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**Original article**

# Enzyme-linked immunosorbent assay for myosin heavy chains in the horse

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**Summary** — The content in slow and fast myosin heavy chains (MHC 1 and MHC 2) of 5 equine muscles was determined using an enzyme-linked immunosorbent assay. The results obtained with this immunoenzymatic method were compared with complementary techniques: electrophoresis and immunohistochemistry. Slices of masseter, diaphragm, tensor faciae latae, semitendinosus and cutaneus trunci were obtained from a 12-year-old saddle horse after slaughter. Muscular proteins were specifically extracted to be analysed by ELISA. The technique used 2 complimentary monoclonal antibodies (MAb). MAb 1 was prepared from a human atrium specimen that reacted specifically against MHC 1. Mab 2 was prepared from myosin of rabbit psoas muscle and reacted against MHC 2. The masseter muscle contained solely MHC 1 (100%) and this was confirmed by electrophoresis and immunohistochemistry. By contrast, the cutaneus trunci was very poor in MHC 1 (1.3%) and was entirely composed of MHC 2 (98.7%) which was confirmed by the other techniques. The diaphragm, tensor fasciae latae and semitendinosus contained 89, 40 and 2% of MHC 1, respectively. It was concluded that this ELISA method made it possible to measure a wide range of MHC contents in equine muscles with a good reproducibility. The results were consistent with those of the other fibre typing techniques. Moreover, this immunoenzymatic method is less time consuming than histological techniques and therefore offers new perspectives for muscle fibre typing in the horse.

**horse / muscle / myosin heavy chain / ELISA**

**Résumé — Méthode de dosage ELISA des chaînes lourdes de la myosine chez le cheval.** *La composition en chaînes lourdes de la myosine lente (MHC 1) et rapide (MHC 2) a été déterminée dans 5 muscles équins par une méthode de dosage ELISA. Les résultats obtenus par cette technique immunoenzymatique ont été comparés avec d'autres méthodes de typage des fibres musculaires : l'électrophorèse et l'immunohistologie. Des tranches de masséter, de diaphragme, de* Tensor fasciae latae, *de* Semitendinosus, *et de* Cutaneus trunci *ont été prélevées sur un cheval de selle de 12 ans euthanasié pour colique. Les protéines musculaires sont extraites par une méthode spécifique et les chaînes*

*lourdes de la myosine sont dosées par une technique ELISA au moyen de 2 anticorps monoclonaux (MAb) complémentaires. MAb 1 a été préparé à partir de muscle cardiaque de l'atrium humain et il réagit spécifiquement avec MHC 1. MAb 2 a été préparé à partir de muscle psoas de lapin et réagit spécifiquement avec MHC 2. Le masséter est exclusivement composé de MHC 1 (100%), ce qui est confirmé par l'électrophorèse et l'immunohistologie. À l'inverse, le* Cutaneus trunci *est presque exclusivement constitué de MHC 2 (98,7% MHC 2). Le diaphragme, le* Tensor fasciae latae *et le* Semitendinosus *contiennent respectivement 89, 40 et 2% de MHC 1. Cette technique de dosage ELISA des chaînes lourdes de la myosine permet de mesurer avec une bonne reproductibilité des compositions très variables en MHC sur les muscles du cheval. Les résultats de dosage sont en accord avec les autres méthodes de dosage. De plus, pour effectuer le typage musculaire, la productivité de cette méthode immunoenzymatique est plus grande que les techniques histologiques, ce qui ouvre de nouvelles perspectives d'applications pour analyser des effectifs importants.*

***cheval / muscle / chaîne lourde de la myosine / ELISA***

## INTRODUCTION

Numerous studies have been performed to demonstrate the various effects of breed (Gunn, 1978; Snow and Guy, 1980), age (Essen *et al*, 1980; Roneus and Lindholm, 1991; Roneus, 1993), sex (Roneus and Lindholm, 1991; Roneus, 1993), training programme and detraining (Gottlieb-Vedi, 1988; Foreman *et al*, 1990; Valette *et al*, 1990; Sinha *et al*, 1991; Lopez-Rivero *et al*, 1991) on muscle fibre typing in the horse. Since the first investigation of equine muscle metabolism performed by Lindholm and Piehl (1974), several classifications of muscle fibre types have been proposed on the basis of metabolic or contractile properties. Sinha and Rose (1992) published a review of the system of muscle fibre classification and their limits in the horse. The histochemical method based on the sensitivity of myosin ATPase activity of different fibre types to various pH has become the most frequently used to identify 3 major types of fibres in humans (Brooke and Kaiser, 1970) and horses: slow-twitch fibres (type 1) and fast-twitch fibres (type 2A and 2B). This provides quantitative information about the percentages and cross-sectional areas of fibre types. Unfortunately, this is a time-consuming method, which reduces the extent of its application to experimental studies. The development of immunohistochemical techniques offers new possibilities of differentiation of fibre types using monoclonal antibodies against tubulin (Horak *et al*, 1991) or myosin heavy chains (Sinha and Rose, 1992). The production of monoclonal antibodies against slow myosin heavy chain and fast myosin heavy chain has been undertaken in other species to develop immunohistological and immunoenzymatic techniques (Winkelmann *et al*, 1983; Danieli Betto *et al*, 1986; Robelin *et al*, 1993).

The purpose of this study was to present the application of an enzyme-linked immunosorbent assay (ELISA) method for measuring the percentage of slow and fast myosin heavy chains (MHC 1 and 2) in 5 equine muscles. The reproducibility of the method was calculated and the results were compared with those obtained by immunohistochemistry and electrophoresis.

## MATERIAL AND METHODS

### *Muscle samples*

Slices of masseter, cutaneus trunci, semitendinosus, tensor fasciae latae and diaphragm were obtained from a 12-year- old horse (gelding, Selle Français), slaughtered for colic which had not

affected muscle fibre. For each muscle, 3 samples less 5 cm from each other were excised (fig 1). Two samples were cut to obtain 4 sub-samples in order to make the MHC analyses and a myosin electrophoresis. One sample for immunochemistry was treated by immersion in isopentane and frozen in liquid nitrogen and stored at –80°C until analysis. The other samples were frozen in liquid nitrogen and stored at –80°C until analysis.

### *Muscle fibre typing by an ELISA method*

For each muscle sub-sample, 1 protein extraction was performed and a triplicate ELISA analysis was performed from the same protein extract. In order to verify the reproducibility of the analyses, the procedure was repeated twice (2 d) on the same extract (fig 1).

### Specific protein preparation

A frozen muscle sub-sample (20–30 mg) was crushed in 3 ml buffer saline solution: 0.5 mol/l NaCl, 20 mmol/l sodium pyrophosphate (NaPPi), 50 mmol/l Tris, 1 mmol/l EDTA, 1 mmol/l dithiothreitol (DTT). After 10 min at 4°C (on ice), the sample was centrifuged for 5 min at 5 000 *g*. The supernatant fluid was then mixed with glycerol at a final concentration of 50% (v/v) and stored at –20°C until use. The protein concentration was measured according to the method of Bradford (1976), using a preparation of bovine serum albumin (BSA) at 1 mg/ml in the same buffer as the standard.

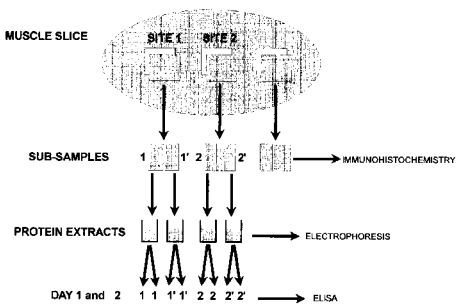


**Fig 1.** Procedure of muscle sampling.

### Myosin heavy chain monoclonal antibodies

In order to determine the percentage of MHC 1 and MHC 2 of various equine muscles, 2 complimentary antibodies were used. The monoclonal antibody MAb 1 (ref F36-5B9 from Biocytex, Marseille, France) reacted specifically against slow myosin heavy chain, MHC 1. It was prepared from a human atrium specimen according to the conditions described by Léger *et al* (1985). The monoclonal antibody MAb 2 (NCL-MHCf, from Novocastra Laboratories Ltd, UK) reacted specifically against fast myosin heavy chain (MHC 2), and was obtained from myosin of rabbit psoas muscle (Ecob-Prince *et al*, 1989).

### ELISA

The method was adapted from the radioimmunoassay used in poultry by Winkelmann *et al* (1983) and then modified for cattle by Picard *et al* (1994). An aliquot of the supernatant fluid stored at –20°C was subsequently diluted in a solution containing 0.02 mol/l Tris HCl, 0.5 mol/l NaCl (pH 7.4) at a working concentration of 2.4 µg of protein/ml. Fifty microlitres were placed in each well of a microtiter plate which was incubated overnight at 4°C. The following day the wells were rinsed 5 times with a solution containing 0.1 mol/l Tris HCl, 0.6 mol/l NaCl (pH 7.4) and 0.1% (v/v) Tween 20. One hundred microlitres of buffer containing 0.02 mol/l Tris HCl, 0.12 mol/l NaCl (pH 7.0), 0.1% (v/v) Tween 20 and 10% (w/v) skimmed milk were added to the wells. After an incubation period of 30 min at room temperature, the plate was rinsed 5 times with the wash solution.

Fifty microlitres of MAb 1 diluted 1/400 fold or 50 µl of MAb 2 diluted 1/20 000, both in phosphate-buffered saline containing 10% skimmed milk (PBS) were then placed in each well. After an incubation period of 90 min at room temperature, 50 µl of the secondary alkaline phosphatase-labeled antibody (ref 315055-003, antimouse IgG from Jackson Immunoresearch, Baltimore, PA, USA) were added to each well and incubated for 90 min at room temperature. This was followed by 5 washes in PBS and 2 washes with 1.5 mol/l NaCl. Fifty microlitres of substrate (*para*-nitrophenylphosphate, Sigma) diluted to 1 mg/ml in a solution containing 9.7% (w/v) diethanolamine, 1 mol/l HCl (pH 9.8) were added to each well. The plate was developed for 1 h at room temperature with the reaction stopped by the addition of

50 µl of 1 mol/l NaOH per well. Absorbance of the final product was read at 405 nm with a microtiter plate reader.

## Calibration curves

A calibration curve of MHC 1 was constructed by mixing equine masseter muscle and bovine serum albumin (BSA, standard for the protein determination), diluted in TBS (Tris buffer). BSA was used to respect a final protein concentration of 2.4 µg/ml. Increasing concentrations of MHC1 were obtained from 0% (100% BSA) to 100% (100% masseter muscle). The calibration points were treated like extracts. The calibration curve is linear up to 80% MHC 1 (80% masseter muscle + 20% BSA) and was used for all the assays to calculate the percentage of MHC 1 in each equine muscle (fig 2).

In the same way, a calibration curve of MHC 2 was constructed by mixing equine cutaneus trunci muscle and bovine serum albumin. Increasing concentrations of MHC 2 were obtained from 0% to 100% (100% cutaneus trunci muscle). The calibration curve is linear up to 100%.

For each muscle sample, the sum of MHC 1 and MHC 2 contents was never exactly equal to 100 because of the systematic error of the method. The percentages of MHC 1 and MHC 2 were calculated so that the sum reached 100%:

$$\%\ \text{MHC 1} = \frac{\text{MHC 1 content}}{(\text{MHC 1 content} + \text{MHC 2 content})}$$

$$\%\ \text{MHC 2} = \frac{\text{MHC 2 content}}{(\text{MHC 1 content} + \text{MHC 2 content})}$$

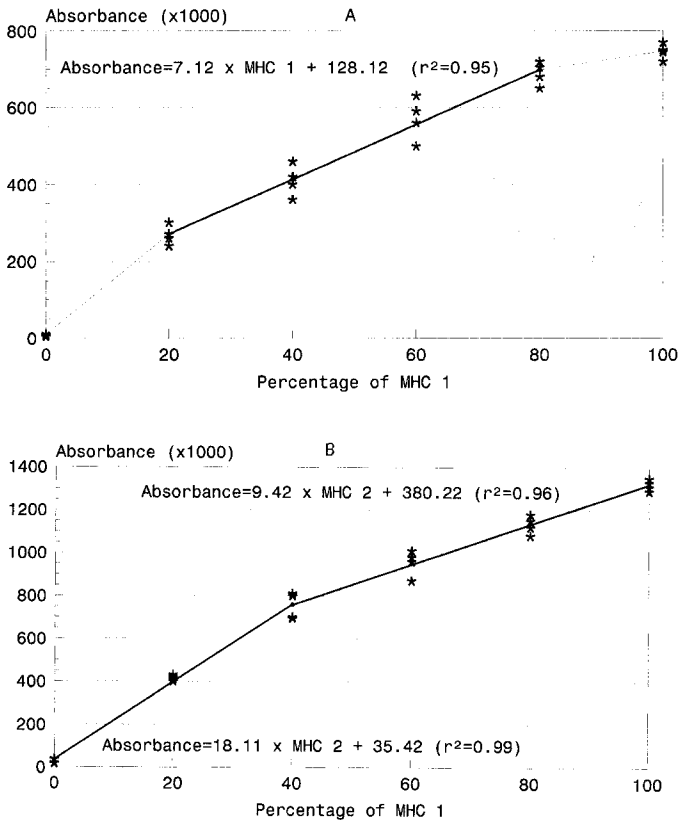


**Fig 2.** Calibration curves of slow (**A**) and fast (**B**) MHC analysis obtained by ELISA method.

### *Electrophoresis*

In order to qualitatively compare the results obtained with the ELISA method, an electrophoresis was performed to identify the slow and fast isoforms of the myosin heavy chain of the 5 equine muscles. For each muscle one protein extract was used to make the electrophoresis. In addition, extracts of bovine masseter and cutaneus trunci were also used as references on the gel to identify the migrating locations of the MHC 1 and MHC 2.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-Page) was performed on plates of 160 x 160 x 1.5 mm by the method described by Laemmli (1970). The separation gel had a 5–8% polyacrylamide gradient (Bär and Pette, 1988) and the stacking gel was at 3.5%. To improve resolution, the separation gel also contained a 40% glycerol gradient (Sugiura and Murakami, 1990).

### *Immunohistochemistry*

Immunohistochemistry was used to show the slow and fast fibres associated with the percentages of slow and fast MHCs in the transverse sections of the muscles. The immunofluorescence technique was described by Pons *et al* (1986). The frozen samples of each muscle were cut perpendicular to the fibre axes with a microtome into 10 µm thick slices.

In order to reveal fast-twitch fibres, a monoclonal antibody reacting specifically against MHC 2 was used (MAb F11315F4). This antibody has the same specificity as MAb 2 and it was effective on frozen tisssus. It was prepared from myosin of rabbit tibialis anterior (Léger *et al*, 1985). The antibody MAb 1 described previously was used to reveal slow fibres. The percentages in fibre type 1 and 2 were determined on microphotographs by counting 150–350 fibres of cross-sectional areas.

### *Statistics*

Descriptive statistics (mean and sd) were used to present the mean percentages of MHC 1 and MHC 2 obtained by the ELISA method in each muscle. A 4-way analysis of variance was applied to the 40 ELISA measurements in order to test the muscle, site, sub-sample and day effects. In order to quantify the reproducibility of the ELISA method, a linear regression was calculated between the results obtained on days 1 and 2 with the same protein extract. The correlation between the percentages of MHCs, obtained by ELISA method, and the percentages of fibre types (1 and 2) estimated by histochemistry were calculated.

## RESULTS

### *ELISA measurements*

Table I indicates the results obtained in the 5 equine muscles, for the 2 sites and the 2 sub-samples. The myosin heavy chain analysis revealed a wide range of MHC 1 and 2 contents from masseter to cutaneus. The masseter was entirely composed of slow twitch fibres (99.2% of MHC 1) and in contrast the cutaneus trunci was entirely composed of fast twitch fibres (98.7% of MHC 2). The diaphragm, tensor fasciae latae and semitendinosus contained 88.8, 40.3 and 2.3% of MHC 1, respectively.

The model used in the analysis of variance was significant at $p < 0.0001$. The muscle effect was highly significant ($p < 0.0001$) but the cutaneus trunci and the semitendinosus were not significantly different ($p > 0.05$). There was also a significant site effect within a muscle ($p < 0.01$) but neither the sub-sample effect ($F = 0.66$) nor the day effect ($F = 0.44$) was significant. Moreover, there was a good linear relationship ($r^2 = 0.996$) between the duplicate measurements obtained on days 1 and 2. These results confirmed an acceptable reproducibility of the ELISA measurements.

The comparison of the percentages of MHC 1 and the composition of fibre type 1 obtained by immunohistochemistry is presented in table II. The correlation between MHC 1 percentages and the composition

of fibre type 1 observed by histochemistry was high at 0.96 ($p < 0.05$). The values were quite similar except for the diaphragm where the MHC 1 content (89%) seemed to be greater than the percentage of fibre 1 (61%).

### *Electrophoresis*

The slowest migrating MHC bands corresponded to the fast myosin isofoms MHC 2 while the fastest migrating band corre-

**Table I.** Percentages of the ELISA measurements associated with the MHC 1 and MHC 2 content of each muscle sub-sample of both sites, measured on days 1 and 2.

| *Muscle* [a] | *Sub-sample* | *Site 1* [a] | | *Site 2* [a] | | *Mean MHCs per muscle* | |
|---|---|---|---|---|---|---|---|
| | | *MHC 1* | *MHC 2* | *MHC 1* | *MHC 2* | *MHC 1 (SD)* | *MHC 2 (SD)* |
| *Masseter* | | | | | | | |
| Day 1 | 1 | 99.9 | 0.0 | 99.0 | 1.0 | | |
| | 1' | 99.9 | 0.0 | 99.4 | 0.6 | | |
| Day 2 | 1 | 99.1 | 0.9 | 98.3 | 1.7 | | |
| | 1' | 99.2 | 0.8 | 99.1 | 0.9 | | |
| Mean per site | | 99.5 | 0.5 | 99.0 | 1.1 | $99.2^{A}$ (0.5) | 0.8 (0.5) |
| *Tensor fascia latae* | | | | | | | |
| Day 1 | 2 | 37.7 | 62.3 | 44.1 | 55.9 | | |
| | 2' | 34.3 | 65.7 | 39.0 | 61.0 | | |
| Day 2 | 2 | 40.3 | 59.7 | 42.9 | 57.1 | | |
| | 2' | 41.9 | 58.1 | 41.8 | 58.2 | | |
| Mean per site | | $38.6^{A}$ | 61.5 | $42.0^{B}$ | 58.1 | $40.3^{B}$ (2.8) | 59.8 (2.8) |
| *Semitendinosus* | | | | | | | |
| Day 1 | 3 | 2.6 | 97.4 | 2.7 | 97.3 | | |
| | 3' | 3.5 | 96.5 | – | – | | |
| Day 2 | 3 | 2.4 | 97.6 | 1.0 | 99.0 | | |
| | 3' | 1.6 | 98.4 | – | – | | |
| Mean per site | | 2.5 | 97.5 | 1.9 | 98.2 | $2.3^{C}$ (0.8) | 97.7 (0.8) |
| *Cutaneus trunci* | | | | | | | |
| Day 1 | 4 | 1.5 | 98.5 | 1.8 | 98.2 | | |
| | 4' | 1.1 | 98.9 | 1.9 | 98.1 | | |
| Day 2 | 4 | 0.9 | 99.1 | 1.0 | 99.0 | | |
| | 4' | 0.9 | 99.1 | 1.0 | 99.0 | | |
| Mean per site | | 1.1 | 98.9 | 1.4 | 98.6 | $1.3^{C}$ (0.4) | 98.7 (0.4) |
| *Diaphragm* | | | | | | | |
| Day 1 | 5 | 84.3 | 15.7 | 90.5 | 9.5 | | |
| | 5' | 84.4 | 15.6 | 92.6 | 7.4 | | |
| Day 2 | 5 | 88.9 | 11.1 | 90.5 | 9.5 | | |
| | 5' | 87.6 | 12.4 | 91.5 | 8.5 | | |
| Mean per site | | $86.3^{A}$ | 13.7 | $91.3^{B}$ | 8.7 | $88.8^{D}$ (2.9) | 11.2 (2.9) |

– Missing value. [a] Muscle and site effects were significant at $p < 0.0001$ and $p < 0.01$, respectively. Letters A to D in the column *Mean MHCs per muscle* indicates significant differences between muscles at $p < 0.01$. Letters A and B in the lines *Mean per site* indicates significant differences between sites 1 and 2 at $p < 0.01$.

sponded to the slow myosin isoform MHC 1 (fig 3). The bovine and equine masseter had only one band corresponding to MHC 1 while the equine and bovine cutaneus trunci showed another double-band of different molecular weight, corresponding to the 2 types of fast myosin heavy chains: MHC 2A and MHC 2B (fig 3). The double-band of fast myosin heavy chains was just separated for the bovine cutaneus trunci but not for the equine muscles. The tensor fasciae latae and diaphragm muscle showed 2 bands corresponding to a mixture of slow and fast MHCs. The intensities of the MHC 2 band was greater than the MHC 1 band for the tensor fasciae latae while the diaphragm had a large MHC 1 band and a light MHC 2 band.

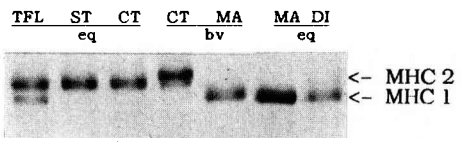


**Fig 3.** Electrophoretic gel of the equine (eq) masseter (MA), cutaneus trunci (CT), diaphragm (DI) and tensor fasciae latae (TFL), semitendinosus (ST). The bovine (bv) masseter (MA) and cutaneus trunci (CT) were used as reference migrating bands. MHC 1 = slow myosin heavy chain; MHC 2 = fast myosin heavy chain.

### *Immunohistochemistry*

Type 1 fibres were lightly stained by reacting with MAb 1 and fluorochrome. Type 2 fibres appeared unstained by reacting with MAb 1 while they were lightly stained by reaction with MAb F11315F4 and fluorochrome.

All the fibres in the transverse section of the masseter were characterized as type 1 (fig 4). In contrast, the transverse section

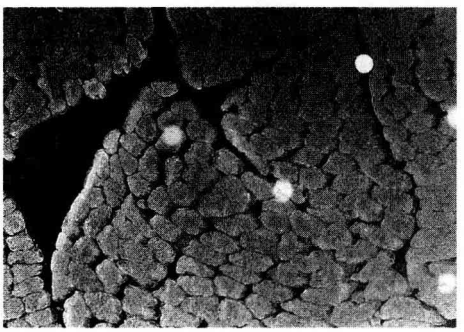

**Fig 4.** Transverse section of the masseter stained by MAb 1 (against MHC 1).

**Table II.** Percentages of MHC 1 obtained by ELISA method and composition in fibre type 1 observed in immunohistochemistry of the equine muscles.

| *Muscle* | *MHC 1 (%)* | *Fibre 1 (%)* |
|---|---|---|
| Masseter | 99 | 100 |
| Diaphragm | 89 | 61 |
| Tensor fasciae latae | 40 | 43 |
| Semitendinosus | 2 | 4 |
| Cutaneus trunci | 1 | 0 |

Correlation between MHC 1 and fibre 1 = 0.96 at $p < 0.05$.

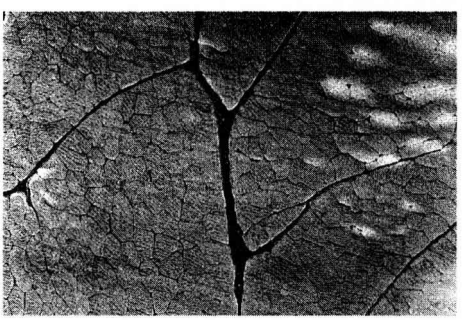

**Fig 5.** Transverse section of the cutaneus trunci stained by MAb F11315F4 (against MHC 2).

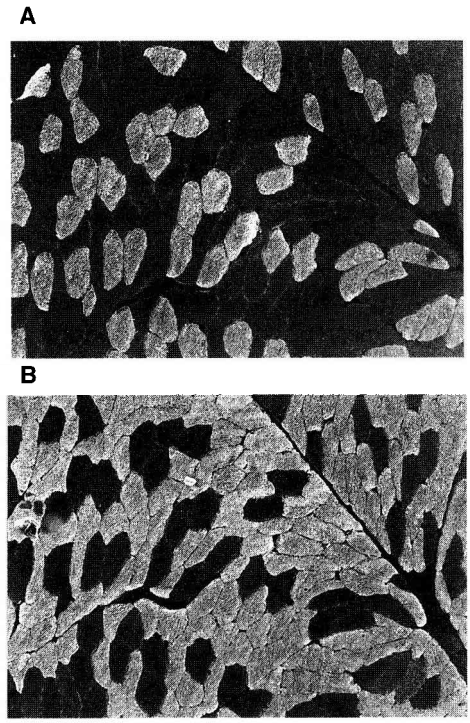


**Fig 6.** Transverse sections of the tensor faciae latae stained by MAb 1 which revealed MHC 1 (**A**) and MAb F11315F4 which revealed MHC 2 (**B**).

of the cutaneus trunci revealed only types 2 fibres (fig 5). The diaphragm, tensor faciae latae (fig 6) and semitendinosus had both fibre types (table II).

## DISCUSSION

The high affinity of the monoclonal antibodies MAb 1 and MAb 2 for slow and fast MHCs ensures a good accuracy of the measurements assuming that each step of the muscular protein extraction and ELISA process is well controlled. According to the non-significant day effect and the high correlation between the duplicate measurements, the reproducibility of the ELISA method was acceptable for quantitative analysis of the slow and fast MHCs in a muscle sample. Moreover, this immunoenzymatic method is less time consuming than histochemical techniques. Thirty muscle samples (with a triplicate measurement of MHC 1 and MHC 2 contents) can be extracted and analyzed by 1 person within 3 d.

The percentages of MHC 1 and MHC 2 were calculated using calibration curves obtained by increasing concentration of 2 reference muscles: equine masseter and cutaneus trunci. In this species, the masseter was entirely composed of slow-twitch fibres, probably because of its masticatory function which requires endurance. The bovine masseter was also shown to be entirely composed of slow-twitch fibres (Suzuki, 1977; Young and Davey, 1981; Picard *et al*, 1994). In the present study, the electrophoresis showed the same MHC 1 content in the equine masseter as in the bovine masseter. In contrast, the cutaneus trunci was almost entirely composed of fast-twitch fibres, which contract suddenly to move the skin of the flanks to repulse insects. The same results were observed for the bovine cutaneus trunci (Picard *et al*, 1994).

The standard was prepared by adding one of the reference muscles (massseter or cutaneus trunci) to a neutral protein in order to keep the myosin concentration constant for the ELISA. In this way, the calibration curves were linear in a large range of MHC contents. However, this mixture seemed to induce a non-linearity for the high percentages of MHC 1 (over 80%). Most of the locomotor muscles usually analyzed for exercise physiology studies have less than 80% of MHC 1 (Valette *et al*, 1995). For example, the gluteus medius contents between 11 and 27% of MHC 1, depending on the depth of sampling (Kline *et al*, 1987).

The results obtained by the 3 techniques of analysis were consistent from a qualitative point of view. It was particularly emphasized for the extreme muscles: masseter and cuta-

neus trunci. In the masseter, 100% of MHC 1 was associated with only one band comigrating with MHC 1 of the bovine masseter, and only slow-twitch fibre types in the transverse section. In the cutaneus trunci, about 98.7% of MHC 2 was associated with a double-band comigrating with MHCs 2A and 2B, and only fast-twitch fibre types in the transverse section. In the other muscles, both types of fibres were observed. The cross-sectional area of the fast-twitch fibres was larger than that of the slow fibres, as already shown in muscles of Standardbreds (Roneus, 1993). According to the mean results of Roneus (1993), the area of a fibre 1 is 3.2 and 4.7 times smaller than that of a fibres 2A and 2B, respectively. Thus, the larger volume of the fast twitch fibres includes a larger quantity of myosin heavy chains. This phenomenon may explain some of the differences observed between the percentages of fibre types and MHC contents.

The comparison between immunological and histochemical techniques has been undertaken previously by several authors. In the horse, the myosin ATPase reaction has been related to electrophoresis of native myosin isoforms (Hermanson *et al*, 1991). The correlation between MHC 1 composition of muscle fibres and ATPase reaction has been established in the rabbit (Staron and Pette, 1986).

The ELISA method of muscle fibre typing is less informative than histological techniques because it only provides a percentage of MHCs without any information about fibre type distribution in space, cross-sectional areas and other characteristics of the tissue such as capillary density. However, the determination of the MHCs percentage is based on the analysis of a larger mass (30–200 mg) of muscle than in histological techniques. Therefore, the ELISA results could be more representative than the percentage calculated by counting the fibre types over 200 fibres of a transverse section.

The coexistence of both MHCs in the same fibre has been demonstrated in the rat and implies that the dominant MHC determines the histochemical type of a muscle fibre (Danieli Betto *et al*, 1986). In this case, the ELISA method should be more appropriate to measure the mean MHCs content of the fibres occurring during different periods of postnatal development.

It may be concluded that the ELISA method makes it possible to accurately measure a wide range of slow and fast MHCs contents in equine muscles. The same method could be developed for measuring the MHC 2A and MHC 2B contents of the muscle by producing other monoclonal antibodies. In order to determine the myosin composition, this immunoenzymatic method is less time consuming than histological techniques and therefore offers new applications for muscle fibre typing in horses. For example, this technique will be applied to large groups of horses for genetic studies.

## ACKNOWLEDGMENT

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## REFERENCES

Bär A, Pette D (1988) Three fast myosin heavy chains in adult rat skeletal muscle. *FEBS Lett* 235,153-155

Bradford MM (1976) A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72, 248-254

Brooke MM, Kaiser KK (1970) Muscle fiber types: how many and what kind? *Arch Neurol* 23, 369-379

Danieli Betto D, Zerbato E, Betto R (1986) Type 1, 2A, and 2B myosin heavy chain electrophoretic analysis

of rat muscle fibers. *Biochem Biophys Res Commun* 138, 981-987

Ecob-Prince M, Hill M, Brown W (1989) Immunocytochemical demonstration of myosin heavy chain expression in human muscle. *J Neurol Sci* 91, 71-78

Essen B, Lindholm A, Thornton J (1980) Histochemical properties of muscle fibre types and enzyme activities in skeletal muscles of Standardbred trotters of different ages. *Equine Vet J* 12, 175-180

Foreman JH, Bayly WM, Allen JR, Matoba H, Grant BD, Gollnick PD (1990) Muscle responses of Thoroughbreds to conentional race training and detraining. *Am J Vet Res* 51, 909-913

Gottlieb-Vedi M (1988) Circulatory and muscle metabolic responses to draught work of varying intensity and duration in Standardbred horses. Thesis, Faculty of veterinary medicine, Swedish University of Agricultural Sciences, Uppsala, Sweden

Gunn HM (1978) Differences in the histochemical properties of skeletal muscles of different breeds of horses and dogs. *J Anat* 127, 615-634

Hermanson JW, Hegemann-Monachelli MT, Daaod MJ, LaFranboise WA (1991) Correlation of myosin isoforms with anatomical divisions in equine musculus biceps brachii. *Acta Anat* 141, 369-376

Horak V, Draber P, Hanak J, Matolin S (1991) Fibre composition and tubulin localization in muscle of Thoroughbred sprinters and stayers. *In: Equine Exercise Physiology 3* (SGB Persson, A Lindholm, LB Jeffcott, eds), ICEEP Publications, Davis, CA, USA, 262-268

Kline KH, Lawrence LM, Novakofski J, Bechtel PJ (1987) Changes in muscle fiber type variation within the middle gluteal of young and mature horses as a function of sampling depth. *In: Equine Exercise Physiology 2* (JR Gillespie, NE Robinson, eds), ICEEP Publications, Davis CA, USA, 271-277

Laemmli UK (1970) Cleavage of structural proteins during the assembly of head of bacteriophage T4. *Nature (Lond)* 227, 680-685

Léger JOC, Bouvagnet P, Pau B, Roncucci R, Léger JJ (1985) Levels of ventricular myosin fragments in human sera after myocardial infarction, determined with monoclonal antibodies to myosin heavy chains. *Eur J Clin Inv* 15, 422-429

Lindholm A, Piehl K (1974) Fibre composition, enzyme activity and concentrations of metabolites and electrolytes in muscles of Standardbred horses. *Acta Vet Scand* 15, 287-309

Lopez-Rivero JL, Morales-Lopez JL, Galisteo AM, Aguera E (1991) Muscle fibre type composition in untrained and endurance-trained horses. *Equine Vet J* 23, 91-93

Picard B, Léger JOC, Robelin J (1994) Quantitative determination of type 1 MHC in bovine muscle with anti-myosin monoclonal antibodies. *Meat Sci* 36, 333-343

Pons F, Léger JOC, Chevally M, Tomé FMS, Fardeau M, Léger JJ (1986) Immunocytochemical analysis of myosin heavy chains in human fetal skeletal muscles. *J Neurol Sci* 76, 151-163

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 eletrophoretic analysis. *Repr Nutr Dev* 33, 25-41

Roneus M (1993) Muscle characteristics in Standardbreds of different ages and sexes. *Equine Vet J* 25, 143-146

Roneus M, Lindholm A (1991) Muscle characteristics in Thoroughbreds of different ages and sexes. *Equine Vet J* 23, 207-210

Sinha AK, Rose R (1992) Muscle fiber typing in the horse: current problems, future directions. *In: Proc Ass Equine Sports Medicin*, Fallbrook CA, 7-11

Sinha AK, Ray SP, Rose R (1991) Effect of training intensity and detraining on adaptations in different skeletal muscles. *In: Equine Exercise Physiology 3* (SGB Persson, A Lindholm, LB Jeffcott, eds), ICEEP Publications, Davis, CA, USA, 223-230

Snow DH, Guy PS (1980) Muscle fibre type composition of a number of limb muscles in different types of horse. *Res Vet Sci* 28, 137-144

Staron RS, Pette D (1986) Correlation between myofibrillar ATPase activity and myosin heavy chain composition in rabbit muscle fibers. *Histochemistry* 86, 19-23

Sugiura T, Murakami N (1990) Separation of myosin heavy chain isoforms in rat skeletal muscles by gradient sodium dodecyl sulfate polyacrylamide gel electrophoresis. *Biomed Res* 11, 87-91

Suzuki A (1977) A comparative histochemical study of the masseter muscle of cattle, sheep, surine, dog, guinea pig and rat. *Histochemistry* 51, 121-131

Valette JP, Wolter R, Zouambi B (1990) Relations entre le type histo-enzymologique des fibres musculaires et les critères physiologiques de l'aptitude sportive chez le poney. *Rec Méd Vét* 166, 765-769

Valette JP, Barrey E, Jouglin M (1995) Determination of slow myosin heavy chain content in various equine muscles by an ELISA method. *Equine Vet J* Suppl 18, 248-251

Winkelmann DA, Lowey S, Press JL (1983) Monoclonal antibodies localize changes on myosin heavy chain isoenzymes during avian myogenesis. *Cell* 34, 295-306

Young OA, Davey CL (1981) Electrophoretic analysis of proteins from single bovine muscle fibers. *Biochem J* 195, 317-327