Journal of Muscle Research and Cell Motility **17**, 657–667 (1996)

Myosin isoform transitions in four rabbit muscles during postnatal growth

F. GONDRET[1*], L. LEFAUCHEUR[2], A. D'ALBIS[3] and M. BONNEAU[2]

[1]*Station de Recherches Cunicoles, INRA, BP 27, 31326 Castanet-Tolosan cedex,* [2]*Station de Recherches Porcines, INRA, St-Gilles, 35590 L'Hermitage, and* [3]*Laboratoire de Biologie Physicochimique, Batiment 433, Unité de Recherche Associée au CNRS no. 1131, Université Paris-Sud 91405, Orsay, France*

Received 12 February 1996; revised 10 June 1996; accepted 1st July 1996

Summary

Four rabbit muscles (i.e. semimembranosus proprius, psoas major, biceps femoris and longissimus lumborum), differing in their fibre type composition in the adult, were investigated during postnatal development. Muscle samples were taken at 1, 7, 14, 21, 28, 35, 49 and 77 days of age. Complementary techniques were used to characterize myosin heavy chain (MHC) isoform transitions, i.e. SDS-PAGE, immunocytochemistry and conventional histochemistry. Good accordance was found between electrophoretic and immunocytochemical techniques. Our results show that rabbit muscles were phenotypically immature at birth. At 1 day of age, perinatal isoform represented 70–90% of the total isoform content of the muscles. Two generations of myofibres could be observed on the basis of their morphology and reaction to specific antibodies. In all muscles, primary fibres expressed slow MHC. In contrast, secondary generation of fibres never expressed slow MHC in future fast muscles, while half of them expressed slow MHC in the future slow-twitch muscle, the semimembranosus proprius. During the postnatal period, all muscles displayed a transition from embryonic to perinatal MHC isoforms, followed by a transition from perinatal to adult MHC isoforms. These transitions occured mainly during the first postnatal month. The embryonic isoform was no longer expressed after 14 days, except in longissimus where it disappeared after 28 days. On the contrary, large differences were found in the timing of disappearance of the perinatal isoform between the four muscles. The perinatal isoform disappeared between 28 and 35 days in semimembranosus proprius and 35 and 49 days in psoas and biceps femoris. Interestingly, the perinatal isoform was still present in 6% of the fibres in longissimus at 77 days, the commercial slaughter age, denoting a great delay in the maturation. Fate of each generation of fibres differed between muscles.

Introduction

Four types of myofibres have been recently identified in adult rabbit limb muscle, using the histochemical classification derived of Brooke and Kaiser (1970): slow twitch type I and fast twitch types IIA, IIB and IIX (or IID) (Hamalainen & Pette, 1993). However, no such clear cut classification can be obtained in fetuses or young animals (Guth & Samaha, 1972). At a molecular level, the content in adult Myosin Heavy Chain (MHC) isoforms is well correlated with the shortening velocity of the motor units (Reiser *et al.*, 1985) and can be used to characterize the functional properties of muscle. In the rabbit, four adult, i.e. I, IIa, IIb and IIx (Aigner *et al.*, 1993; Janmot & d'Albis, 1994), and two developmental, i.e. embryonic and perinatal (Hoh & Yeoh, 1979; d'Albis *et al.*, 1991), MHCs isoforms have been identified. During growth, adult-type isoforms gradually replace developmental myosins (Schiaffino *et al.*, 1989; La Framboise *et al.*, 1991). The profile of developmental and adult isoforms is then a useful marker to study maturation of muscle fibre types in growing rabbits.

Few systematic studies have investigated developmental changes in rabbit myofibre characteristics, particularly concerning myosin heavy chain isoform transitions. The major studies were performed on native myosin and myosin light chains (Hoh & Yeoh, 1979; d'Albis *et al.*, 1991; Briand *et al.*, 1993).

The aim of the present study was to investigate the developmental changes in the different myosin

*To whom correspondence should be addressed at: Station de Recherches Porcines, INRA, St-Gilles, 35590 L'Hermitage, France.

heavy chains isoforms during the postnatal period, from birth to commercial slaughter age in four rabbit muscles, selected according to their adult fibre type composition. Electrophoretic analysis in SDS-Page was used to separate and quantify the perinatal and the four adult MHCs isoforms. Histochemical and immunocytochemical studies, using specific monoclonal antibodies raised against MHCs, were used to localize the different MHC isoforms expressed in the different fibres.

Materials and methods

Animals and muscles sampling

A total of 40 New Zealand male rabbits were rendered unconscious by electric stunning and exsanguinated at 1, 7, 14, 21, 28, 35, 49 or 77 days of age. Five animals were analysed at each stage. Samples were taken from the following muscles: m. semimembranosus proprius (SMP) i.e. a slow-twitch muscle), m. psoas major (PS) (i.e. a fast-twitch muscle), m. biceps femoris (BF), and m. longissimus lumborum (LD) (i.e. two mixed muscles). The samples were frozen in isopentane cooled with liquid nitrogen.

Myosin preparation

Frozen muscles were cut into small pieces on ice and washed with a solution of 20 mM NaCl, 3.4 mM PO_4H_2Na, 1.6 mM PO_4HNa_2, 1 mM EGTA (pH 7). After centrifugation at 12 000 ×g, myosin was extracted with three volumes 100 mM sodium pyrophosphate, 5 mM EGTA, 1 mM dithiothreitol. The mixture was shaken during 30 min and then centrifugated at 12 000 ×g for 10 min. The supernatant was mixed with glycerol at a final concentration of 50% (v/v) and stored at −20° C until further analysis (d'Albis *et al.*, 1979).

Electrophoresis of myosin heavy chains (MHC) isoforms

Electrophoresis was performed according to the method described by Talmadge and Roy (1993) and modified by Janmot and d'Albis (1994). The entire gel unit was placed in a styrofoam box containing ice to maintain the temperature below 10° C during the 28 h run. Gels were stained with Coomassie Blue R-250 and quantification of the different isoforms was obtained by densitometry.

Cytochemical analysis

For immunocytochemistry, cryostat serial cross-sections (10 μm) were reacted with monoclonal antibodies specific to different MHC isoforms. Four monoclonal antibodies purchased from Novocastra (France) were used: Anti-Slow (AS), Anti-Fast (AF), Anti-Perinatal (AP) and Anti-Embryonic (AE). Three of them (AS, AF and AP) have been raised against rabbit muscles. The AE Ab has been raised against rat muscle. The antibodies have been shown to be specific of the corresponding MHC isoforms in skeletal muscle (Ecob-Prince *et al.*, 1989; Franchi *et al.*, 1990; d'Albis *et al.*, 1994). After fixation in acetone, sections were rinsed in a 10 mM Na-K-Phosphate Buffer Solution (PBS) (pH 7.4), containing 137 mM NaCl and 2.7 mM KCl. They were then incubated overnight in a humid chamber at 4° C with the first antibody, diluted 20-fold (AS, AF), tenfold (AP) or fivefold (AE). Specific antibody binding was revealed by the avidin biotin peroxidase technique (Vectastain ABC kit, Vector Laboratories, Burlingame, CA).

For actomyosin ATPase cytochemistry, the remaining serial cross-sections (10 μm) were stained after preincubations (pH 4.3 and 10.4) (Guth & Samaha, 1970). Myofibres were classified in type I, IIA, and IIB + IIX (Brooke & Kaiser, 1970). Additional serial sections were reacted for succinate dehydrogenase (SDH) activity (Nachlas *et al.*, 1957) in order to investigate the energy metabolism of the fibres (oxidative vs non-oxidative).

Statistical analysis

Means and SDs of the means were calculated from individual values by the usual procedures (SAS-package, 1985). The influence of age on the proportion of the various isoforms was tested by one-way analysis of variance. For each stage, the mean proportion of one isoform was compared with the value of the same isoform at 77 days (Dunnett test). Differences were considered to be significant at $p < 0.05$.

Results

ELECTROPHORESIS OF MHC ISOFORMS

Electrophoretic mobility of the different MHC isoforms

Five isoforms could be identified on the gels (Fig. 1). The electrophoretic mobilities of these isoforms increased in the order MHC-P, MHC-IIa, MHC-IIx, MHC-IIb and MHC-I (Aigner *et al.*, 1993; Janmot & d'Albis, 1994). The embryonic isoform (MHC-E) could not be separated from MHC-IIa because these two isoforms have been shown to have the same electrophoretic mobility (Janmot & d'Albis, 1994).

Changes in the isoform contents from birth to 77 days of age (Fig. 2)

The influence of age on the proportion of each isoform was highly significant during the 1–21 days period in the SMP and the 1–35 days period in PS, BF and LD muscles. At day 1, all muscles contained mainly MHC-P. In comparison to other muscles, SMP exhibited a lower proportion of MHC-P (73% vs 89–93%; $p = 0.01$). Thereafter, MHC-P steadily declined. This isoform was barely detectable and non significantly different from 0% to 28 days in SMP, 35 days in PS and BF, and 49 days in LD.

Muscle specific changes in the adult isoform content occured during the postnatal period, as shown in Fig. 2. In SMP, MHC-I represented 7% of total isoforms at day 1. This proportion increased rapidly during the first postnatal week (54% at day 7) and reached 100% from day 28 onwards, in parallel with the disappearance of developmental

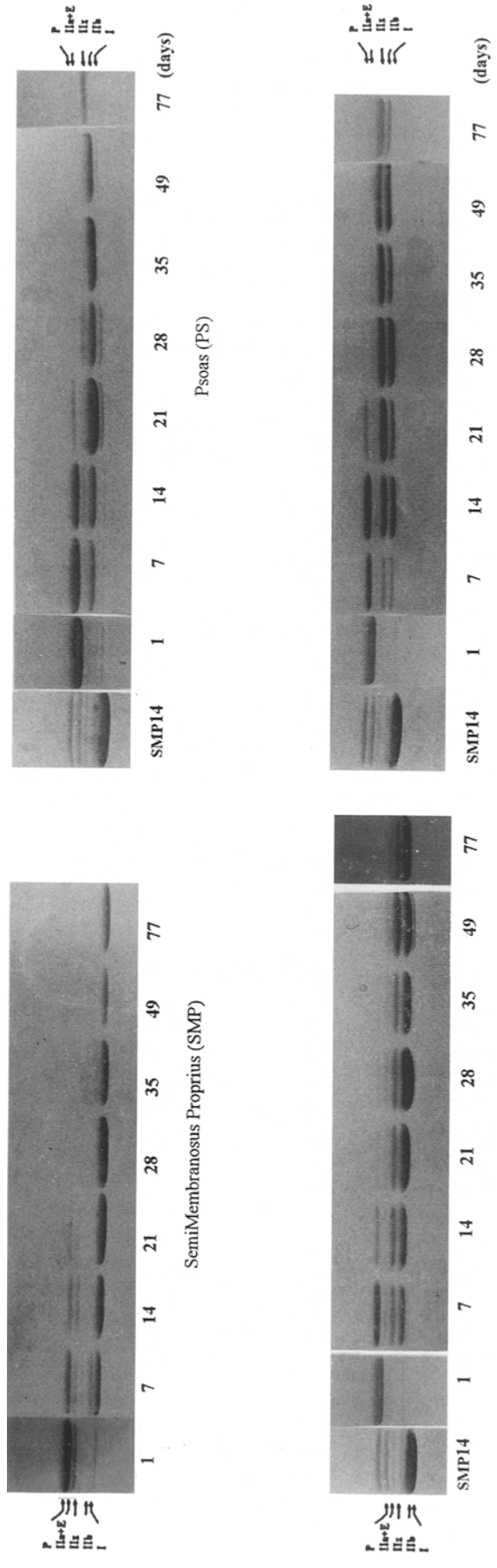


Fig. 1. Characterization of the different isoforms of MHC by electrophoresis (SDS-Page) from day 1 to day 77 in four muscles. SMP at day 14 (SMP14) was used as standard. MHC-IIa and MHC-E could not be separated and the corresponding band was noted IIa+E. The different isoforms migrated in the order: P, IIa+E, IIx, IIb, I.

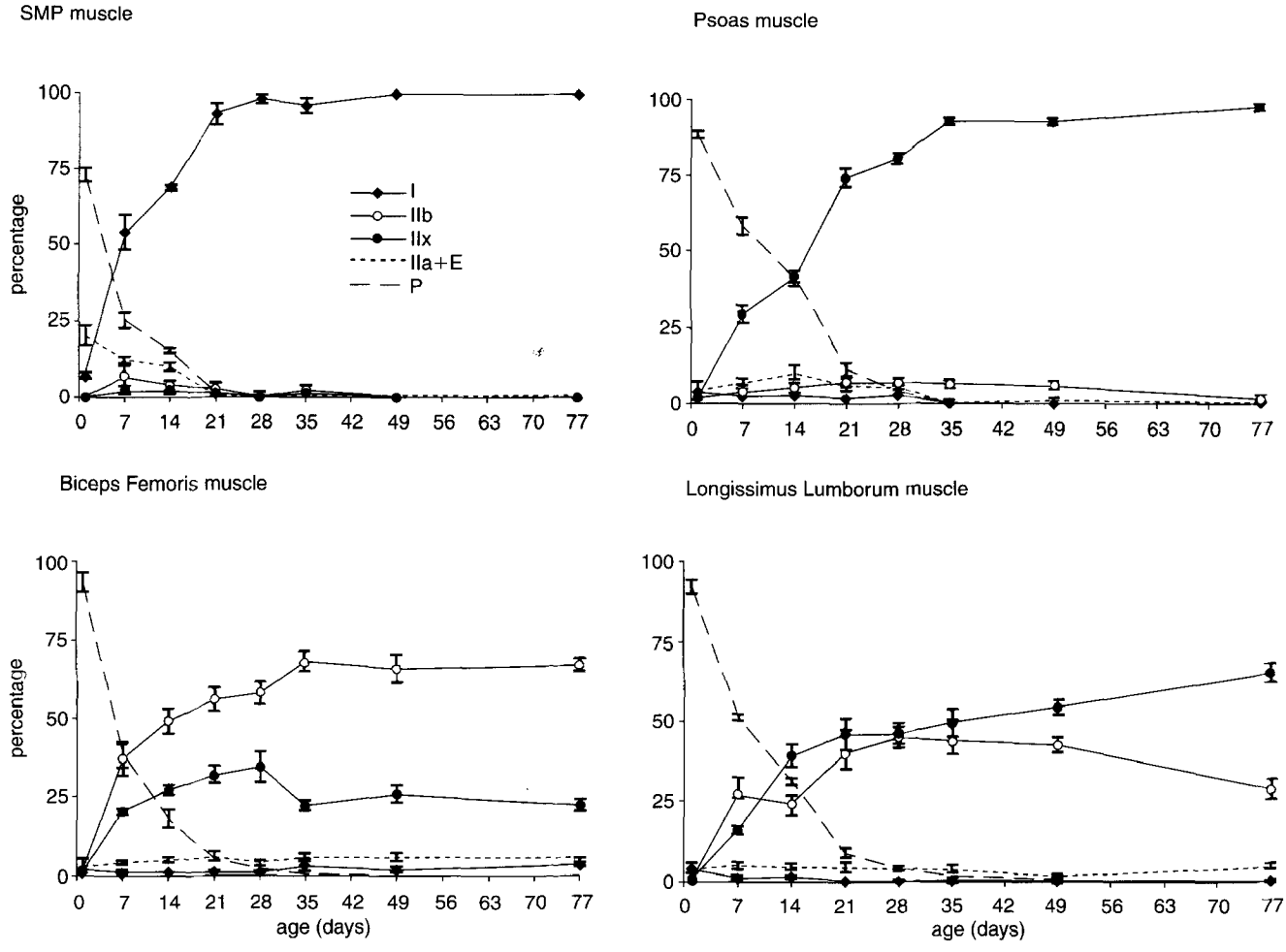


Fig. 2. Age-related changes in the proportion of the different MHC isoforms in the muscles.

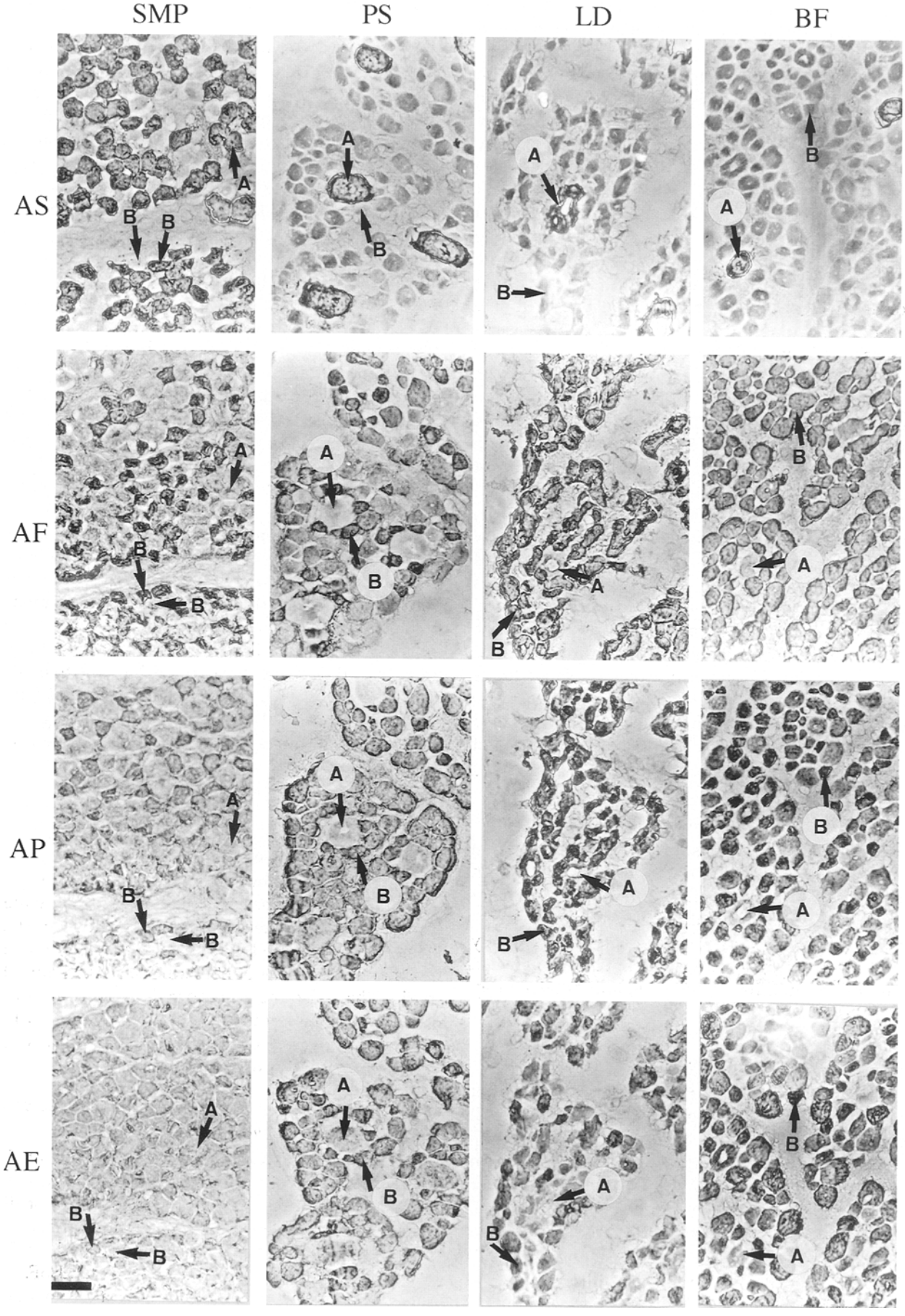
and adult fast isoforms. At day 77, SMP consisted only of MHC-I. In PS, MHC-I and MHC-IIa+E were very low (< 4%) from birth to 28 days of age and disappeared from 35 days onwards. MHC-IIb remained constant (2%) from birth to day 77. In BF, MHC-I remained more or less constant (2%) from birth to day-28 and then increased slowly up to 4% at day 77. The proportion of MHC-IIx and MHC-IIb rose gradually from 2% at birth to 34% and 58%, respectively, at day 28. Thereafter, the proportion of MHC-IIx decreased to reach 22% at day 77, in parallel with an increase in MHC-IIb (68% at day 77). In LD, MHC-I decreased from 4% at birth to a faintly detected level (1%) from day 21 onwards. The proportion of MHC-IIa+E remained about constant (4–5%) from birth to day 77. The proportion of MHC-IIx and MHC-IIb rose gradually from a barely detectable level at birth (1%) to 45 and 46%, respectively, at day-30. After day 35, the proportion of MHC-IIx increased to reach 66% at day 77, in parallel with a decrease of MHC-IIb (29% at day 77).

IMMUNOHISTOLOGY

Cell location of the adult slow and fast isoforms

Two categories of myofibres coexisted in all muscles at day 1: isolated primary myotubes, surrounded by small secondary fibres in larger number (Fig. 3). In all muscles, first generation fibres reacted

Fig. 3. Immunological analysis of transverse serial sections of four muscles with antibodies raised against slow MHC (AS), fast MHC (AF), perinatal MHC (AP) and embryonic MHC (AE) at the age of 1 day. (A) Cells of the first generation. (B) Cells of the second generation. Slow MHC was expressed only in first generation cells in PS, BF and LD muscles, while half of the second generation cells also expressed this isoform in SMP muscle. Scale bar = 50 μm.


SMP
PS
LD
BF
AS
AF
AP
AE
A
B

strongly with AS and negatively with AF Abs. The characteristics of the secondary generation differed between muscles. In SMP, 40% of the secondary fibres reacted strongly with AS and negatively with AF Abs. The other fibres (60%) were labelled by AF Ab. In contrast, in PS, BF and LD, all the secondary fibres reacted with AF Ab and were not labelled by AS Ab.

Thereafter, the two generations of fibres could not be distinguished on the basis of their size and morphology. The changes in the proportion of the fibres labelled by AS or AF Abs were similar to the changes in the proportion of the corresponding isoforms (MHC-I and MHC-II, respectively) in the electrophoretic diagramm. In SMP, the number of fibres labelled by AF Ab decreased progressively and fast myosin was no longer detected at day 28. In parallel, an increasing number of fibres were labelled only by AS Ab, to reach 100% of fibres from day 28 onwards. In PS, the number of fibres labelled by AS Ab rapidly decreased, with none of them labelled after day 14, while all fibres stained positively with AF Ab from day 7 onwards. In BF, some isolated fibres kept expressing only slow MHC throughout development, they represented 5% of the fibres at day 77. The proportion of fibres reacting with AF Ab increased during growth and reached 95% of total fibres at day 77. In LD, the number of fibres labelled by AS Ab decreased from birth to day 77, when only few isolated fibres (1% of the fibres) reacted with AS Ab. The percentage of fibres reacting with AF Ab increased during development to reach 99% of total fibres at day 77.

Temporal expression of the embryonic isoform

At birth, the embryonic isoform was expressed in most of the small secondary fibres (Fig. 3). In contrast, most of the primary myotubes were not labelled by AE Ab. Thereafter, the number of fibres stained with AE Ab decreased progressively throughout development and disappeared between day 14 and 21 in SMP, PS and BF, and between day 28 and 35 in LD.

Temporal disappearance of the perinatal MHC isoform

At birth, expression of the perinatal isoform was not detected in most of the large primary myotubes. All secondary fibres were labelled by AP Ab in PS, BF and LD (Fig. 3). In contrast, only 60% of them reacted with AP Ab in SMP. Thereafter, the number of fibres labelled by AP Ab decreased in all muscles, during development. There were no more fibres reacting with AP Ab at day 35 in SMP and at day 49 in PS and BF. In contrast, 6% of the fibres still weakly reacted with AP Ab at day 77 in LD.

Histoenzymology

At birth, all fibres in all muscles were stained with the same intensity for the ATPase reaction after preincubation at pH 4.3 (data not shown). Thereafter, heterogeneous staining intensities could be seen from day 7 to day 28, but clear cut classification was not possible. From day 35 onwards, type I fibres, as identified by AS Ab, exhibited a high staining intensity, while type II fibres (labelled by AF Ab) showed a weaker staining intensity. Clear distinction between type IIA (low staining intensity) and type IIB+IIX (intermediate staining intensity) was seen only after day-35. At day 77, fibres could be classified in I, IIA and IIB+IIX types. In SMP, all fibres were classified as type I fibres. In PS, about 100% of the fibres were classified as IIB+IIX, except in a small area at the periphery of the muscle where 3% of type IIA were observed. In BF, the ATPase staining showed that the muscle was composed of 5% type I, 9% type IIA and 86% type IIB+IIX. LD muscle consisted on 1% type I, 6% type IIA and 93% type IIB+IIX.

At birth, an evenly distributed, coarse granular SDH staining was observed in all muscles (Fig. 4). Different metabolic types of myofibres could not be distinguished at birth. In PS, LD and BF muscles, distinction between SDH negative and positive fibres was possible from 21 days onwards. In these muscles, the percentage of SDH positive fibres decreased from 100% at birth to reach 2% in PS, 10% in LD and 20% in BF at day 77 (Fig. 4). In SMP, all the fibres were positively stained from birth to day 77.

Discussion

Our results showed that rabbit muscles were phenotypically immature at birth and acquired gradually their mature phenotype, following a muscle specific pattern of transition between MHCs. This study stressed the importance of the first postnatal month for rabbit muscle maturation. This period has also been reported as critical for the determination of total number of myofibres, DNA content (Nouges, 1972, 1973) and the energetic metabolism (Bacou & Vigneron, 1976).

Electrophoretic pattern and histological data give complementary information

MHC-IIa and MHC-E could not be separated by their electrophoretic mobilities, in accordance with Janmot and d'Albis (1994). Specific staining of fibres with AE Ab demonstrated that embryonic isoform was present at birth in most of the secondary fibres and disappeared by day 21 in SMP, PS and BF. From this result, it can be deduced that, after this stage,

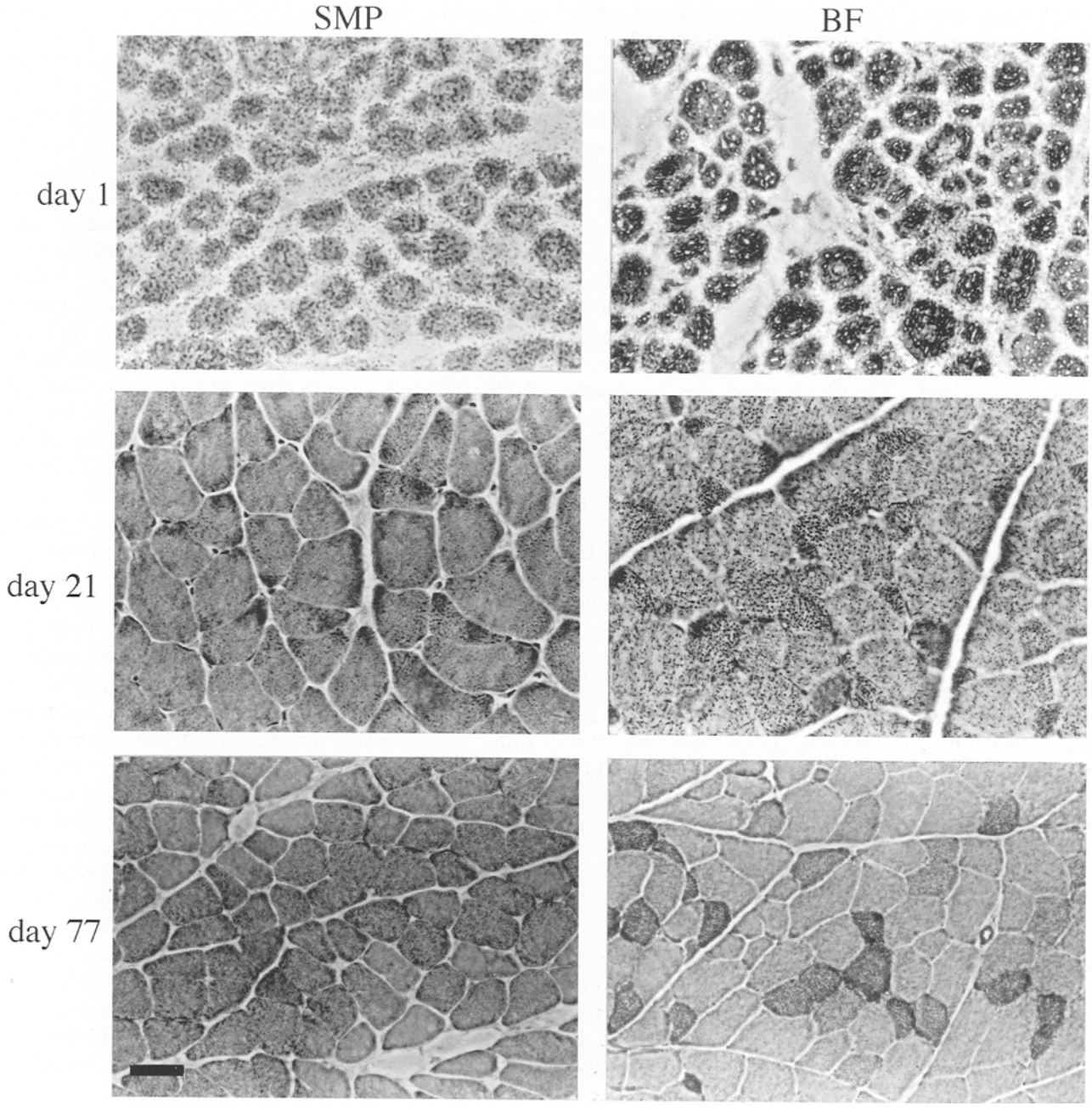


Fig. 4. Histochemical analysis of transverse serial sections of SMP and BF muscles after treatment with SDH (succinate dehydrogenase) at the age of 1 (scale bar = 75 μm), 21 (scale bar = 50 μm) and 77 days (scale bar = 25 μm). Distinction between negative and positive fibres was possible only from 21 days onwards in BF. In SMP, all fibres were positively stained from birth to day 77.

the band corresponding to both MHC-IIa+E in the electrophoretic pattern, was MHC-IIa. The same conclusion can be drawn after day 35 in LD. Good matching was generally found between electrophoretic and immunohistological methods for identifying changes in the MHC isoform content. If we assume that ATPase activity of developmental isoforms is intermediate between that of adult and slow isoforms, changes in MHC polymorphism (present study) are consistent with changes in ATPase activity previously reported by Syrovy (1977) and Syrovy and Gutmann (1977).

Rabbit muscle is immature at birth

At 1 day of age, all the investigated muscles were characterized by a very immature phenotype. Two populations of myotubes (i.e. primary and secondary generation) were biochemically and morphologically

distinct. In contrast, these two generations of cells were not distinguishable after 210 days of gestation in bovine muscles (Picard *et al.*, 1994), denoting a more mature state in bovine than in rabbit. Our results showed that most of the fibres still expressed developmental MHC isoforms (i.e. embryonic and perinatal). Depending on the muscle, 70–90% of total MHC was perinatal at birth. The perinatal isoform was still present until at least 4 weeks of age in all muscles. This contrasts with cattle and pig where it disappears during the last period of gestation (210–250 days of gestation) in cattle (Picard *et al.*, 1994) and during the two postnatal weeks in pig (Lefaucheur *et al.*, 1995) but is similar to the rat (d'Albis *et al.*, 1989). On the basis of this criterion, immaturity of these species at birth could be expressed as rabbit = rat > pig > cattle. At birth, we observed in all muscles an evenly distributed coarse granular SDH staining, as noticed in pig (Lefaucheur & Vigneron, 1986). A clear-cut classification between SDH negative or positive fibres in future fast muscles was possible only from 21 days onwards. This contrasts with cattle where this distinction is possible shortly before birth (Robelin *et al.*, 1991). Thus, the maturation of rabbit muscle fibres, as determined by contractile and metabolic aspects, is delayed in comparison to cattle. The evenly distributed coarse granular staining we observed at birth after the SDH reaction corresponds to a high oxidative metabolism, as previously reported by Bacou and Vigneron (1976) and Briand and colleagues (1993).

The temporal disappearance of developmental isoforms is muscle dependent

The developmental myosins disappeared progressively during postnatal development. A transition from embryonic to perinatal MHC isoforms was observed, followed by a transition from perinatal to adult MHCs isoforms. The major transitions were observed during the first 5 postnatal weeks. Several studies have shown that thyroid hormones inhibit synthesis of developmental myosins and activate adult fast myosin synthesis in rabbit (D'Albis *et al.*, 1987) and rat (Gambke *et al.*, 1983; Butler-Browne *et al.*, 1984). Therefore, the switch from developmental to adult isoforms we observed is consistent with the peak of serum thyroid hormones occuring at 7 postnatal days (2.73 ng ml^{-1}) as noted by Devaskar and colleagues (1986) in rabbit. The immaturity of rabbit muscles at birth could be related the low degree of rabbit thyroid gland morphological development (Mach, 1977).

Large differences in the timing of MHC transitions were observed between muscles. These differences affected more the perinatal to adult transitions than the embryonic to perinatal transitions, as previously noticed in rat (d'Albis *et al.*, 1989). However, a delay in the disappearance of the embryonic isoform was observed in the LD muscle (between 28 and 35 days) in comparison to the other three muscles (between 14 and 21 days), denoting a delay in the maturation of LD muscle. Differences between the muscles were more important when the perinatal isoform was considered. This isoform disappeared between 28 and 35 days in SMP, 35 and 49 days in PS and BF, while it was still present in 6% of the fibres in LD at 77 days. Our results showed that the rank order of maturation of the selected muscles is SMP > PS = BF > LD. A persistance of developmental MHC isoforms has been previously evidenced in adult rat Masseter (d'Albis *et al.*, 1986). However, the present study is the first one to demonstrate the presence of the perinatal isoform in rabbit LD muscle at 77 days of age, the commercial slaughter age.

Our results show that each muscle was subjected to its own programme of MHC transition. A difference of precocity between muscles has also been observed in rabbit (d'Albis *et al.*, 1991), cattle (Picard *et al.*, 1994) and rat (d'Albis *et al.*, 1989). The difference of maturation between muscles could be the result of muscle physical activity. Indeed, it is of interest to note that SMP, the earliest maturing muscle, is involved in the posture and therefore is required to function just after birth. On the contrary, muscles involved in movements (PS, BF and LD muscles) are gradually used during the first postnatal weeks, as mobility of the animals is increasing. Another mechanism could involve thyroid hormones since each muscle do not respond to the same way to these hormones (Izumo *et al.*, 1986; d'Albis *et al.*, 1990). Type I muscle seemed to exhibit a higher sensitivity to thyroid hormones (Van Hardeveld & Kassenaar, 1978, in rat; Dainat *et al.*, 1986, in chickens), which would contribute to explain the earlier maturation of SMP muscle, a slow-twitch muscle.

Transitions between slow and fast adult MHC isoforms occur during the postnatal period

Up to day 7 or 14, all muscles contained a low proportion of both fast and slow adult type MHCs. In all muscles, the slow isoform was synthesized very early during development. Similar results have been obtained in rat (La Framboise *et al.*, 1991; Hughes *et al.*, 1993), chicken (Stockdale & Miller, 1987), cattle (Robelin *et al.*, 1993) and pig (Lefaucheur *et al.*, 1995). The slow isoform was initially expressed only in primary myotubes in rabbit future fast muscles and in primary and a subpopulation of secondary myotubes in rabbit future slow muscles, as noticed previously in rat (Rubinstein & Kelly, 1981) or cat (Hoh *et al.*, 1988).

After the disappearance of the developmental isoforms, our results demonstrated a muscle-specific transition between adult isoforms during the postnatal period. A schematic representation of the fate of the different generations of fibres is shown in Fig. 5. Briefly, a fast-to-slow myosin transition occured in adult slow muscles, whereas a slow-to-fast myosin transition occured in predominantly adult fast muscles. The transitions from slow-to-fast isoforms in PS and LD muscles or from fast-to-slow isoforms in SMP are consistent with the age-related changes in myosin ATPase activity observed by Briand and colleagues (1993) in rabbit muscles. Indeed, these authors have shown that myosin ATPase activity increases in future fast muscles up to adulthood whereas it remains low in slow muscle.

The postnatal transitions between slow and fast isoforms are likely influenced by innervation, and especially the pattern of activity it imposes on the muscle fibres. The general view is that muscle fibres turn to a fast phenotype in a neurally independant programm of transitions, unless they receive a neural signal that induces the expression of slow-type MHCs and the inhibition of fast type myosin expression (Butler-Browne & Whalen, 1984; Swynghedaw, 1986; Jakubiec-Puka *et al.*, 1990). However, the opposite situation could be present in the rabbit. Indeed, denervation studies have shown that innervation appears to be necessary to maintain the fast phenotype, but not the slow phenotype (d'Albis *et al.*, 1994). This controversy shows that more studies are needed to determine the effect of innervation on normal maturation of fibres during development in rabbit.

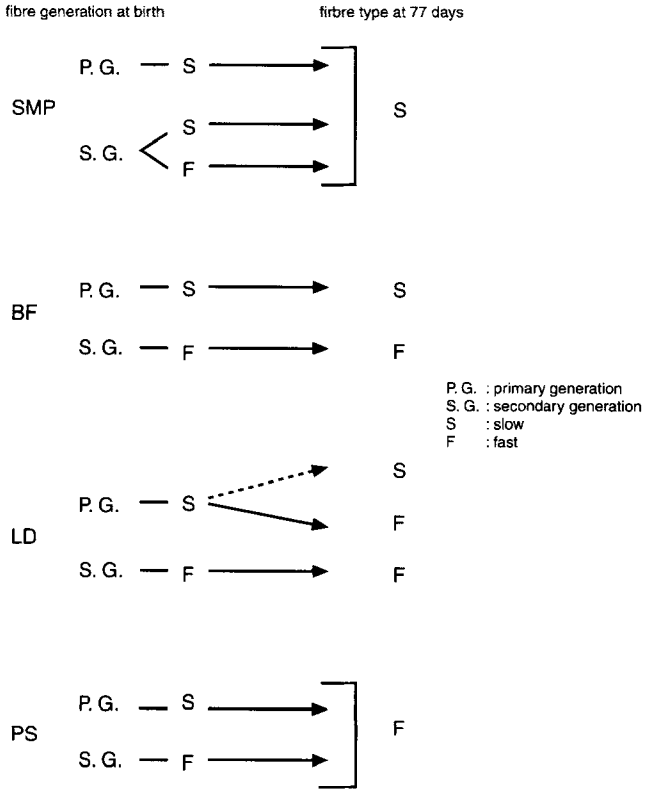


Fig. 5. Proposed simplified lineage of fibre generation after birth, on the basis of the expression of MHC isoforms evidenced by immunocytochemistry.

Conclusion

Based on the proportions of developmental MHC isoforms, our study showed that rabbit muscles were very immature at birth. Dramatic changes affected the MHC pattern during the first postnatal month. Each muscle expressed a specific programme of transition from birth onwards and matured in the following order SMP > PS = BF > LD. Immaturity of rabbit skeletal muscles at birth and during the first postnatal month could lead to a high plasticity of muscle characteristics during this period, which could allow a manipulation of muscular characteristics by nutritional, environmental and hormonal factors.

References

AIGNER, S., GOHLSCH, B., HÄMÄLÄINEN, N., STARON, R. S., UBER, A., WEHRLE, U. & PETTE, D. (1993) Fast myosin heavy chain diversity in skeletal muscles of the rabbit: heavy chain IId, not IIb predominates. *Eur. J. Biochem.* **211**, 367–72.

BACOU, F. & VIGNERON, P. (1976) Evolution périnatale des voies métaboliques glycolytique et oxydative de divers types de muscles squelettiques du lapin et du poulet. *Ann. Biol. Anim. Biochim. Biophys.* **16**, 675–86.

BRIAND, M., BOISSONNET, G., LAPLACE-MARIEZE, V. & BRIAND, Y. (1993) Metabolic and contractile differenciation of rabbit muscles during growth. *Int. J. Biochem.* **25**, 1881–7.

BROOKE, M. H. & KAISER, K. K. (1970) Muscle fiber types: how many and what kind? *Arch. Neurol.* **23**, 369–79.

BUTLER-BROWNE, G. S. & WHALEN, R. G. (1984) Myosin isozyme transition occuring during the postnatal development of the rat soleus muscle. *Dev. Biol.* **102**, 324–34.

BUTLER-BROWNE, G., HERLICOVIEZ, D. & WHALEN, R. G. (1984) Effects of hypothyroidism on myosin isozyme transitions in developing rat muscle. *FEBS Lett.* **166**, 71–5.

DAINAT, J., BRESSOT, C., REBIERE, A. & VIGNERON, P. (1986) Ontogenesis of triiodothyronine nuclear receptors in three skeletal muscles in male and female chicks. *Gen. Comp. Endocrinol.* **62**, 479–84.

D'ALBIS, A., PANTALONI, C. & BECHET, J. J. (1979) An electrophoretic study of native myosin isozymes and of their subunit content. *Eur. J. Biochem.* **99**, 261–72.

D'ALBIS, A., JANMOT, C. & BECHET, J. J. (1986) Comparison of myosins from the masseter muscle of adult rat, mouse and guinea-pig. *Eur. J. Biochem.* **156**, 291–6.

D'ALBIS, A., LENFANT-GUYOT, M., JANMOT, C., CHANOINE, C., WEINMAN, J. & GALLIEN, C. L. (1987) Regulation by thyroid hormones of terminal differenciation in the skeletal dorsal muscle. *Dev. Biol.* **123**, 25–32.

D'ALBIS, A., COUTEAUX, R., JANMOT, C. & ROULET, A. (1989) Specific programs of myosin expression in the postnatal development of rat muscles. *Eur. J. Biochem.* **183**, 583–90.

D'ALBIS, A., CHANOINE, C., JANMOT, C., MIRA, J. C. & COUTEAUX, R. (1990) Muscle-specific response to thyroide hormone of myosin isoform transitions during rat postnatal development. *Eur. J. Biochem.* **193**, 155–61.

D'ALBIS, A., JANMOT, C. & COUTEAUX, R. (1991) Species and muscle type dependence of perinatal isomyosin transitions. *Int. J. Dev. Biol.* **35**, 53–6.

D'ALBIS, A., GOUBEL, F., COUTEAUX, R., JANMOT, C. & MIRA, J. C. (1994) The effect of denervation on myosin isoform synthesis in rabbit slow-type and fast-type muscle during terminal differenciation. Denervation induces differenciation into slow-type muscle. *Eur. J. Biochem.* **223**, 249–58.

DEVASKAR, U. P., DEVASKAR, S. U., SADIQ, H. F. & CHECHANI, V. (1986) Ontogeny of plasma-free thyroxine and triodothyronine concentrations during the perinatal period and maternofoetal transfer of thyroid hormones in the rabbit. *Dev. Pharmacol. Ther.* **9**, 115–23.

ECOB-PRINCE, M., HILL, M. & BROWN, W. (1989) Immunocytochemical demonstration of myosin heavy chain expression in human muscle. *J. Neurol Sci.* **91**, 71–8.

FRANCHI, L. L., HILL, M., BROWN, W. E., MAYNE, C. N., ELLIOTT, L. & SALMONS, S. (1990) Subcellular localization of newly incorporated myosin in rabbit fast skeletal muscle undergoing stimulation-induced type transformation. *J. Muscle Res. Cell Motil.* **11**, 227–39.

GAMBKE, B., LYONS, G. E., HASELGROVE, J., KELLY, A. M. & RUBINSTEIN, N. A. (1983) Thyroidal and neural control of myosin transitions during development of rat fast and slow muscles. *FEBS Lett.* **156**, 335–9.

GUTH, L. & SAMAHA, F. J. (1970) Procedure for the histochemical demonstration of actomyosin ATPase. *Exp. Neurol.* **28**, 365–70.

GUTH, L. & SAMAHA, F. J. (1972) Erroneous interpretations which may result from application of the 'myofibrillar ATPase' histochemical procedure to developing muscle. *Exp. Neurol.* **34**, 465–75.

HAMALAINEN, N. & PETTE, D. (1993) The histochemical profiles of fast fiber types IIB, IID and IIA in skeletal muscles of mouse, rat and rabbit. *J. Histochem. Cytochem.* **41**, 733–43.

HOH, J. F. Y. & YEOH, G. P. S. (1979) Rabbit skeletal myosin isoenzymes from fetal, fast-twitch and slow-twitch muscles. *Nature* **280**, 321–2.

HOH, J. F. Y., HUGHES, S., HALE, P. T. & FITZSIMMONS, R. B. (1988) Immunocytochemical and electrophoretic analyses of changes in myosin gene expression in cat limb fast and slow muscles during postnatal development. *J. Muscle Res. Cell Motil.* **9**, 30–47.

HUGHES, S. M., CHO, M., KARSCH-MIZRACHI, I., TRAVIS, M., SILBERSTEIN, L., LEINWAND, L. A. & BLAU, H. M. (1993) Three slow myosin heavy chains sequentially expressed in developing mammalian skeletal muscle. *Dev. Biol.* **158**, 183–99.

IZUMO, S., NADAL-GINARD, B. & MADHAVI, V. (1986) All members of the MHC multigene family respond to thyroid hormone in a highly tissue-specific manner. *Science* **231**, 597–600.

JAKUBIEC-PUKA, A., KORDOWSKA, J., CATANI, C. & CARRARO, U. (1990) Myosin heavy chain isoform composition in striated muscle after denervation and self-reinnervation. *Eur. J. Biochem.* **193**, 623–8.

JANMOT, C. & D'ALBIS, A. (1994) Electrophoretic separation of developmental and adult rabbit skeletal muscle myosin heavy chain isoforms: example of application to muscle denervation study. *FEBS Lett.* **353**, 13–15.

LA FRAMBOISE, W. A., DAOOD, M. J., GUTHRIE, R. D., SCHIAFFINO, S., MORETTI, P., BROZANSKI, B., ONTELL, M. P., BUTLER-BROWNE, G. S., WHALEN, R. G. & ONTELL, M. (1991) Emergence of the mature myosin phenotype in the rat diaphragm muscle. *Dev. Biol.* **144**, 1–15.

LEFAUCHEUR, L. & VIGNERON, P. (1986) Postnatal changes in some histochemical and enzymatic characteristics of three pigs muscles. *Meat Sci.* **16**, 199–216.

LEFAUCHEUR, L., EDOM, F., ECOLAN, P. & BUTLER-BROWNE, G. S. (1995) Pattern of muscle fiber type formation in the pig. *Dev. Dyn.* **203**, 27–41.

MACH, Z. (1977) Postnatal changes in the thyrotropic hormone potency of the pig and the rabbit pituitary gland. *Folia Biol.* **25**, 328–39.

NACHLAS, M. M., TSOU, K., DE SOUZA, E., CHENG, C. & SELIGMAN, A. M. (1957) Cytochemical demonstration of succinic deshydrogenase by the use of a new p-nitrophenyl substituted ditretrazole. *J. Histochem. Cytochem.* **5**, 420–36.

NOUGES, J. (1972) Etude de l'évolution du nombre des fibres musculaires au cours de la croissance postnatale du muscle chez le lapin. *C. R. Soc. Biol. Montpellier.* **166**, 165–72.

NOUGES, J. (1973) Etude histologique de la croissance postnatale des muscles soleus et accessorius latissimi dorsi chez le lapin commun. *Ann. Biol. Anim. Biochim. Biophys.* **13**, 37–50.

PICARD, B., ROBELIN, J., PONS, F. & GEAY, Y. (1994) Comparison of the foetal development of fibre types in four bovine muscles. *J. Muscle Res. Cell Motil.* **15**, 473–86.

REISER, P. J., MOSS, R. L., GIULAN, G. G. & GREASER, M. L. (1985) Shortening velocity in single fibers from adult rabbit soleus muscles is correlated with myosin heavy chain composition. *J. Biol. Chem.* **260**, 9077–80.

ROBELIN, J., LACOURT, A., BECHET, D., FERRARA, M., BRIAND, Y. & GEAY, Y. (1991) Muscle differenciation in the bovine fetus: a histological and histochemical approach. *Growth Dev. Aging* **55**, 151–60.

ROBELIN, J., PICARD, B., LISTRAT, A., JURIE, C., BARBOIRON, C., PONS, F. & GEAY, Y. (1993) Myosin expression

in semitendinosus muscle during fetal development of cattle: immunocytochemical and electrophoretic analyses. *Reprod. Nutr. Dev.* **33**, 25–41.

RUBINSTEIN, N. A. & KELLY, A. M. (1981) Development of muscle fiber specialization in the rat hindlimb. *J. Cell Biol.* **90**, 128–44.

SAS User's Guide (1985) Statistical Analysis System Institute. Inc., Cary, NC.

SCHIAFFINO, S., GORZA, L., SARTORE, S., SAGGIN, L., AUSONI, S., VIANELLO, M., GUNDERSEN, K. & LOMO, T. (1989) Three myosin heavy chain isoforms in type 2 skeletal muscle fibres. *J. Muscle Res. Cell Motil.* **10**, 197–205.

STOCKDALE, F. E. & MILLER, J. B. (1987) Review the cellular basis of myosin heavy chain isoform expression during development of avian skeletal musculature. *Dev. Biol.* **123**, 1–9.

SWYNGHEDAW, B. (1986) Developmental and functional adaptation of contractile proteins in cardiac and skeletal muscles. *Physiol. Rev.* **66**, 710–71.

SYROVY, I. (1977) Myosin-characteristics of the semitendinosus and extensor digitorum longus muscle of new-born and adult rabbit. *Int. J. Biochem.* **8**, 847–8.

SYROVY, I. & GUTMANN, E. (1977) Differenciation of myosin in soleus and extensor digitorum longus muscle in different animals species during development. *Pflügers Arch.* **369**, 85–9.

TALMADGE, R. G. & ROY, R. R. (1993) Electrophoretic separation of rat skeletal muscle myosin heavy-chain isoforms. *J. Appl. Physiol.* **75**, 2337–40.

VAN HARDEVELD, C. & KASSENAAR, A. A. H. (1978) Thyroid hormone uptake and T4 derivated T3 formation in different skeletal muscle types of normal and hyperthyroid rats. *Acta Endocr.* **88**, 306–20.