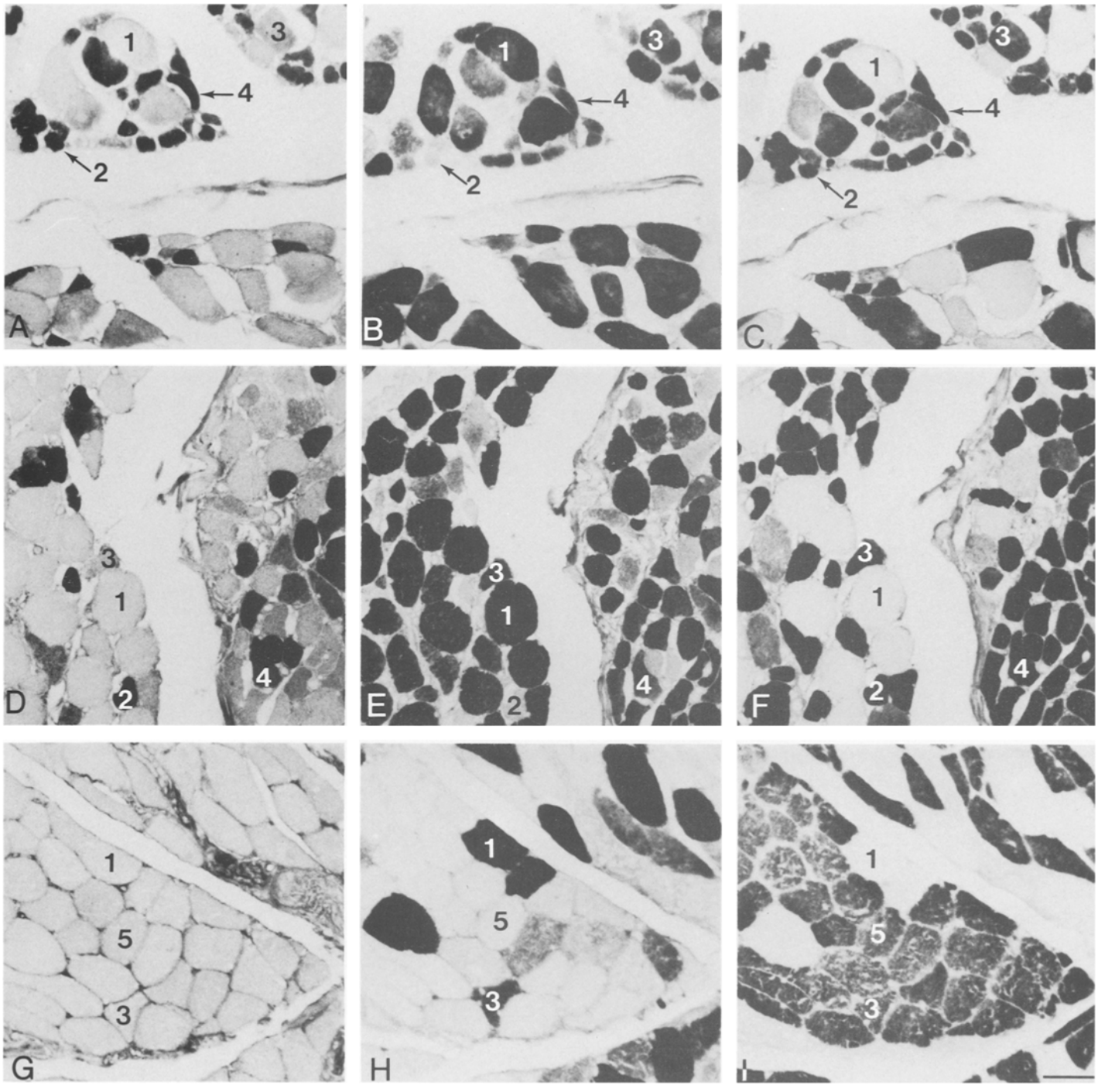


**Fig. 6.** Immunohistochemical analysis of serial sections of adult human masseter (posterior-mid part) (A–C), temporalis (D–F), and digastric (G–I) muscles. The sections were immunohistochemically stained with anti-α MHC (A,D,G), anti-slow MHC (B,E,H) and anti-fast MHC (C,F,I). Fibres containing slow MHC (1), 'cardiac' α and fast MHC (2), slow and fast MHC (3), 'cardiac' α, slow and fast MHC (4) and fibres containing fast MHC (5) are indicated. The scale bar represents 36 μm.

and that in small species (like rabbit) the slow jaw muscle fibres have become faster. Information from more species will be essential in order to draw this conclusion.

Recently we have found that 'cardiac' α MHC occurs in other cranial muscles also, such as the human and rabbit extraocular muscles (Asmussen *et al.*, in preparation), intrinsic and extrinsic tongue muscles and facial and infrahyoideal muscles in the rabbit (Bredman *et al.*, 1990b). Combined ATPase/immunohistochemistry reactions showed that 'cardiac' α MHC occurred in some ATPase defined type

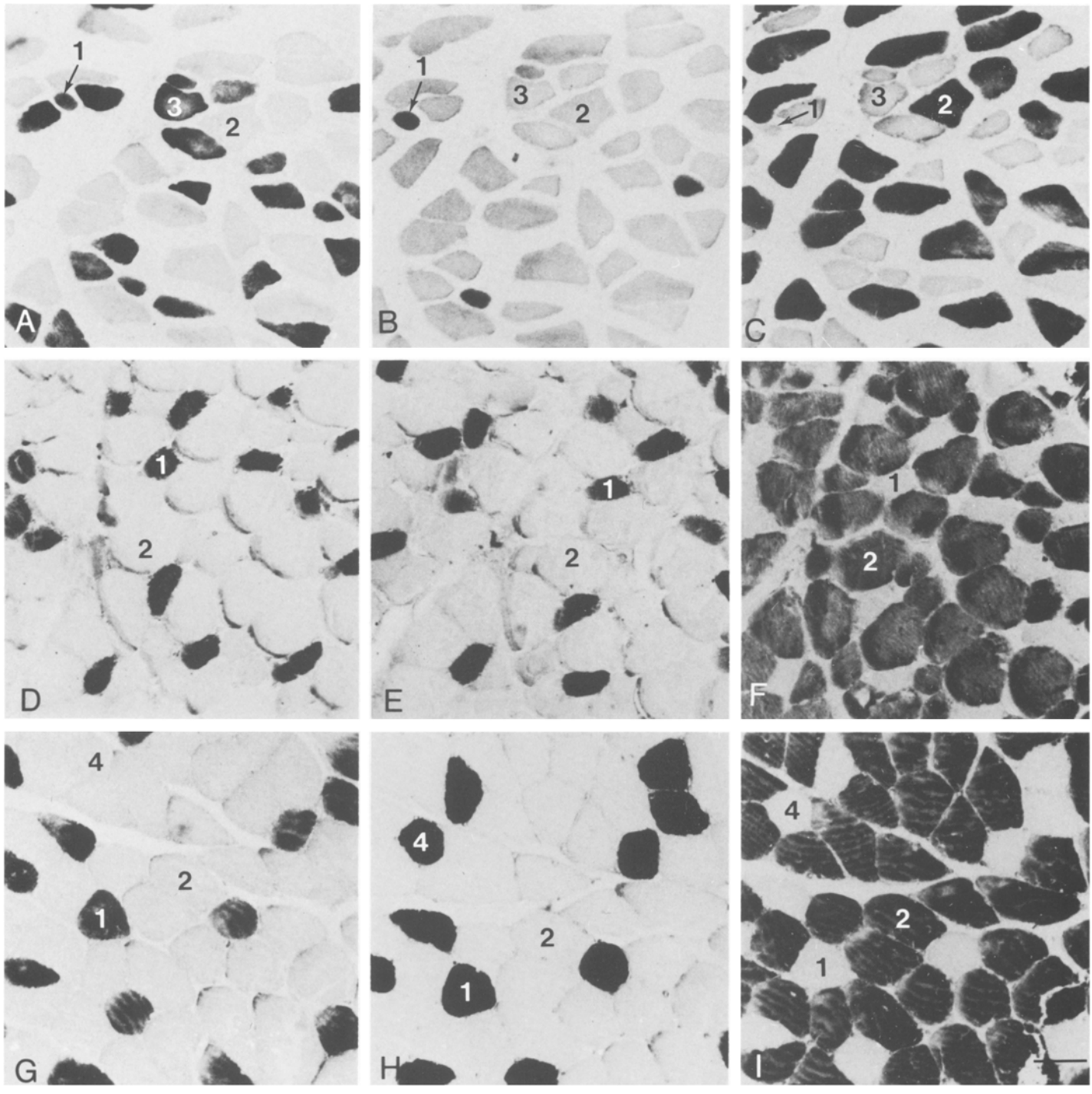


**Fig. 7.** Immunohistochemical analysis of serial sections of adult rabbit masseter (superficial MSS1 part; Bredman *et al.*, 1990a) (A–C), temporalis (D–F) and digastric (G–I) muscles. The sections were immunohistochemically stained with anti-α MHC (A,D,G), anti-slow MHC (B,E,H) and anti-fast MHC (C,F,I). Fibres containing slow and 'cardiac' α MHC (1), fast MHC (2), 'cardiac' α MHC (3) and fibres containing slow MHC (4) are indicated. The scale bar represents 45 μm.

IIC and IIC' fibres (see Table II). On the other hand, 'cardiac' α MHC containing muscle fibres were found to be absent in the rabbit cervical muscles, sternocleidomastoideus and trapezius muscles and in all limb muscles investigated. The fact that all 'cardiac' α-containing muscles are found in the head area (note that the heart originates from the most cranial part of the embryo) might indicate that this expression pattern is determined by the developmental history of the muscles concerned. Interestingly, there is a marked correspondence between the muscles reacting with anti-α MHC and the head and neck muscles in birds

which are known to have their connective tissue derived from embryonic neural crest cells (Noden, 1983a). As it is known that these cells form the connective tissue of the muscle and influence the pattern of differentiation and growth of the subsequently arriving myoblasts (Noden, 1983b) it is possible that there might be a role for these cells in inducing the expression of specific MHCs in skeletal muscle. More muscles from the head/neck region should be investigated to substantiate this idea.

The qualitative data presented in this paper clearly shows that 'cardiac' α is present in jaw muscles. However, its distribution shows species-specific variation. First, the fibre type in which it is expressed is different in human and rabbit jaw muscles (*vide supra*). Second, the 'cardiac' α MHC is present in rabbit but not in human digastric muscle. Previously we have shown there is a marked difference in types and proportions of fibres within and between the various muscle compartments of the rabbit masseter muscle (Bredman *et al.*, 1990a). It should be stressed, however, that in view of the heterogeneity and the size of these muscles it is possible that we have overlooked the 'cardiac' α MHC in the human digastric muscle. These findings make a re-evaluation by a quantitative, combined immuno- and enzyme-histochemical (ATPase) analysis necessary. This work is currently being undertaken.

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