

Contractile Differentiation of Bovine Masseter Muscle

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Abstract

In ruminants the masseter muscle is composed entirely of slow-twitch fibres in the adult. Previous work showed that its contractile differentiation occurs late compared with limb muscles throughout foetal development. To extend these findings we studied the evolution with age of the bovine masseter muscle at four stages: 270 days postconception, 30 days postnatal, 170 days postnatal in weaned and in suckling animals and 490 days postnatal in bulls, steers and cows. The choice of these groups of age was to precise the development of the Masseter with the age of animals, more specifically during the weaning period, with the transition of the activity of the Masseter from suckling to chewing, and finally to study the influence of sexe and androgen on the fibres composition of this muscle.

The contractile characteristics of the muscle were determined immunohistochemically using antibodies specific to slow (I), fast (IIA and IIB), foetal, embryonic and alpha-cardiac myosin heavy chain (MHC) isoforms. To investigate the effects of weaning, this study also included biochemical analyses of DNA and protein content, and measurement of the oxidative and glycolytic activities of the muscle. The results obtained show the presence of heterogeneous fibres containing several MHC isoforms at 270 days postconception, and at 30 and 170 days postnatal in suckling animals. The foetal and alpha-cardiac isoforms present in the muscle during the foetal stage vanish at birth. In contrast, the embryonic isoform and the type IIC fibres disappear only at weaning. The muscle thus acquires its slow-twitch adult character as an adaptive response to rumination. Just before birth it contains approximately 20% of adult type I fibres, this proportion increases with age (45% at 30 days pn, 53% at 170 days in suckled animals) and from weaning it represents 100% both in bulls, steers and cows.

Key words: masseter, myosin, muscle, bovine.

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The masseter (Ma) muscle of the jaw presents specific contractile characteristics in different animals (for review: [38, 32, 35, 21]). In adult ruminants, which masticate slowly and continuously, it is composed solely of slow oxidative type I fibres. In contrast, in rodents such as rat, mice and rabbit, which have much more rapid mastication patterns, it contains both type I and fast oxido-glycolytic IIA fibres. In guinea pigs it consists exclusively of type IIA fibres. In carnivores such as cat and dog, and also in primates, it includes an unusual fibre type containing a myosin heavy chain (MHC) termed superfast MHC associated with the aggressive bite of carnivores [30, 14]. In omnivores including humans it is made up of both slow and fast fibres, with slow fibres predominating [3]. The masseter muscle thus presents specific characteristics that suggest an adaptation to elicited patterns of contractile activity.

The fibre composition of this muscle is also different from that of other muscles [7]. In several species, it has been shown to contain large amounts of intermediate fibres composed of several myosin types. In humans, Butler-Browne et al. [9], have shown that a masseter fibre can simultaneously express up to five different MHCs, while oro-facial and limb muscles contain only one or two types. If fibres are classified according to their MHC composition up to five different fibres can be distinguished in oro-facial and limb muscles and up to eight types in the masseter [36]. Among the specific MHCs in the masseter, two developmental isoforms; embryonic MHC (emb) and foetal or neonatal MHC (foet) have been found in various species after birth, in particular in humans [9, 13], rabbits [11] and chickens [10]. An embryonic type myosin light chain (MLCemb), similar to cardiac MLC [4] also persists in the

human adult masseter [9, 35]. An alpha-cardiac MHC is also present in the adult masseter in rabbits [12] and humans [6, 7, 5, 24]. The presence in the adult of these MHC emb, foet and alpha-card isoforms observed in the other muscles during foetal growth but vanishing after birth, may indicate late differentiation of the masseter. This delay may be due to the different embryonic origin and innervation of this muscle. The persistence of developmental isoforms has been observed in both the Masseter which is of branchial origin and in the extraocular muscle which is of somitic origin both of which are innervated by cranial nerves, this suggests a common neuronal regulation of the expression of these isoforms [35]. Indeed Soussi Yanicostas *et al.* [35], have shown that in fast muscle the transition from foetal to fast MHC is independent of innervation, but in slow muscle the nerve is essential for the expression of a slow phenotype. Some workers have reported that the masseter is less differentiated at birth than the limb muscles in several species [28, 11, 35]. This is the case in bovine, in which the differentiation of the masseter involves two generations of cells, primary and secondary, as in other muscles, with a delay in the evolution of both of these generations throughout the life of the foetus [26]. At birth, this muscle, which is composed solely of type I fibres in the adult, still contains large proportions of fast and IIC fibres. The purpose of the work described here was to monitor the differentiation of this muscle after birth, especially at the time of weaning, when the contractile activity of the muscle changes from suckling activity to chewing activity. Antibodies were used to determine whether, as in other species, the MHC emb, foet and alpha-card isoforms are still present after birth in the bovine masseter.

Material and Methods

Animals and muscle samplings

The muscle Masseter was taken at the ages: 270 days postconception, just before birth, at 30 days postnatal, at 170 days and at 490 days. At the two first stages 3 calves were analysed. Stage 170 days concerned more specifically the influence of weaning on contractile and metabolic characteristics of masseter. Two groups of 7 calves were slaughtered at 170 days. One group was progressively weaned between 107 and 128 days, the other group was suckled until slaughter. At 490 days postnatal, the muscle was taken on 12 bulls and on 12 steers castrated at the age of 4 months. To precise the differences between sexes, the masseter was taken on 3 cows.

After slaughter, samples used for biochemical measurements were directly frozen in liquid nitrogen, stored at -80°C and then ground. Specimens used for histological investigations were frozen progressively in isopentane, then in liquid nitrogen and were stored at -80°C until used.

Histochemistry and immunohistochemistry

The metabolic type of fibres was studied by the revelation of succinate dehydrogenase activity (SDH), enzyme of

oxidative metabolism, according to the method of Pearse [23].

The myosins present at this stage of foetal life were evidenced by immunofluorescence on serial frozen sections. The procedure used was that described by Pons *et al.* [27]. The muscle sections were incubated with the monoclonal antibody for 30 minutes at 37°C. After washings with PBS, the second antibody (rabbit anti mouse IgG labelled with dichlorotriazinylaminofluorescein, Interchim) diluted in PBS was applied for 30 minutes at 37°C. After washings with PBS, the sections were fixed with mowiol (Calbiochem, 475904). We used 5 monoclonal antibodies (Biocytex, Marseille) raised against different isoforms of myosin heavy chain. The antibody called S (slow) was prepared from an adult human auricle specimen. It recognized the isoform slow (MHC 1). The antibody called R (rapid) was specific for fast MHC (MHC 2a and MHC 2b). It was obtained from adult rabbit Tibialis. The antibody called F (foetal) was obtained from muscle of a 22 weak-old bovine foetus. It was specific of a MHC present only during the foetal period, called MHC foetal. The antibody called E (embryonic) was obtained from adult human atrium, it was specific of a MHC observed during the foetal period called MHC embryonic. The last antibody called A (alpha cardiac) was raised against human cardiac muscle. The specificity of these antibodies has been described in early works [27, 22]. Their cross-reactions with myosin from foetal and adult cattle have been analysed [29].

Protein preparation

Two hundred mg of frozen muscle were ground in 5 ml of buffer solution: 0.5 M NaCl, 20 mM sodium pyrophosphate, 50 mM Tris, 1 mM EDTA, 1 mM dithiothreitol (DTT). After 10 min at 4°C (on ice), the sample was centrifuged for 5 min at 2500 g. The supernatant was then mixed with glycerol at a final concentration of 50% (v/v) and stored at -20°C for several months. These protein preparations were used for electrophoresis.

Electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed on plates of 160 x 160 x 1.5 mm by the method of Laemmli [18]. The separation gel was a 5-10% polyacrylamide gradient and the stacking gel was at 3.5%. To improve resolution, the separation gel also contained a 30-40% glycerol gradient, according to Sugira and Murakami [37].

The samples stored at -20°C were diluted 50% (v/v) in a solution containing 34% (w/v) SDS, 20% (v/v) 1 M Tris pH 6.8, 17% (v/v) glycerol, 17% (w/v) Pyronin Y and 7.5% (v/v) mercaptoethanol. They were incubated for 10 min at room temperature and then at 90°C for 10 min. Wells were probed with 5 µg of protein. Migration was performed at a constant voltage, at a temperature of 4°C. This last stage is essential to obtain a clear separation of the different MHC bands. For a uniform penetration of the proteins in the stacking gel, migration was begun at 50 V for 30 min

and then continued for 110 V for 18 hours. The gel was stained in a solution of R250 Coomassie Blue.

Determination of protein content

The protein content of muscles was determined by the method of Bradford [19] with a preparation of cattle albumin serum at 1 mg/ml as standard. The muscle samples were first homogenized in NaOH 1N and kept for 20 h at 30°C to make the proteins soluble.

Measurement of DNA content

Muscle samples were homogenized in a buffer (TNE: 10 mM Tris, 2 M NaCl, 2 mM EDTA, pH 7.5) rich in salt to dissociate the DNA from the chromatin proteins. The DNA concentration ($\mu\text{g/g}$ of muscle) was determined on homogenates according to the method of Labarca and Paigen [17], with a fluorimeter. The method is based on the increase in the fluorescence of DNA-linked Hoescht H33258 compound. Calf thymus DNA at 10 $\mu\text{g/ml}$ was used as standard.

The ration proteins/DNA was calculated to estimate hypertrophy of muscle.

Determination of muscle metabolism

The muscle samples were ground and homogenized in a 140 mM sucrose, 50 mM triethanolamine buffer pH 7.5 according to Ansay [1]. The homogenate was centrifuged at 3.500 g for 15 min at 4°C. The supernatant, containing the soluble enzymes and the mitochondria, was used to measure enzyme activity. Anaerobic glycolytic metabolism was studied by measuring the lactate dehydrogenase (LDH) activity in the transformation of pyruvate into lactate. This activity was measured by following the disappearance of nicotinamide adenine dinucleotide, reduced from (NADH) at 340 nm in the reaction medium: 50 mM triethanolamine, 5 mM EDTA, 2 mM pyruvate, 0.234 mM NADH, pH 7.5 [1]. Aerobic oxidative metabolism was studied by measuring isocitrate dehydrogenase (ICDH) NADP activity according to Briand et al. [8]. This activity was measured by following the reduction of nicotinamide adenine dinucleotide phosphate (NADP) at 340 nm in the reaction medium: 38.8 mM Na_2HPO_4 , 0.5 mM MnCl_2 , 0.05% Triton X100, 0.36 mM NADP, 1.38 isocitrate, pH 7.5. Enzyme activity was expressed in $\mu\text{mol/min/g}$ of muscle.

Statistical analysis

The influence of weaning was studied by a variance analysis with the G.L.M. procedure of SAS package [33] on biochemical data.

Results

Specific features of the masseter

From weaning, the bovine masseter consists of fibres of homogeneous size and type (Fig. 1b). Its metabolism is totally oxidative (Fig. 1c). Comparison with other muscles shows that these fibres have a specific size. At 170 days, for example, the average area of all the Ma muscle fibres

(909 μm^2) is significantly lower than that of *Semitendinosus* (ST) (1706 μm^2) and *Longissimus thoracis* (LT) (1218 μm^2) fibres. Conversely, the type I fibres are largest in the Ma (1706 μm^2 against 743 μm^2 in the LT and 1466 μm^2 in the ST). Similarly, the type IIC fibres have a significantly larger surface area in the Ma (1348 μm^2 against 238 μm^2 in LT and 834 μm^2 in ST). Figure 1 (b and c) shows that connective tissue occupies a greater proportion of the Ma compared with ST muscle. This proportion calculated by image analysis from sections of this two muscles on weaned animals shown $49.8\% \pm 6.8$ for Ma and $23.5\% \pm 3.8$ for ST. The same difference was found on suckled animals. Protein content is significantly lower than that of other muscles (155.6 mg/g of muscle versus 186.3 mg/g in LT and 182.5 mg/g in ST). In contrast, its DNA content is significantly higher (1160 $\mu\text{g/g}$ of muscle versus 397 $\mu\text{g/g}$ in LT and 447 $\mu\text{g/g}$ in ST). Hence the protein / DNA ratio is significantly lower in the Ma (135 versus 470 in LT and 407 in ST). Owing to its pure type I fibre composition, the Ma has an oxidative activity significantly higher than that of other muscles (7.42 versus 1.09 in LT and 1.13 in ST). Correspondingly, it has the lowest glycolytic activity (212.5 versus 1357 in LT and 1213 in ST).

Evolution with age

Birth (270 days)

Just before birth, a high proportion of cells were recognised by fast (R) antibody (Fig. 2a). Some of these R+ cells were also recognised by slow (S) antibody, but not all. At this stage, Ma contained approximately 20% type I fibres, 35% type II fibres and 45% type IIC fibres (R+ and S+). SDH activity testing (Fig. 1a) showed that all the fibres had an oxidative metabolism at 270 days postconception. No cells were recognised by alpha-cardiac (A) antibody, and very few by foetal (F) antibody, whereas many were still recognised by embryonic (E) antibody (Fig 2a). Hence the MHC isoform profile showed that just before birth the Ma contains five types of fibre composed respectively of MHC1, MHC1 + MHC2, MHC1 + MHC2 + MHCemb, MHC1 + MHC2 + MHCfoet + MHCemb, and MHC2 + MHCemb. These will be called I, IIC, IICemb, IICemb-foet and Iemb.

30 days

At this stage, almost all the Iemb fibres had vanished (Fig 2b). All the cells were recognised by the S antibody, but among these many were also recognised by R antibody, corresponding to a high proportion of type IIC fibres. Many were still recognised by E antibody, but none were recognised by either F or A antibodies. At this stage four cell types were therefore recognisable: I, Iemb, IIC and IICemb. Compared with the previous stage, the Iemb and IICemb-foet fibres have disappeared, and the IIC and Iemb fibres are present in a greater proportion.

170 days

In suckling calves (Fig 3a) many fibres were still recognised by both R and S: IIC, and also many E+ cells were

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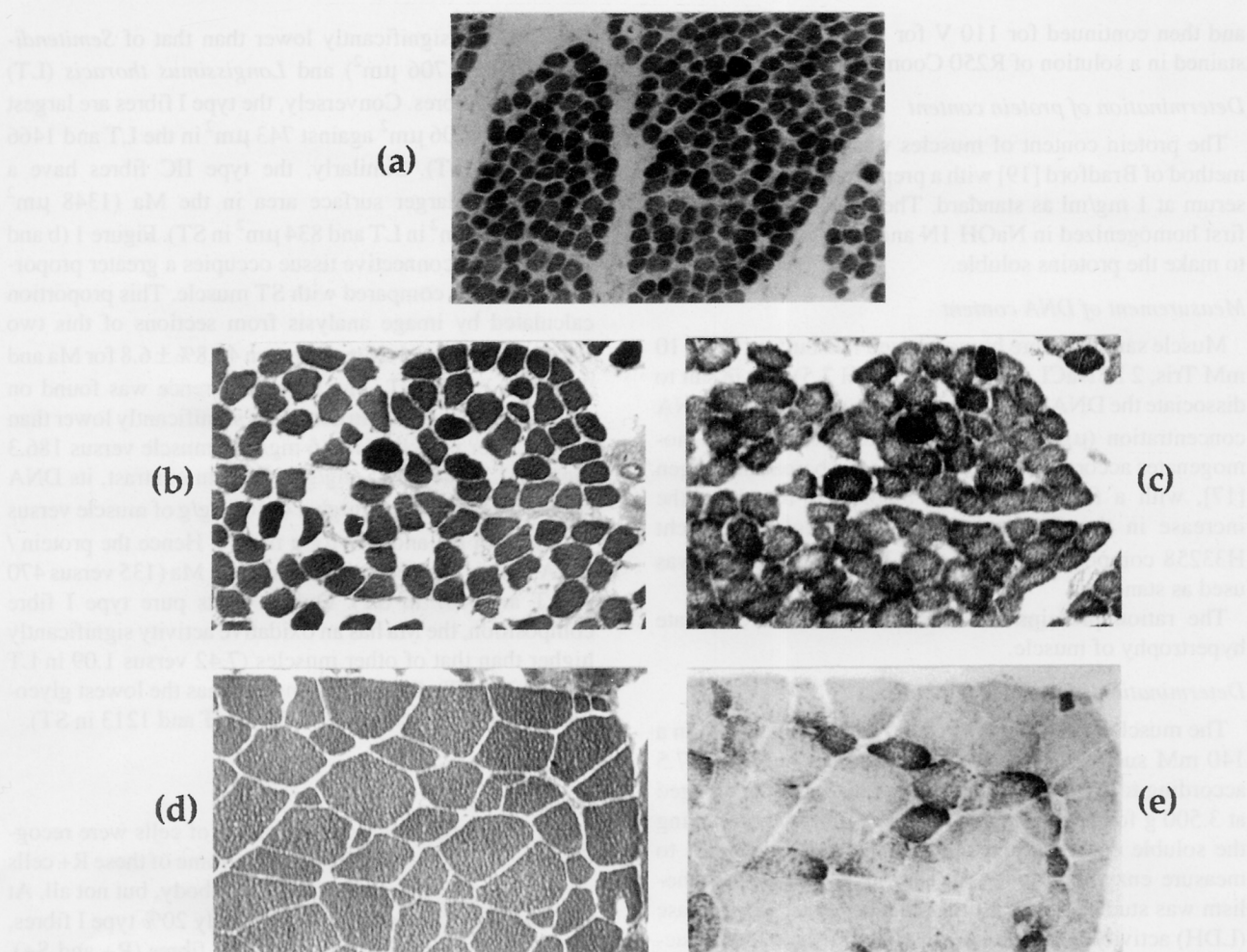


Figure 1. Structure and oxidative activity of Masseter comparing to Semitendinosus muscle (x 125): (a) - SDH activity of masseter at 270 days postconception (just before birth), (b) - Azorubine coloration of cells of masseter at 170 days postnatal (weaned), (c) - SDH activity of masseter at 170 days postnatal (weaned), (d) - Azorubine coloration of cells of semitendinosus at 170 days postnatal (weaned), (e) - SDH activity of semitendinosus at 170 days postnatal (weaned).

still present. As in the previous stage, four cell types can be recognised: MHC1, MHC1 + MHCE, MHC1 + MHC2 and MHC1 + MHC2 + MHCE, corresponding respectively to fibres: I, Iemb, IIC, and IICemb (Table 1). Comparatively to the previous stage the relative proportion of Iemb fibre have increased and the IIC and IICemb fibres have decreased.

In weaned calves (Fig. 3b) most of the fibres contained only MHC1, i.e. were type I fibres. The Ma contained some R+ cells in only one out of seven weaned calves, and the response to R antibody was much weaker than in the suckling calves. The Ma of this calf therefore contained a very small proportion of type IIC fibres, whereas these had disappeared in all the others. Similarly, the response to E antibody was positive in only two calves out of seven, and was very weak.

These modifications of MHC composition on weaning are accompanied by other changes (Table 2). The muscle

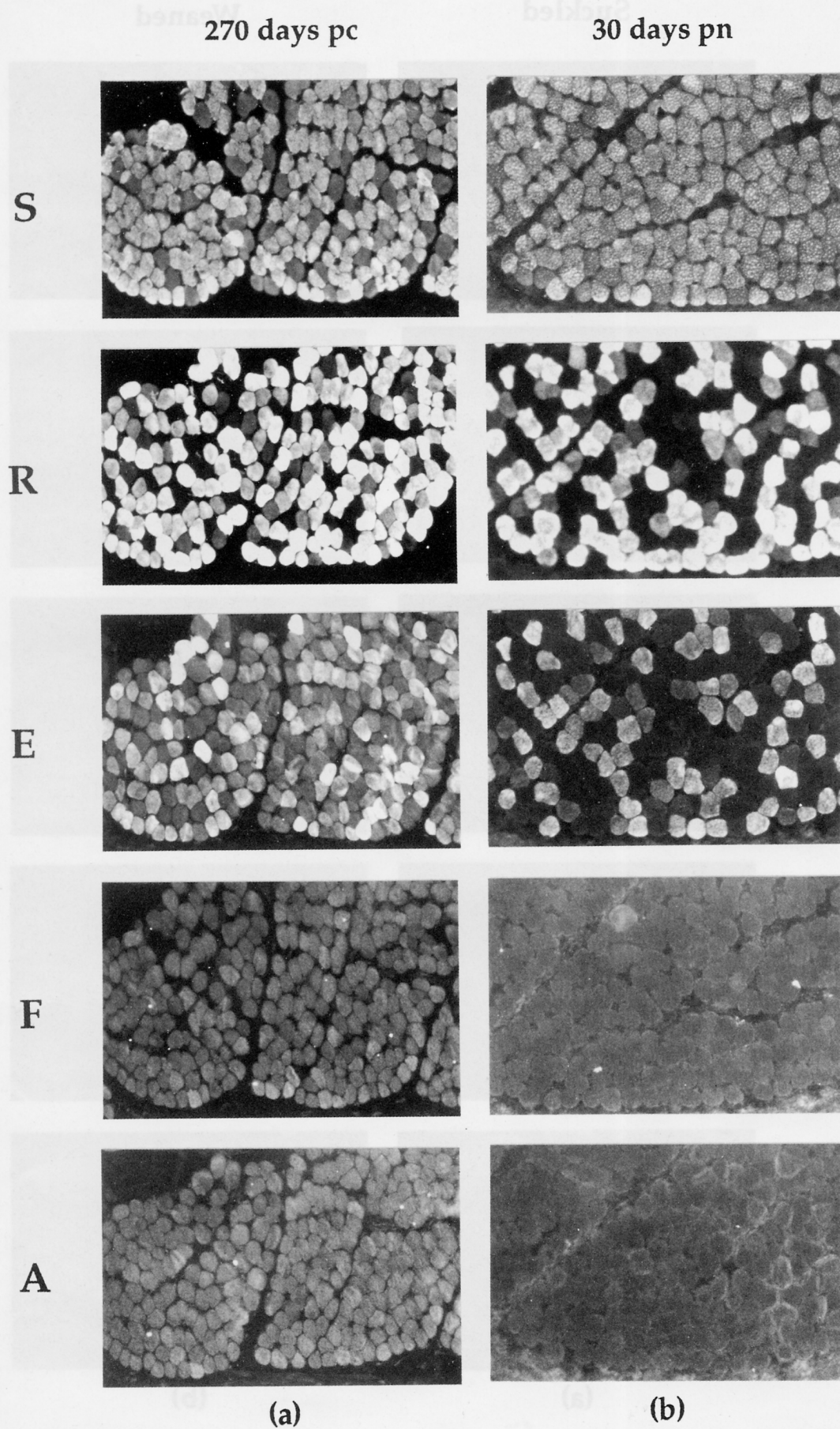
Figure 2. Immunocytochemical analysis of transverse serial sections (x 125) of the muscle Masseter with antibodies directed against slow MHC (S), fast MHC (R: 2a and 2b), foetal MHC (F), embryonic MHC (E), and alpha cardiac MHC (A) at: (a) - 270 days postconception, (b) - 30 days postnatal.

protein content is not significantly modified. Although the DNA content is significantly lower in the Ma of weaned calves, neither the protein/DNA ratio nor the average fibre surface area is significantly modified. ICDH activity was significantly stronger in the Ma of weaned calves, consistent with a higher proportion of type I fibres. The very weak glycolytic activity (LDH) showed no significant difference.

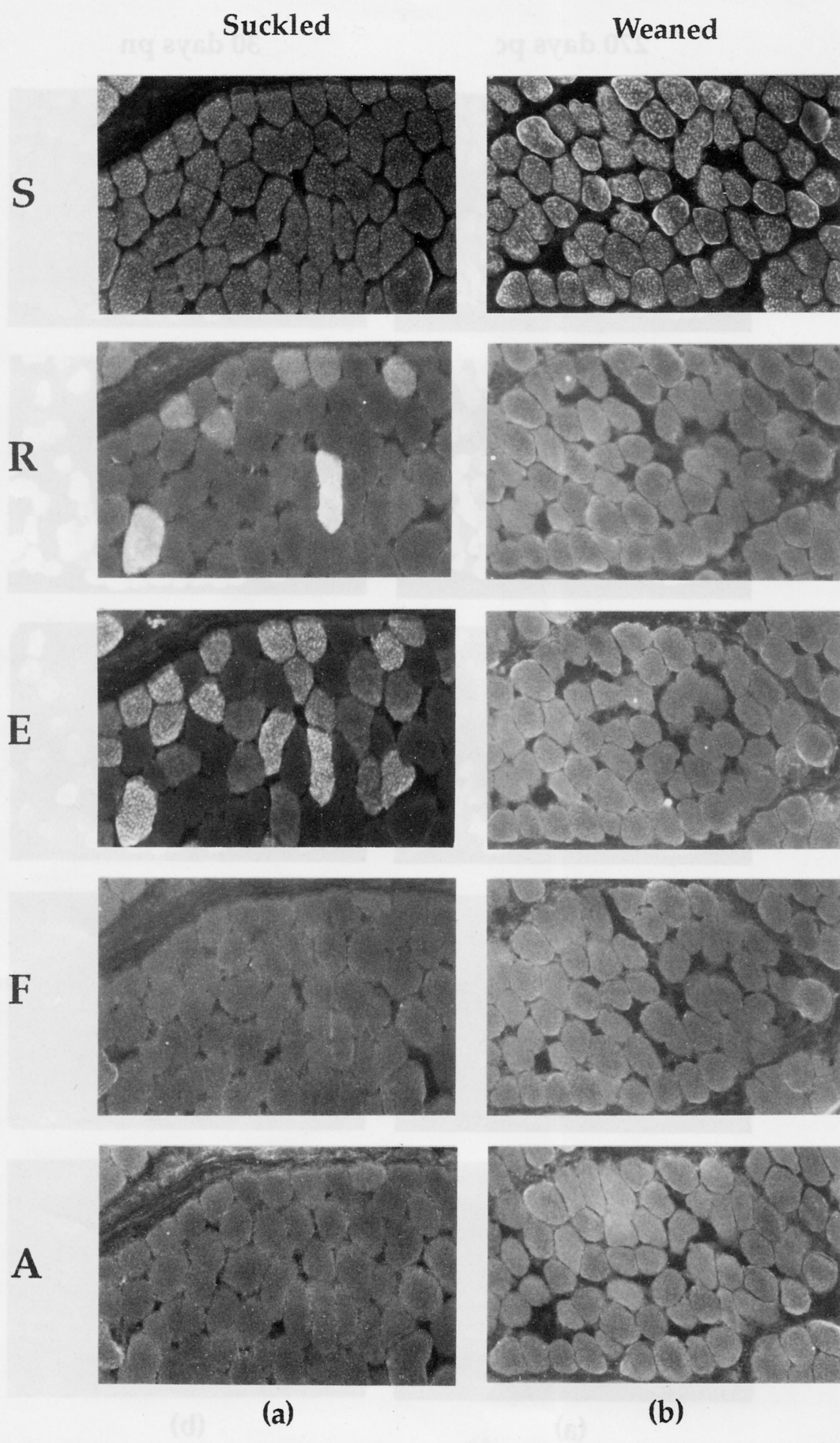
490 days

At 16 months, the Ma in the great majority of the male

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Table 1. Different cells of Masseter at 270 days pc, 30 and 170 days pn, with this composition: Fibre I: MHC 1; fibre Iemb: MHC 1 and MHC emb; fibre IIemb: MHC 2a and MHC emb; fibre IIC: MHC 1 and MHC 2a; fibre IIC emb: MHC 1 and MHC 2a and MHC emb; fibre IICembfoet: MHC 1 and MHC 2a and MHC emb and MHC foet.

Days	Fibres					
	I	Iemb	IIemb	IIC	IICembfoet	IICemb
270 pc	20%	-	35%	3%	2%	40%
30 pn	45%	10%	-	15%	-	30%
170 suckled	53%	40%	-	5%	-	2%
170 weaned	99%	-	-	-	-	1%

Figure 3. Immunocytochemical analysis of transverse serial sections ($\times 125$) of the muscle Masseter with antibodies directed against slow MHC (S), fast MHC (R: 2a and 2b), foetal MHC (F), embryonic MHC (E), and alpha cardiac MHC (A) at 170 days postnatal on: (a) - suckled calves, (b) - weaned calves.

calves studied, whether castrated or not, showed 100% type I fibres (Fig. 4a and b). However, approximately 25% of these animals (castrated and uncastrated) had an Ma containing a very small number (1/100 on average) of cells recognised by S, R, F and A antibodies but very weakly or not at all by E antibody (Fig. 5). No such cells were observed in the Ma of female calves, which all contained 100% type I fibres (Fig 4c).

Analysis by electrophoresis (Fig. 6) confirms the above results. At 270 days postconception the Ma contained MHC2a and MHC1. At the subsequent stages, 30 days and 170 days suckling, the concentration of MHC2a had fallen, completely vanishing after weaning. At 170 days after weaning and at 490 days, the Ma in either sex contained only isoform MHC1.

Discussion

Specific features of the masseter

Besides its specific fibre composition, the bovine masseter differs from the other muscles in the size of its fibres. The type I and IIC fibres are larger than those of the same type in other muscles. Such differences in fibre size have also been observed in other species [21, 34]. The type II fibres are smaller than the type I fibres in the human masseter, whereas the opposite is the case in the other muscles. It may be supposed that these size differences are due to the activity of the Ma muscle, which is more sustained than that of the other muscles in ruminants.

The Ma also differs from the other muscles in its very wide-ranging fibre composition among different species. In bovine the alpha-cardiac MHC isoform, which occurs in the adult muscle in several other species, is absent after

Table 2. Effect of weaning on biochemical characteristics of Masseter.

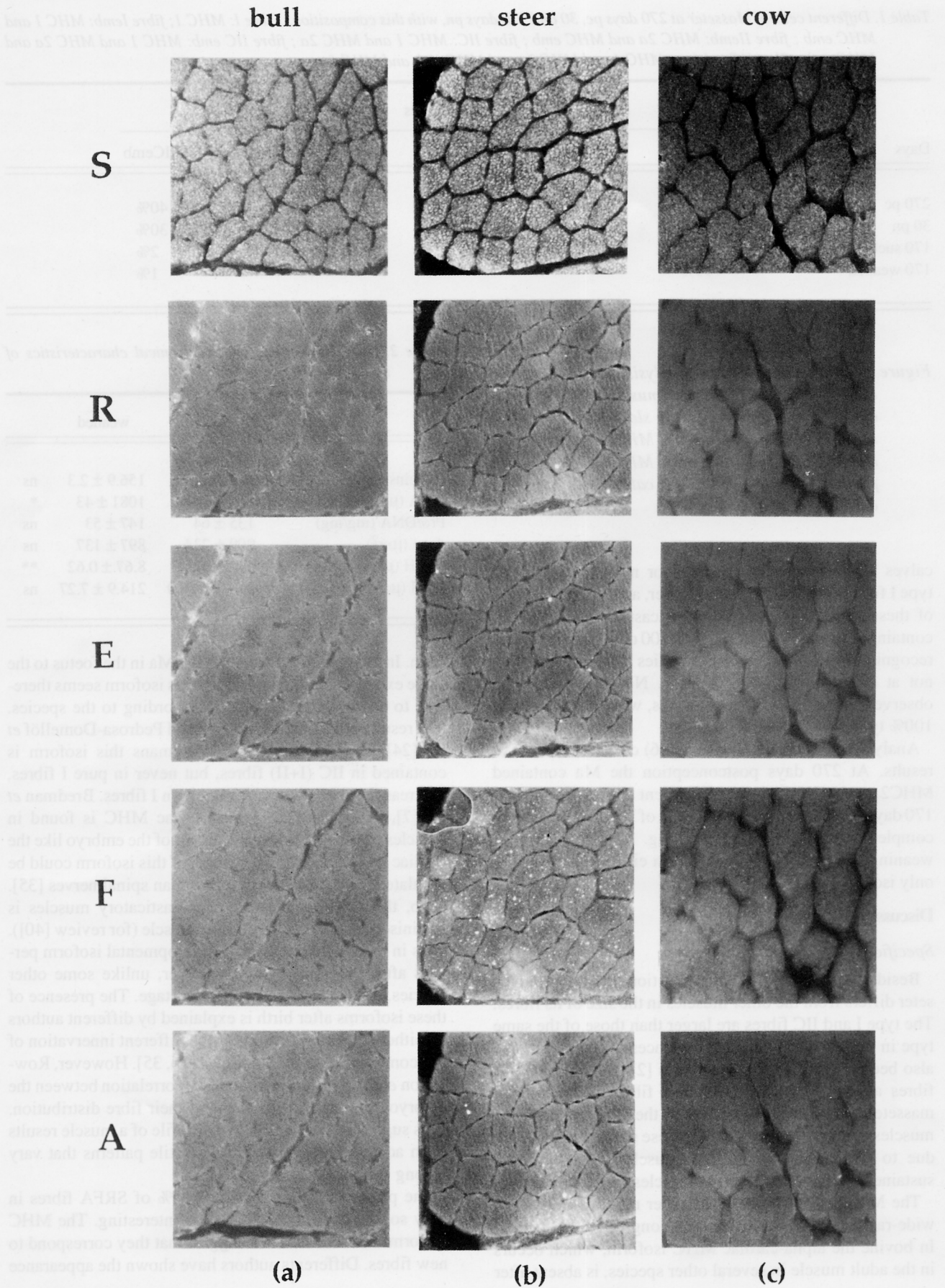
	suckled	weaned	
Proteins (mg/g)	155.6 $\pm$ 3.0	156.9 $\pm$ 2.3	ns
DNA (μ g/g)	1160 $\pm$ 48	1081 $\pm$ 43	*
Pro/DNA (mg/mg)	135 $\pm$ 64	147 $\pm$ 53	ns
Area (μ m ²)	909 $\pm$ 234	897 $\pm$ 137	ns
ICDH (μ mol/min/g)	6.17 $\pm$ 0.64	8.67 $\pm$ 0.62	**
LDH (μ mol/min/g)	210.2 $\pm$ 16.0	214.9 $\pm$ 7.27	ns

birth. In contrast, it is present in the Ma in the foetus to the same extent as in other muscles. This isoform seems therefore to be regulated differently according to the species. The results of Bredman *et al.* [6], and Pedrosa-Domellöf *et al.* [24], support this, since in humans this isoform is contained in IIC (I+II) fibres, but never in pure I fibres, whereas in rabbits it occurs mainly in I fibres. Bredman *et al.* [7], suggested that alpha cardiac MHC is found in muscles originated from cranial part of the embryo like the cardiac muscle. So the expression of this isoform could be regulated by cranial nerves rather than spinal nerves [35]. Also, the high plasticity of the masticatory muscles is reminiscent of that of the cardiac muscle (for review [40]).

As in many other species, a developmental isoform persists after birth in bovine. However, unlike some other species, bovine lose it by the adult stage. The presence of these isoforms after birth is explained by different authors by either embryonic origin, or the different innervation of Ma compared with limb muscles [16, 35]. However, Rowleson *et al.* [31], failed to find any correlation between the embryonic origin of muscles and their fibre distribution. This suggests that the fibre type profile of a muscle results from adaptation to specific contractile patterns that vary among species.

The presence of approximately 1% of SRFA fibres in only some calves at 16 months is interesting. The MHC isoforms of these muscles suggest that they correspond to new fibres. Different authors have shown the appearance

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Figure 4. Immunocytochemical analysis of transverse serial sections ($\times 125$) of the muscle Masseter with antibodies directed against slow MHC (S), fast MHC (R: 2a and 2b), foetal MHC (F), embryonic MHC (E), and alpha cardiac MHC (A) at 490 days postnatal on: (a) - bulls, (b) steers, (c) cows.

of new fibres under the influence of a particular stress, as compensatory growth, stretch or exercise (for review [2]). Generally these small new fibres express developmental MHC. The presence of ventricular MHC in these small fibres has been reported in ALD of chicken [2]. The fact that in bovine, muscle cells in early foetal growth contain

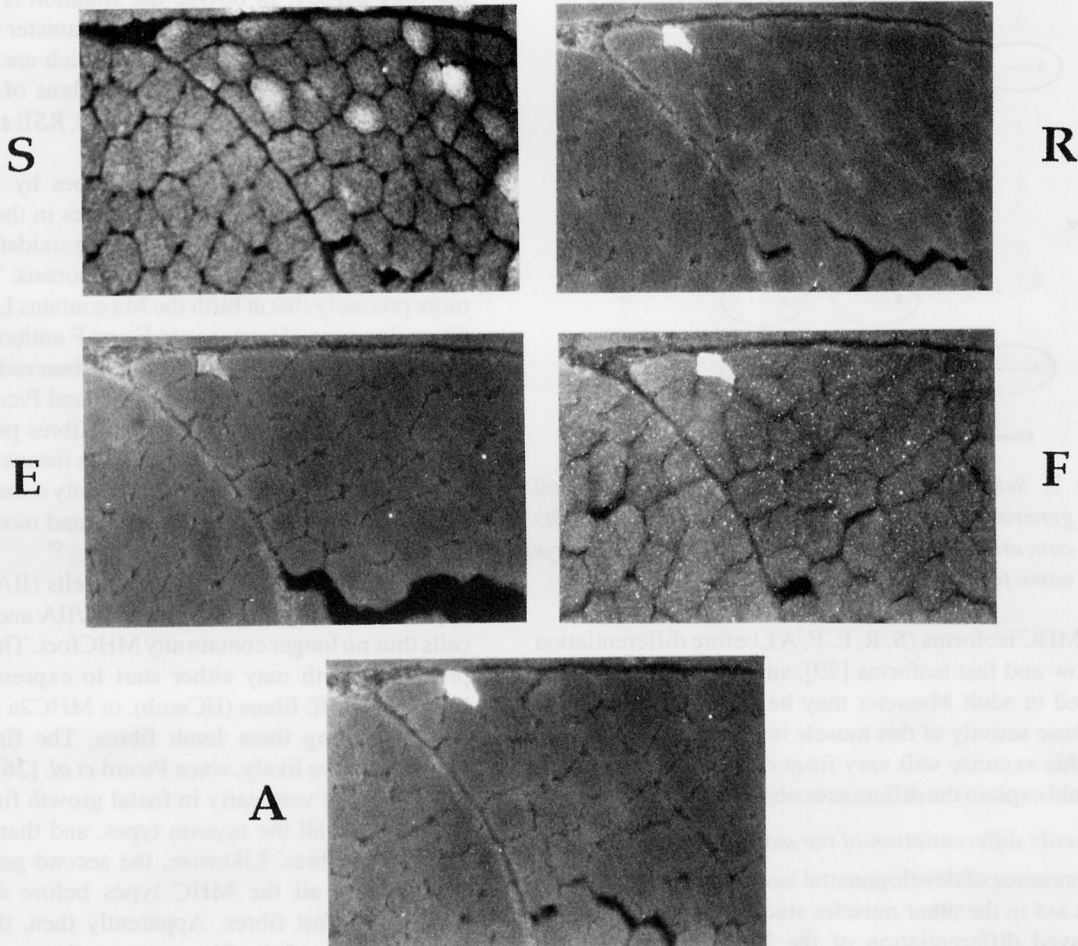


Figure 5. Cells recognized by both S, R, F, A and a little by E antibodies, in a very small proportion of animals at 490 days ($\times 125$). All the cells are recognized by antibody S (a), only one cell is recognized by R, F, A and a little by E antibodies.

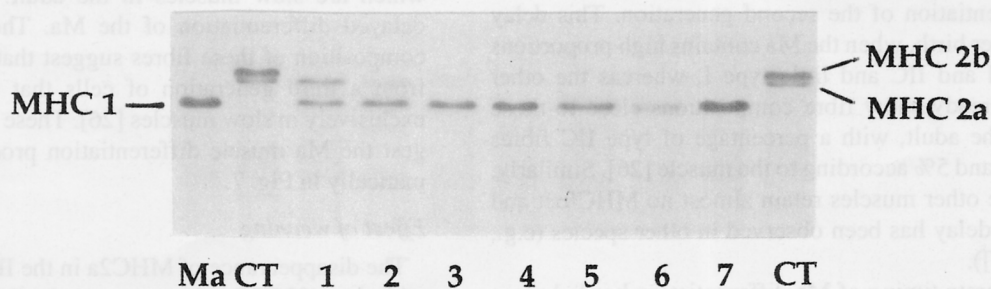


Figure 6. Electrophoretic separation (SDS-PAGE) of MHC isoforms of bovine masseter at different age: (1) 270 days pc, (2) 30 days pn, (3) 170 days pn suckled, (4) 170 days pn weaned, (5) 490 days on bulls, (6) 490 days on steers, (7) 490 days on cows. CT and Ma are used as standards.

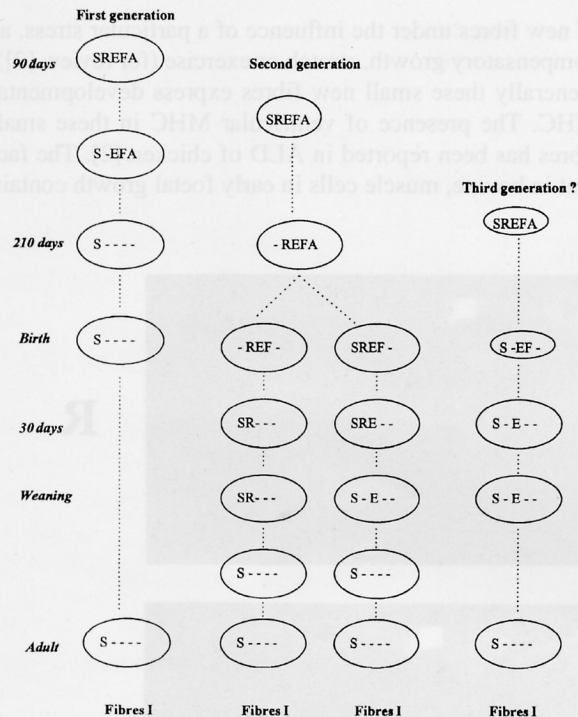


Figure 7. Schematic development of the different cell generations in the bovine masseter. The results concerning the foetal stages from 90 to 270 days come from Picard *et al.*, 1994 [26].

all the MHC isoforms (S, R, E, F, A), before differentiation into slow and fast isoforms [29], suggest that these fibres observed in adult Masseter may be formed as a result of the intense activity of this muscle in ruminant. The intensity of this exercise will vary from one animal to another, and could explain the differences observed among animals.

Contractile differentiation of the masseter

The presence of developmental isoforms after birth in the Ma but not in the other muscles studied, may be the result of delayed differentiation of the Ma throughout foetal growth and after birth. Picard *et al.* [26], have shown that compared with *Semitendinosus*, *Cutaneus trunci*, *Longissimus thoracis*, and *diaphragma* muscles, the bovine Ma displays delayed differentiation of the first generation of myotubes in early stages, followed by late appearance and differentiation of the second generation. This delay persists after birth, when the Ma contains high proportions of types II and IIC and little type I, whereas the other muscles already show fibre compositions close to those found in the adult, with a percentage of type IIC fibres between 0 and 5% according to the muscle [26]. Similarly, at birth the other muscles retain almost no MHCfoet and emb. This delay has been observed in other species (e.g., [11, 34, 28]).

This separate timing of Ma differentiation has led some workers to suggest that jaw and limb muscles belong to two distinct allotypes. This does not seem to be the case in bovine, since the two generations of cells involved in the

contractile differentiation are the same as in the other muscles [26]. In humans, Barbet *et al.* [3], have also shown that during foetal growth the Ma, like other muscles, derives from two successive generations of fibres. A specific phenotype appears only after birth. It is characterised by the persistence of MHCemb and MHCfoet, and by two populations of fibres: one of small diameter coexpressing MHCemb, foet and fast, and one of large diameter containing only MHC1. In bovine the situation is similar; after birth, we find type I fibres of large diameter that no longer contain MHCemb, foet and fast, which are derived from the first generation of cells [26]. Fibres of smaller sizes distributed in different categories: RE, RSE and RSEF may also be observed.

Comparison of different fibre types by SDH activity testing suggests that all the fast fibres in the Ma are type IIA fibres, as they all display strong oxidative activities. This result is confirmed by electrophoresis. This indicates more precisely that at birth the Ma contains I, IIA and I/IIA fibres. In terms of response to E and F antibodies, I, IIA/E, IIA/I/E and IIA/I/E/F fibres can be observed. On the basis of the results of Robelin *et al.* [29], and Picard *et al.* [26], it can be concluded that the type I fibres present at birth derive from the first generation. This means that of all the slow fibres present in the adult Ma, only a small proportion is derived from the first generation, and most derive from the second generation.

At 30 days postnatal, the all-fast cells (IIA/E) have disappeared, leaving only types I, I/E, I/IIA and I/IIA/E. The cells thus no longer contain any MHCfoet. The IIA/E fibres present at birth may either start to express MHC1 and become IIA/I/E fibres (IICemb), or MHC2a is replaced by MHC1 making them Iemb fibres. The first possibility seems the more likely, since Picard *et al.* [26], have shown in bovine that very early in foetal growth first-generation cells contain all the myosin types, and then differentiate into type I fibres. Likewise, the second-generation cells also contain all the MHC types before differentiating mostly into fast fibres. Apparently then, the contractile differentiation of the fibres occurs via a compulsory IIC fibre stage. This has been demonstrated in other species [7]. In addition, late in foetal growth Picard *et al.* [26] have observed small-sized cells recognised by S, E and F antibodies in the Ma and S and E antibodies in the diaphragm, which are slow muscles in the adult. This also shows delayed differentiation of the Ma. The size and MHC composition of these fibres suggest that they are derived from a third generation of cells that form slow fibres exclusively in slow muscles [26]. These observations suggest the Ma muscle differentiation process shown schematically in Fig. 7.

Effect of weaning

The disappearance of MHC2a in the IIC fibres, together with that of MHCemb, occurs gradually with age, ending with weaning, in response to a change in the speed of contraction of the muscle due to the replacement of a liquid by a solid diet, or by evolution from a pre-ruminant to the

ruminant stage. During this period the activity of the muscle is very modified. Swanson et Harris [39], have shown that the activity of ingestion that involves the jaw muscles is double, the rumination activity which implies also these muscles is multiplied by a factor 4. On the contrary, the period of rest is divided by 1.5. The final differentiation of the Ma is therefore an adaptive response to a change in the calf's pattern of activity. Such changes in Ma composition at weaning have also been observed in other species. In rabbits, alpha-cardiac MHC rises and MHC2a falls at weaning [5]. Maeda *et al.* [20] and Herring [15] have shown that the peak of IIA/A fibres in mammals corresponds to the suckling-to-chewing transition. Our results are consistent with these observations. The changes brought about in the Ma by weaning are essentially functional in origin, while the other muscles (ST, LT) react to new food intake on weaning by changes in metabolism. ST and LT muscles in weaned animals contain fewer type I fibres and more type IIA fibres, and have more glycolytic and less oxidative metabolisms, consistent with the marked decrease in fatty acids and glucose in the ruminant diet [25]. These reactions are in sharp contrast to those of the Ma, suggesting that the contractile differentiation of the muscles is regulated by different mechanisms.

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