J. Anat. (1990), **168**, pp. 31–47
With 7 figures
Printed in Great Britain

Histochemical and functional fibre typing of the rabbit masseter muscle

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(*Accepted* 30 *May* 1989)

INTRODUCTION

Compared to most leg muscles the rabbit masseter muscle is architecturally complex and it also shows regional differences in masticatory activity (Weijs & Dantuma, 1981). Because of the heterogeneity in both form and function the question arises whether the fibre type composition of this muscle is adapted to local mechanical functions such as speed of contraction.

Leg muscles have already been examined in this respect. In some cat leg muscles English & Letbetter (1982) and Chanaud, Pratt & Loeb (submitted for publication) found neuromuscular compartments in which fibre types and function were correlated. In the chewing muscles, histochemical compartmentalisation has often been described (see, for example, Herring, Grimm & Grimm, 1979; Clark & Luschei, 1981; Eriksson & Thornell, 1983; Gorniak, 1986). However, fibre type composition has rarely been related to quantified mechanical functions. Moreover, previous histochemical findings on jaw-closing muscles have been based mostly upon analysis of small samples obtained from distinct anatomical regions or compartments and may therefore not be valid for the functionally different parts (or compartments) of the entire masseter.

The aim of this study is to investigate the distribution of fibre types in the rabbit masseter and relate it to its anatomical and functional compartmentalisation. To describe the types we have made use of enzyme histochemistry. The myosin-ATPase staining method of Staron & Pette (1986) was used to acquire an estimation of contraction speeds and the succinate dehydrogenase method to estimate the aerobic capacity of the fibres. The role of the muscle during normal functioning has been described previously (Weijs & Dantuma, 1981; Weijs, Brugman & Klok, 1987; Weijs, Brugman & Grimbergen, 1989). An indication of contraction speed differences within the muscle was obtained by combining the above-mentioned data with data on sarcomere length changes during normal mastication (Weijs, unpublished).

MATERIALS AND METHODS

Animals and dissection

Seven adult male New Zealand rabbits (1900–2200 g) were anaesthetised by an intravenous injection of sodium pentobarbitone, The masseter muscle was quickly freed from overlying tissue. Prior to muscle removal, the level and direction of three future sections were indicated by means of small stitches. After removal, using a sharp blade, stitches were placed in both ends of the muscle and attached to an adjustable

metal clamp. This clamp was used to stretch the muscle to a little beyond its resting length and to hold it during freezing. The stretched muscle was then coated with 5% glycerin (Meade, 1987), quenched in liquid nitrogen-cooled liquid Freon-22 (monochlorodifluoromethane) and stored at −70 °C.

The posterior deep portion of the masseter muscle (MPPO; Weijs *et al.* 1987) was removed separately because it has an orientation different from the rest of the masseter. After removal of the muscles the rabbits were killed with an overdose of the anaesthetic.

Histochemistry

The frozen masseter was bisected in a direction, perpendicular to its fibres with a cooled knife. The muscle halves were mounted on chucks and serial transverse sections (10–12 μm) were cut parallel to the plane of bisection on a cryostat microtome and mounted on glass slides. The sections were taken in three different regions: halfway through the belly of the masseter, about 9 mm above this level and halfway through the belly of the MPPO (Fig. 1, Lines I, II and III).

Preliminary 12 μm sections were fast-stained with Paragon (a mixture of alkaline fuchsin, toluidine blue and 96% alcohol) to check for tissue damage and fibre orientation.

The sections were incubated for Ca^{2+}-activated adenosine triphosphatase (ATPase) at pH 9·4 (Staron, Hikida & Hagerman, 1983), after three pre-incubations: pH 4·4, pH 4·6 and pH 10·5.

Using these procedures seven histochemical fibre types can be identified (Staron & Pette, 1986) (see Table 1 and, for example, Fig. 2). Type I fibres (slow contracting) show alkaline-labile (pH 10·5) and acid-stable ATPase activity (pH 4·4–4·6). Type IIA fibres (fast contracting) show alkaline-stable, acid-labile ATPase activity (pH 4·4–4·6). Staron & Pette (1986) furthermore describe five intermediate types showing a continuum of staining intensities between Type I and Type IIA fibres. In the rabbit masseter the most prevalent fibres are Type I and Type IIA. With the staining method of Staron & Pette (1986) Types I and IIA can be distinguished very clearly. The intermediate fibres are found in relatively low numbers and are less important.

In all rabbits succinate dehydrogenase activity (SDH) was demonstrated in serial sections according to van Noorden, Bhattacharya & Vogels (1983) (the incubation time varied between 10 and 12 minutes at room temperature). The level of this enzyme is a measure of the aerobic capacity and fatiguability of the fibre.

Morphometric techniques

In sections of the masseter the following anatomical units or compartments (Weijs *et al.* 1987) can be defined (Fig. 1): (i) The superficial masseter 12 (MSS1 and 2) originates from a conspicuous crest at the anterior root of the zygomatic arch; fibres diverge to the margin of the anterior two thirds of the angular process. (ii) The superficial masseter 3 (MSS3) lies deep to the MSS1 and MSS2, with which it shares its origin; fibres insert on the lateral surface of the angular process. (iii) The superficial masseter 4 (MSS4) is situated posterior to the MSS1 and 2; fibres originate from the lateral surface of the anterior third of the zygomatic arch and insert on the margin of the posterior third of the jaw angle. (iv) The middle masseter (MSM12) lies medial to the superficial masseter, originates from the ventral margin of the zygomatic arch, and attaches to the lateral side of mandible. (v) The anterior deep masseter (MPAN) and posterior deep masseter (MPPO) originate from the medial aspect of the zygomatic arch and attach to the upper part of the lateral surface of the mandible. The deep masseters are separated by the masseteric nerve.

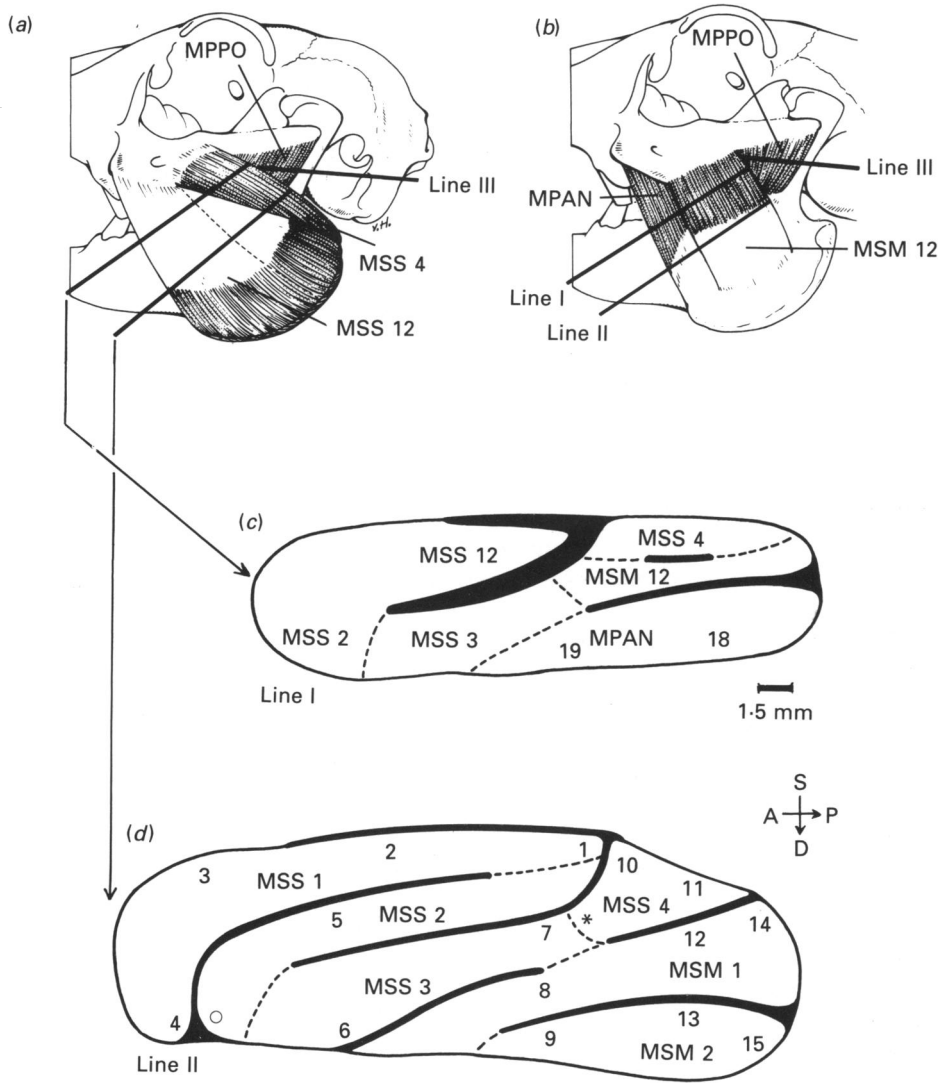

Fig. 1 (*a–d*). Lateral view of the superficial masseter (*a*) and the middle and posterior masseter (*b*). Lines I, II and III indicate the level of the selected sections. (*c*) and (*d*) show the position of the different samples in cross-sections I and II. Heavy solid lines indicate the aponeuroses, dashed lines divide the compartments. * Site of Figure 4, ○ site of Figure 6. A, anterior; P, posterior; S, superficial; D, deep. Other abbreviations in text.

As it is uncertain whether the compartments are histochemically homogeneous, samples were taken in two directions: one from the anterior to the posterior side of the muscle and another from lateral (superficial) to medial (deep) side. A total of nineteen sample sites were selected in this way, one to four in each anatomical compartment. Thus the sample sites could be arranged in rows and columns, independent of anatomical compartments (Fig. 1).

Because all compartments (except MPAN and MPPO) were represented in Plane II, fifteen samples were measured in that plane. The selected sample sites were close to the aponeuroses or near the exterior border of the muscle section to ensure that the site

Table 1. *Fibre types distinguished by Staron & Pette (1986) on the basis of mATPase stain with pre-incubation at variable pH*

Fibre type	pH 10·5	pH 4·3	pH 4·6
I	−	+	+
IIA	+	−	−
IC	+/−	+	+
IIC	+	+/−	+
IIC	+	+/−−	+/−
IIAB	+	−	+/−
IIB	+	−	+

+, Intensive reaction; −, no reaction.

belonged to an anatomically defined compartment. The anterior deep masseter was mainly represented in Plane I. Two samples were taken in this plane. In the separately dissected and sectioned MPPO two samples were taken near the middle of the mid-belly section. Sections from the available serial sections from different animals were selected on the basis of the presence and configuration of a number of aponeuroses.

Photomicrographs using Nomarski optics were taken of each sample area for the three ATPase stainings and the SDH activity. The micrographs were printed and a group of 120 adjacent fibres was selected randomly. The ATPase reaction was graded into dark and light; a small number of fibres did not fall into either of these categories and their reaction was therefore marked as intermediate. By this procedure each of the 120 fibres of a sample site was assigned to one of the types described by Staron & Pette (1986).

In some compartments, areas occur with more than 90% acid-stable (at pH 4·4–4·6) fibres. These areas were marked roughly on a map to give an indication of their position and to describe their pattern of distribution. Only areas exceeding 0·1% of the complete masseter area were treated in this way.

The SDH reaction was subsequently determined; only positive and negative fibre categories were distinguished.

Statistical analysis

Analysis of paired Student's t test for comparison of two samples and two-way analysis of variance for comparison of more than two samples were used to test the hypothesis of no difference of: (i) fibre type composition within each compartment; (ii) fibre type composition between the different compartments; and (iii) fibre type composition between rows and columns.

The Spearman rank correlation coefficient (R_S) was used to define a correlation in the area measurements. The null hypothesis was rejected at the 0·05 level of significance.

RESULTS

Fibre types (Fig. 2)

In most portions of the masseter muscle fibre Types I and IIA together constitute more than 85% of the fibre population. Both types show a positive SDH reaction. Of the other types, fibre Type IIB was seen in one sample only. The two Types IIC have been lumped into a single category and show, like Type IC, a moderate to strong SDH reaction. The posterior deep (MPPO) and posterior superficial (MSS4) masseter are

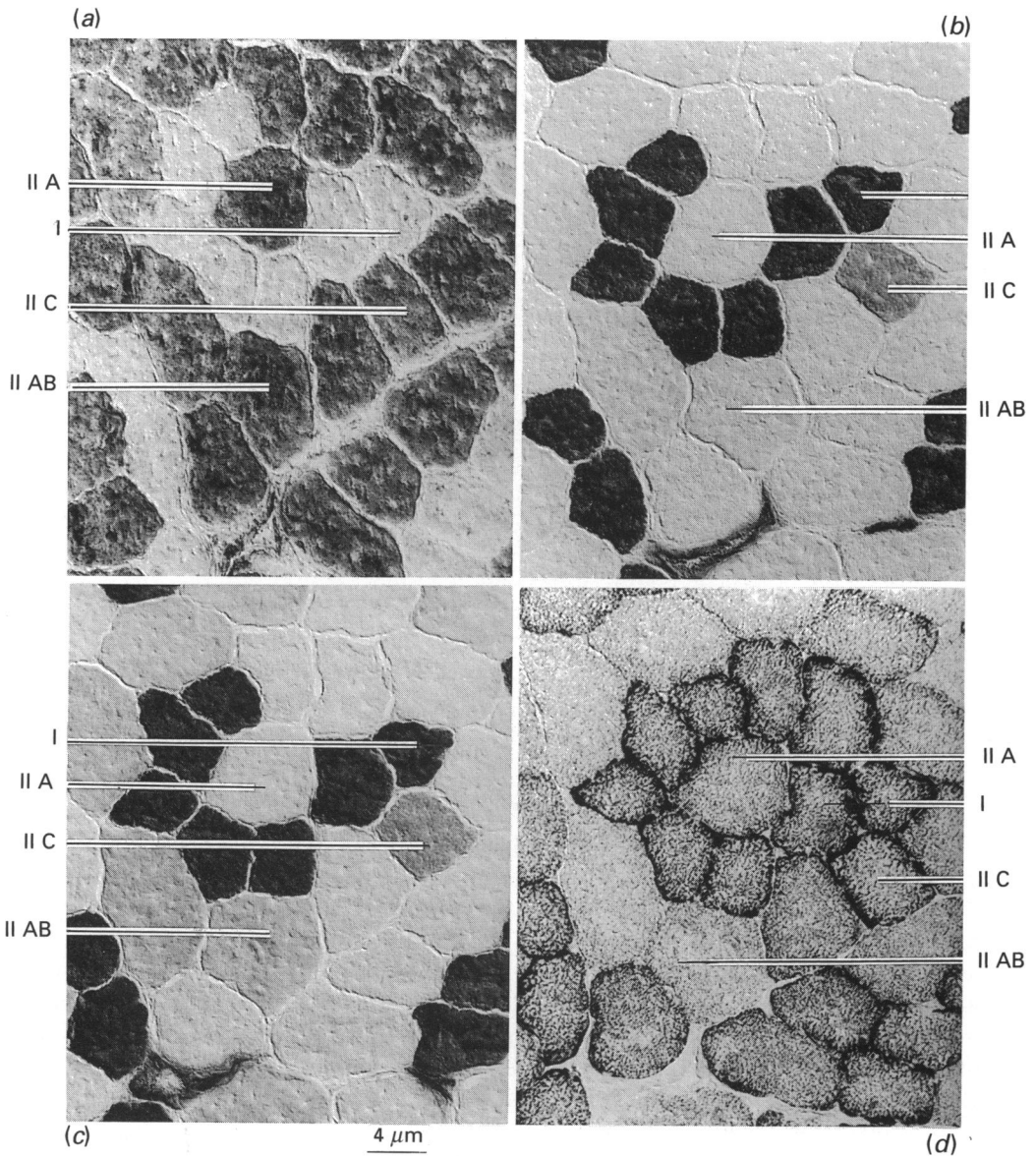

Fig. 2(*a–d*). Serial transverse sections of a region of the masseter at Line II. (*a*) Alkali-stable ATPase (pH 10·6 pre-incubation). (*b*) Acid-stable ATPase (pH 4·4 pre-incubation). (*c*) Acid-stable ATPase (pH 4·6 pre-incubation). (*d*) SDH. Type I fibres (I) have low alkali-stable ATPase (*a*), high acid-stable ATPase (*b* and *c*), and high SDH activity (*d*). Type II A fibres (II A) have high alkali-stable ATPase (*a*), low acid-stable ATPase (*b* and *c*), and moderate SDH activity (*d*). Type II AB fibres (II AB) have low alkali-stable ATPase (*a*), low acid-stable ATPase pH 4·4 (*b*), moderate acid-stable ATPase pH 4·6 (*c*), and low SDH activity (*d*). Type II C fibres (II C) have high alkali-stable ATPase (*a*), moderate to high acid-stable ATPase (*b* and *c*).

relatively rich in Type II AB fibres. In these fibres SDH activity could not be detected. Consequently, these regions of the masseter are the only ones displaying a mosaic reaction pattern for SDH. In the following four sections all Types except I and II A are discussed as a single category, intermediate (I M). A separate section describes the different frequencies of I M fibre types.

Table 2. *The results of a two-way analysis of variance carried out to test the possible significance of any variation in observed fibre type distributions which might be attributable to differences between animals and/or differences between sample places, resulting from the use of ATPase staining for fibre type. This is done for the total muscle*

Compartment	Fibre type	*F* animal	*F* sample
Total muscle	I	32·27*	30·24*
	IIA	16·74*	10·29*
	IM	2·41*	7·37*

F animal and *F* sample are variance ratios. * Means that a ratio is significant at the $P \leqslant 0{\cdot}05$ level.

Distribution of fibre types

The mean percentages of numbers of Type I, Type IIA and Type IM fibres at all sample sites are shown in Figure 3. The Figure shows that posterior regions contain a higher proportion of Type IIA and lower proportions of Type I fibres than anterior regions and that superficial regions also contain a higher proportion of Type IIA and a lower proportion of Type I fibres than deep regions. The MPPO contains more Type IIA than Type I fibres and the MPAN contains about the same portion of Type I and Type IIA fibres. The Type IM fibres are especially abundant in the posterior part of the superficial (MSS4) and in the posterior deep masseter (MPPO); they occur in low numbers in the rest of the masseter muscle.

Differences between animals and sample sites

The individual animals differed markedly with respect to the relative number of slow and fast fibres. For instance the 'slowest' animal had an average (over all samples) of 55% slow fibres in the masseter, the 'fastest' animal of 31%. Because of these inter-individual differences a two-way analysis of variance with animal and sample site as factors was applied to the data (Table 2). Both animal and sample site effect were highly significant. This indicates that the fibres are heterogeneously distributed throughout the masseter according to a consistent pattern and that there are consistent inter-individual differences. The animal effect and sample site effect accounted for approximately the same amount of variation in the data. The results were similar for analysis of the proportion of Type I and for the proportion of Type IIA fibres in the samples.

Differences within anatomical compartments

Figure 4 is an example of differences in fibre content between compartments. The MSS4 compartment shows a characteristic picture with a few small Type I fibres and many large Type IIA fibres. In the adjacent MSS3 compartment the Type I fibres are larger and occur in greater number; the Type IIA fibres are smaller than in MSS4 and occur in smaller number.

To investigate whether anatomical compartments (see methods) are homogeneous with respect to fibre type distribution an analysis of variance (in case of three or more samples) or a paired Student's *t* test (in case of two samples) was performed for sites within individual compartments.

Within the MSS1, MSS2, MSS4, MSM2, MPPO and MPAN significant differences

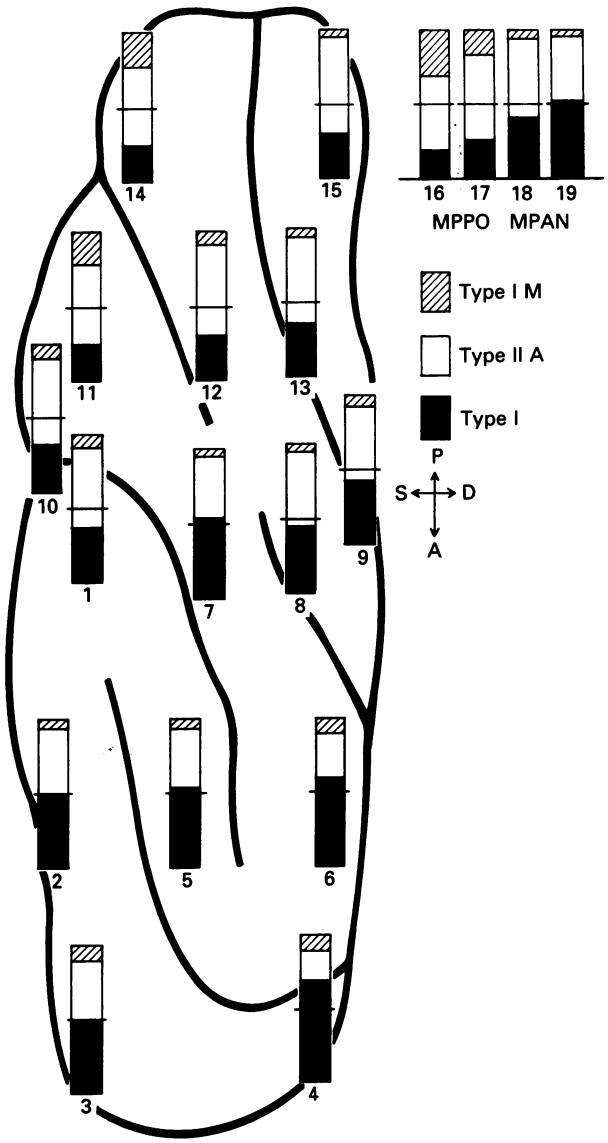

Fig. 3. Mean percentage distribution of the different fibre types at the sample sites.

were found between the sample sites for at least one fibre type (Table 3). Thus these compartments were non-homogeneous with respect to fibre-type distribution.

Differences within rows and columns

The chosen sample sites can be arranged in rows (superficial–deep) and columns (anterior–posterior) (Fig. 3).

The percentage of fibre types along the rows and columns are illustrated in Figure 5. Together with Table 4 it shows that progression from superficial to deep involves a significant increase of Type I and a significant decrease of Type II A fibres. (Sample 7 is an exception with an extremely high percentage of Type I fibres.) The row with

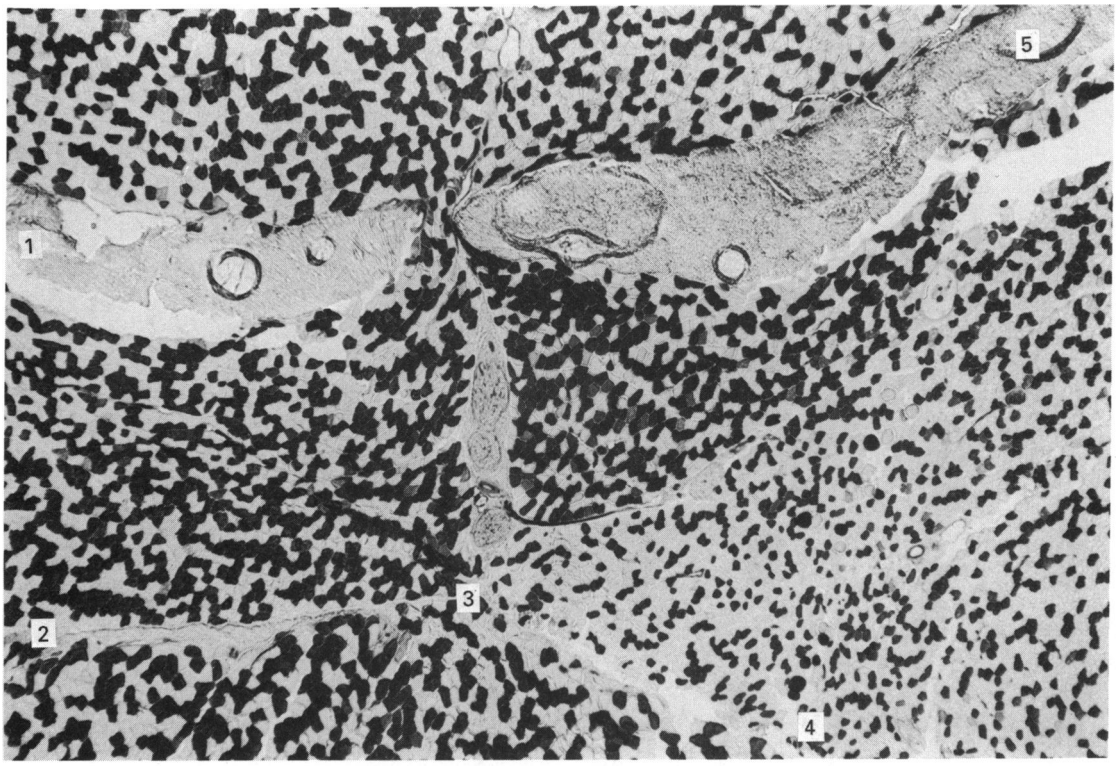

Fig. 4. Transverse section of the masseter at line II. Four different compartments (MSS2, MSS3, MSS4 and MSM1) and their characteristic fibre structures are shown. MSS2 is the part that lies above the line connecting 1 and 5. MSS3 lies between lines 1–4 and 2–3–4. MSS4 lies to the right of line 4–3–5. MSM1 lies below the line 2–3–4.

Samples 11 to 13 shows a decrease of IM fibres. From anterior to posterior there is a significant decrease of Type I, a significant increase of Type IIA and in two columns a significant increase of Type IM fibres.

Hence Type I fibres predominate in deep regions and decrease progressively in prevalence towards the posterior part of the muscle; Type IIA fibres show the reverse trend. Type IM fibres predominate in the posterior–lateral regions.

Distribution of intermediate fibres

So far Types IC, IIC and IIAB fibres have been treated as one category, Type IM. In Table 5 the distribution of the three intermediate fibre types is shown. There appears to be a different distribution of the three types over the compartments. The compartments MSS1, MSS2 and MSS3 show a predominance of Type IIC fibres, compartments MSS4 and MPPO a predominance of Type IIAB fibres and compartments MSM1, MSM2 and MPAN equal proportions of Type IC and IIC fibres.

Table 3. *Differences within anatomical compartments between percentages of I, IIA and IM fibre types*

Compartment	Fibre type	*F* animal†	*F* sample†	D.F.	*t* ratio‡
MSS1	I	9·53*	35·48*		
	IIA	8·81*	29·79*		
	IM	1·75	0·90		
MSS2	I	—	—	6	+5·88*
	IIA	—	—	6	−2·75*
	IM	—	—	6	−1·06
MSS3	I	—	—	6	−0·86
	IIA	—	—	6	+1·26
	IM	—	—	6	−1·76
MSS4	I	9·73*	7·03*		
	IIA	4·93*	0·24		
	IM	3·62*	4·31*		
MSM1	I	—	—	6	−1·95
	IIA	—	—	6	+0·13
	IM	—	—	6	+0·79
MSM2	I	—	—	6	−2·22
	IIA	—	—	6	+2·60*
	IM	—	—	6	+0·13
MPPO	I	—	—	4	+1·86
	IIA	—	—	4	+1·84
	IM	—	—	4	−2·84*
MPAN	I	—	—	6	+3·13*
	IIA	—	—	6	−3·00*
	IM	—	—	6	−0·97

* Significant $P \leqslant 0{\cdot}05$. D.F., degrees of freedom.
† Two-way ANOVA is used for compartments with more than two sites.
‡ Student's *t* test for compartments with two sites.

Areas with almost exclusively slow fibres

For each rabbit the total area occupied by regions with more than 90% of Type I fibres ('slow' areas) was computed (Fig. 6). The total area occupied by these 'slow' areas in different animals varies between 0 and 6% of the area of Plane II. The areas are especially localised in the deep part of compartments MSS1 and MSS2 and in the deep and middle part of compartment MSS3, areas that already have the largest percentages of slow fibres. The nineteen sample sites were always situated outside the 'slow' areas. This indicates that the difference between sample sites would be even larger if the 'slow' areas had been taken into account.

The grand mean for Type I fibres of all samples was computed for every animal and compared with the size of the 'slow' area by means of Spearman rank correlation coefficient (R_S) (Glantz, 1987). The correlation was significant ($R_S = 0{\cdot}82$, $P \leqslant 0{\cdot}05$). Hence rabbits with a high Type I predominance show a greater percentage of 'slow' areas.

DISCUSSION

Distribution of fibre types

The distribution of muscle fibres with high, low and intermediate myosin ATPase activity throughout the masseter muscle is not homogeneous. Type I (slow-twitch, oxidative) fibres predominate in the deep regions, Type IIA (fast-twitch, oxidative) fibres predominate in the superficial regions. Type I fibres increase and Type IIA

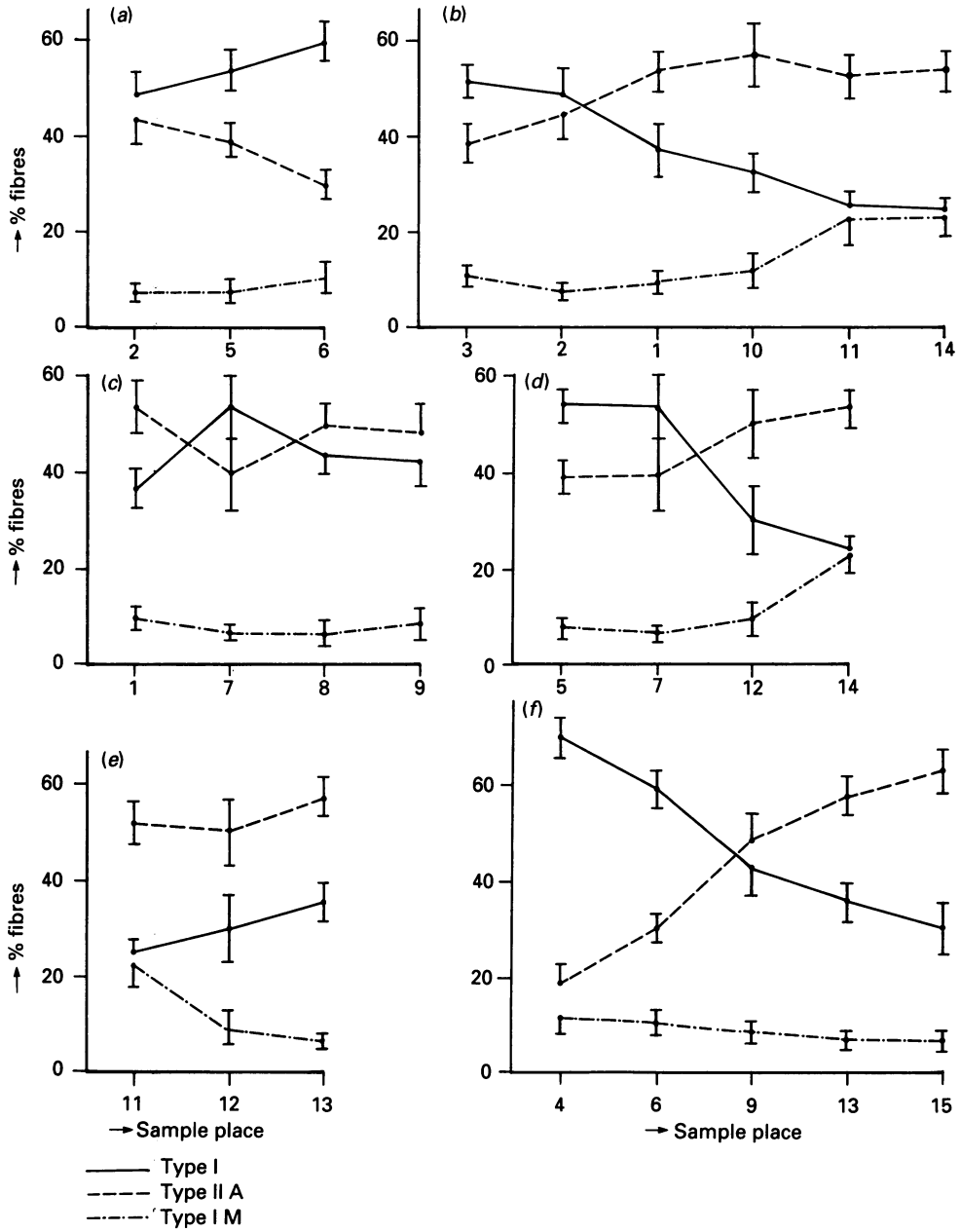

Fig. 5(*a–f*). Percentage of three different fibre types, at the different sample places, according to rows and columns. In (*a*), (*c*) and (*e*) the row goes from superficial to deep. In (*b*), (*d*) and (*f*) the column goes from anterior to posterior.

decrease in relative numbers going from posterior to anterior in the muscle. Intermediate fibres, including Type IIAB (fast-twitch, glycolytic) are relatively uncommon, except in the posterior portions of the superficial and in the deep masseter. In agreement Ringqvist (1973) reported the localisation of intermediate fibres in restricted areas in the human masseter.

Table 4. *Differences within sample areas arranged in rows (S–D) and columns (A–P) according to Figure 3*

Axis	Fibre type	Source of variation	*F* animal†	*F* sample†	D.F.	*t* ratio
S–D	I	3–4	—	—	6	+4·48*
	IIA		—	—	6	−6·34*
	IM		—	—	6	+0·44
S–D	I	2–5–6	11·17*	7·99*		
	IIA		4·58*	7·35*		
	IM		1·57	1·16		
S–D	I	1–7–8–9	16·53*	9·84*		
	IIA		23·05*	7·79*		
	IM		6·34*	1·33		
S–D	I	11–12–13	6·61*	12·21*		
	IIA		4·22*	1·14		
	IM		0·34	3·17		
S–D	I	14–15	—	—	5	+1·05
	IIA		—	—	5	+1·63
	IM		—	—	5	−4·39*
A–P	I	3–2–1–10–11–14	22·14*	42·63*		
	IIA		6·36*	4·09*		
	IM		2·01	4·93*		
A–P	I	5–7–12–14	6·85*	23·12*		
	IIA		5·32*	3·59*		
	IM		2·84*	10·25*		
A–P	I	4–6–9–13–15	12·48*	47·94*		
	IIA		6·10*	43·11*		
	IM		4·06*	1·21		

* Significant $P \leqslant 0{\cdot}05$. A, anterior; P, posterior; S, superficial; D, deep. D.F., degrees of freedom.
† 2-way ANOVA and *t* tests are used to test for differences within rows and columns consisting of more than two and of two samples, respectively.

Table 5. *Mean (± S.D.) distribution of the various categories of intermediate (IM) fibres in the muscle compartments*

	IC		IIC		IIAB		IM*	
Compartment	*x*	S.D.	*x*	S.D.	*x*	S.D.	*x*	S.D.
MSS1	21·3	28·9	50·2	36·8	24·0	31·9	11·8	4·3
MSS2	39·6	35·2	60·3	35·1	0	0	8·3	5·7
MSS3	26·0	31·9	66·4	31·0	4·7	12·1	10·1	6·0
MSS4	15·1	23·0	15·8	24·5	65·6	39·0	21·6	10·5
MSM1	39·2	32·4	30·1	25·2	2·3	7·7	9·4	5·5
MSM2	36·3	27·8	34·3	30·9	3·3	8·8		
MPPO	24·8	30·0	16·8	16·5	46·5	44·8	31·5	11·9
MPAN	42·7	31·9	43·5	32·5	9·8	16·6	9·0	6·2

* Total mean percentage distribution of the three intermediate fibre types.

Fibre types

In this paper the fibre type characterisations I, IIA, IIB, IIAB, IC and IIC, according to the definition of Staron & Pette (1986), are used. The last four of these types are referred to as intermediate (IM). The basis of this method is the use of three different pre-incubation pH values. The use of three pH values for incubation circumvents some of the problems of the ATPase method such as variation in time

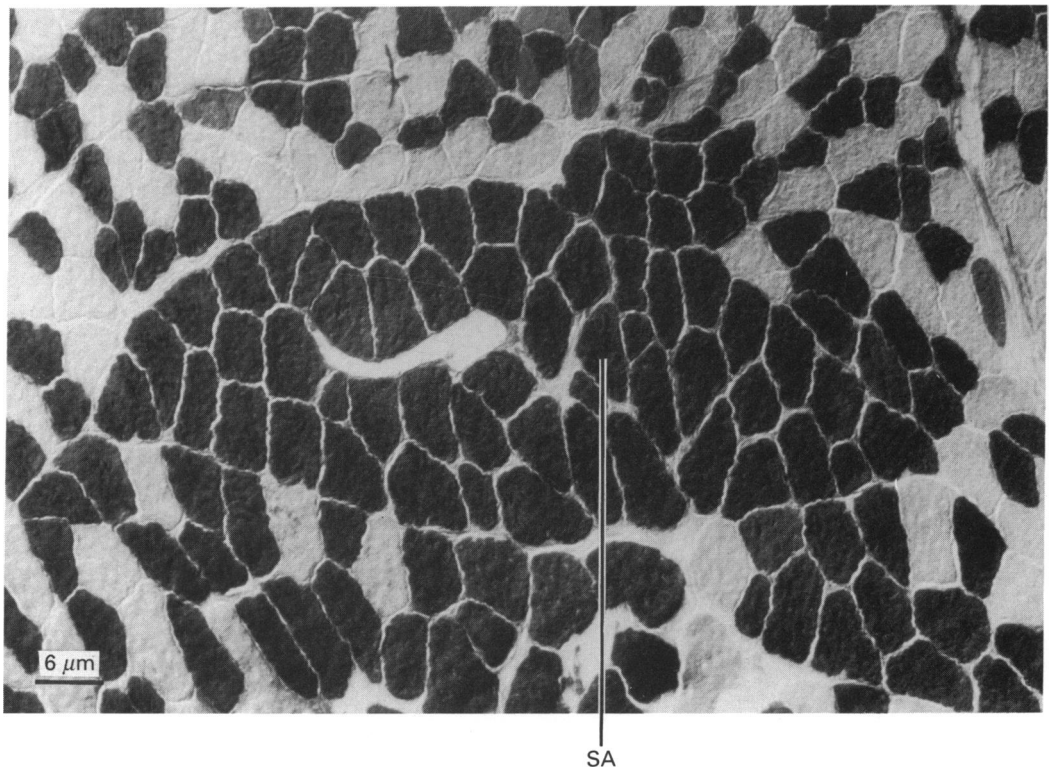

Fig. 6. Transverse section of the MSS2 compartment in a rabbit masseter at Line II. A 'slow' area with more than 90% Type I fibres is indicated (*SA*). ATPase staining at pH 4·6.

(Guth & Yellin, 1971; English & Wolf, 1982) and in pH optimum (Davis, 1986). It allows the positive identification of a fibre population with intermediate properties. However, the variety of methods and their technical difficulties hamper comparison of our results with data from the literature. The latter show not only differences within and between masseters of mammals, but also inconsistencies between different authors working on the same muscle. Specifically, caution is necessary if fibre classification is based on staining intensity since seemingly small alterations of technique have been shown to produce dramatic changes in relative colour intensities. In this study the staining method of Staron & Pette (1986) was used because these authors correlate their histochemical fibre types directly with the myosin heavy chain composition of the fibres. For instance, Type I fibres contain exclusively heavy chain I (HCI) and Type IIA contain only HCIIa myosins.

SDH activity shows that both Types I and IIA can be classified as oxidative. Consequently, Type I fibres must be slow-twitch oxidative and Type IIA fast-twitch oxidative. These types have been indicated as SO and FOG (Peter *et al.* 1972). The intermediate fibres (Type IM) belong to different physiological categories. Staron & Pette (1986) showed that they have varying ratios of heavy chain myosins. The posterior portions of the superficial (MSS4) and deep (MPPO) masseter are rich in IM fibres belonging almost exclusively to Type IIAB (Table 5). The Type IIAB fibres display a coexistence of the HCIIa and HCIIb in varying ratios (Staron & Pette, 1986). In these fibres the SDH activity is absent or very low and hence they must be fast-twitch glycolytic fibres.

The remaining intermediate types are IC, IIC and IIB. Type IIB was hardly ever found in the rabbit masseter. Gauthier (1986) used a slightly different ATPase method (according to Brooke & Kaiser, 1970). She found that 2C fibres in some species (including man) showed stable ATPase activity at pH values even below 4·4. Her Type 2C is probably similar to our Type IIC. Staron & Pette (1986) showed that Type IC and IIC fibres represent a histological continuum between the Types I and IIA. The intermediate C-fibres are characterised by the coexistence of both HCI and HCIIa myosins in varying ratios.

Fibre typing in the masseter

Many authors have underestimated the heterogeneity of the masseter and describe its fibre composition on the basis of one or two samples (Tamari *et al.* 1973; Taylor, Cody & Bosley, 1973; Schiaffino, 1974; Taylor, 1976; Hiraiwa, 1978; Mabuchi *et al.* 1984; Bubb & Sims, 1986). This can explain the enormous discrepancies found in the literature in the description of differences in fibre type composition. As far as generalisations are possible, the combined data show that larger animals, with low chewing rates, have smaller proportions of fast fibres. This observation has also been made for other muscles (Hiraiwa, 1978; Suzuki, 1977; Gauthier, 1986).

Differences between superficial and deeper layers of the masseter are also described for the pig (Herring *et al.* 1979), rat (Hiiemae, 1971; Rokx, van Willigen & Jansen, 1984), macaque monkeys (Clark & Luschei, 1981) and man (Eriksson & Thornell, 1983). It appears that slow fibres are more prevalent in the deep regions of the masseter. However, some studies (Serratrice, Pellissier, Vignon & Baret, 1976: man; Gorniak, 1986: cat) have failed to demonstrate such differences. Differences between anterior and posterior masseter muscle segments have been described for man (Eriksson & Thornell, 1983), rhesus monkey (Maxwell *et al.* 1979) and bat (de Gueldre, 1988). They found, as we did, that the posterior regions contain more fast fibres than the anterior regions. However, Herring *et al.* (1979) found no differences between the posterior and anterior regions of the pig masseter. Whether it is possible to find systematic differences between animals of the same size with different dietary habits cannot be answered because of the differences between authors in their staining methods and choice of measurement sites.

Relation to function

What is the functional significance of the observed fibre type distribution? For locomotion, a recruitment pattern has been established in which the muscles or muscle regions, high in slow fibres such as the semitendinosus muscle, are recruited prior to those with low slow fibre content (Walmsley, Hodgson & Burke, 1978; Burke, 1981; Burke, 1986). Muscles can be 'histochemically regionalised', i.e. characterised by a non-uniform fibre type distribution but may also be 'mechanically heterogeneous', i.e. contain regions with different contributions to contraction speed and/or force. Chanaud *et al.* (submitted for publication) and English (1985) showed that 'mechanically heterogeneous' hindlimb muscles of the cat are not uniformly active although they have a uniform fibre composition. The muscles consist of several 'neuromuscular compartments' each of which is recruited in a different way; within a compartment the normal recruitment order of motor units is present. For example, the slow units are recruited at lower force threshold levels than the fast ones, in accordance with the 'size principle' (Loeb, 1984).

Specific functions of the masseter muscle

The rabbit masseter is a more complicated muscle than the leg muscles. According to Chanaud *et al.* (submitted for publication) it is 'histochemically regionalised' and also 'mechanically heterogeneous'. Fibre directions range from predominantly forward (protractive) via vertical to backward (retractive); the distance of the muscle fibres to the temporomandibular joint varies from 0–5 cm in the adult rabbit (Weijs & Dantuma, 1981; Weijs *et al.* 1987). The muscle consists of a number of anatomically discrete compartments, each of which not only has a different mean fibre composition, but also shows an internal gradient in fibre composition.

Weijs & Dantuma (1981) found that the different anatomical compartments fire at different times during the chewing cycle. At the working side of the jaw the deep portion of the masseter starts firing, the more superficial portions gradually follow. At the balancing side of the jaw a reverse firing order is observed. There is also a difference in firing between the posterior (MPPO) and anterior deep masseter (MPAN). During jaw closing the MPPO fires strongly on the working side and prior to the MPAN. But on the balancing side both fire simultaneously.

Using a 3-dimensional model (Weijs *et al.* 1987) in conjunction with X-ray cinematographic data of mastication (Weijs *et al.* 1989) we calculated maximum contraction speeds for different masseter compartments of the rabbit during normal mastication of pellet, hay or carrot (unpublished). In Figure 7 the maximally attained contraction speed during normal mastication is plotted against the percentage of fast fibres in the different compartments. It shows that *within* a compartment there seems to be a positive correlation between the percentage of fast fibres and the maximum contraction speed. However *between* the compartments no such relationship is found.

There are indications that the anatomical compartments of the masseter in the pig correspond to physiological compartments with a specific innervation (Wineski & Herring, 1983). This might be true for the rabbit too because EMG activities differ between compartments (Weijs & Dantuma, 1981). The different contraction speeds measured in the masseter compartments, however, are not related to differences in fibre composition. Perhaps the masseter compartments are differentially involved only in other, more strenuous efforts, such as long-lasting gnawing activities. At present, evidence for this is lacking. The efficiency of the different fibres in generating an isometric bite force is linearly related to the length of the moment arm of their force, relative to the temporomandibular joint. Measurements show a negative correlation ($R_S = -0{\cdot}81$, $P \leqslant 0{\cdot}05$) between the percentage fast fibres (IIA) and the moment arm. The fibres with the longest moment arm have the highest slow fibre content and may be the first to become active during a slowly increasing bite force.

Within compartments the EMG activation is uniform and recruitment might occur according to the 'size principle'. As the histochemical differences within the compartments are relatively small it is conceivable that the EMG measurements are too crude to demonstrate differential recruitment inside a compartment.

Regional variation of fibre types

It is a common observation, for both jaw and limb muscles, that slow fibres usually lie deeply and fast fibres superficially. As shown above, regional variation cannot be explained by normal contraction speeds or activity patterns. Such a fibre regionalisation might be correlated with the blood flow in the muscles. Armstrong & Laughlin (1983) have shown that, in rats, blood flow to the deep, 'red' oxidative portions of leg extensor muscles increases during treadmill walking to above the pre-

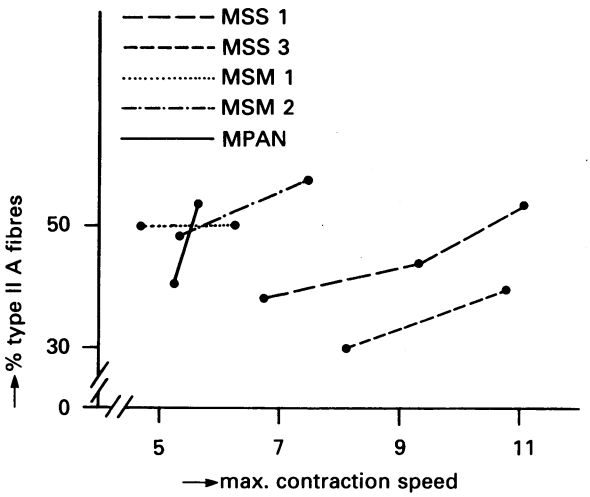

Fig. 7. Maximum contraction speed (μm/sarcomere/second) versus mean percentage Type II A fibres for the compartments MSS1, MSS2, MSM1, MSM2 and MPAN.

exercise level, while flow to the peripheral 'white' portions of the same muscles decreases significantly. With increasing treadmill speed, blood flow in the whole muscle mass increases. At all treadmill speeds the increase in blood flow is directly proportional to the number of fast-twitch oxidative fibres in the muscles. In the rabbit masseter, most fibres are oxidative but there might still be an analogous difference in blood flow to the deep, slow oxidative and superficial, fast oxidative fibres.

'Slow' areas

In some masseter regions, areas with more than 90% Type I fibres ('slow' areas) were observed. Eriksson, Eriksson, Ringqvist & Thornell (1982) found, in jaw muscles of healthy young human adults, large groups of densely packed muscle fibres of a similar histochemical type. Stålberg, Eriksson, Antoni & Thornell (1986) assume that clusters of fibres with similar histochemical types in jaw muscles are not likely to be a sign of re-innervation but are probably composed of fibres from different motor units. Gans (1982) explains this phenomenon by motor unit localisation. He showed that, in pinnate muscles, fibres of one motor unit are more localised than in muscles with parallel fibres. The masseter is strongly pinnate and compartmentalised. These two facts make it likely that fibres of a single motor unit are restricted to a limited territory so that, by chance, motor units of identical fibre types form continuous areas.

This study emphasises the need for careful selection of sampling sites for, *inter alia*, the histochemical characterisation of a muscle so that a precise description can be made of the correlation between its histochemical, anatomical and biomechanical properties. We have shown that with the present information, complex fibre type distribution cannot be explained by biomechanical factors alone.

SUMMARY

The fibre-type distribution of the masseter muscle of the rabbit was studied by means of the myosin-ATPase and succinate dehydrogenase reactions. Six different

fibre types were found and these were unequally distributed between and within the anatomical compartments of the muscle.

Most of the masseter consists of slow- and fast-twitch oxidative fibres. The slow fibres increase in numbers in the deeper and more anterior regions of the muscle. Fast-twitch glycolytic fibres were almost exclusively found in the most posterior portions of the superficial and deep masseter. The fibre composition within the sagittally orientated anatomical compartments was found to be correlated with maximal contraction speeds during natural mastication as estimated from a mechanical model. However, the differences in fibre composition between the anatomical compartments (and hence between superficial and deep layers) appeared not to be correlated with contraction speed.

The regional and compartmental specialisation within the masseter permits the muscle to perform many different functional roles in the generation and control of the jaw movements, jaw position and bite forces.

We would like to thank T. M. G. J. van Eijden for his comments on the manuscript, A. van Horssen and J. van Horssen-Medema for the art work, H. Rolleman-ter Heurne for preparation of the Tables, C. Hersbach for the photographic work and P. J. Sijses for her computer assistance.

REFERENCES

ARMSTRONG, R. B. & LAUGHLIN, M. H. (1983). Blood flows within and among rat muscles as a function of time during high speed treadmill exercise. *Journal of Physiology* **344**, 189–208.

BROOKE, M. H. & KAISER, K. K. (1970). Muscle fiber types: how many and what kind? *Archives of Neurology* **23**, 369–379.

BUBB, W. J. & SIMS, M. H. (1986). Fiber type composition of rostral and caudal portions of the digastric muscle in the dog. *American Journal of Veterinary Research* **47**, 1834–1842.

BURKE, R. E. (1981). Motor units: anatomy, physiology, and functional organization. In *Handbook of Physiology: The Nervous System*, vol. 2, pp. 345–422. Bethesda: American Physiological Society.

BURKE, R. E. (1986). Physiology of motor units. In *Engel and Banker's Myology Basic and Clinical*, pp. 419–443. New York: McGraw-Hill Book Co.

CLARK, R. W. & LUSCHEI, E. S. (1981). Histochemical characteristics of mandibular muscles of monkeys. *Experimental Neurology* **74**, 654–672.

DAVIS, H. L. (1986). Compatibility of different histochemical techniques for the demonstration of ATPase for muscle fiber typing. *Journal of Histotechnology* **9**, 187–188.

ENGLISH, A. W. (1985). Limbs vs. jaws: Can they be compared? *American Zoologist* **25**, 351–363.

ENGLISH, A. W. & LETBETTER, W. D. (1982). A histochemical analysis of identified compartments of cat lateral gastrocnemius muscle. *Anatomical Record* **204**, 123–130.

ENGLISH, A. W. & WOLF, S. L. (1982). The motor unit, anatomy and physiology. *Journal of the American Physical Therapy Association* **62**, 1763–1772.

ERIKSSON, P. O., ERIKSSON, A., RINGQVIST, M. & THORNELL, L. E. (1982). Histochemical fibre composition of the human digastric muscle. *Archives of Oral Biology* **27**, 207–215.

ERIKSSON, P. O. & THORNELL, L. E. (1983). Histochemical and morphological muscle-fibre characteristics of the human masseter, the medial pterygoid and the temporal muscles. *Archives of Oral Biology* **28**, 781–795.

GANS, C. (1982). Fiber architecture and muscle function. *Exercise and Sport Sciences Reviews* **10**, 160–207.

GAUTHIER, G. F. (1986). Skeletal muscle fiber types. In *Engel and Banker's Myology Basic and Clinical*, pp. 255–283. New York: McGraw-Hill Book Co.

GLANTZ, S. A. (1987). In *Primer of Biostatistics*, 2nd ed. New York: McGraw-Hill Book Co.

GORNIAK, G. C. (1986). Correlation between histochemistry and muscle activity of jaw muscles in cats. *Journal of Applied Physiology* **60**, 1393–1400.

GUELDRE DE, G. (1988). Functionele morfologie van het kauwapparaat van Pteropus giganteus (*Megachiroptera*). Thesis, University of Antwerpen, pp. 65–103, 139–149.

GUTH, L. & YELLIN, H. (1971). The dynamic nature of the so-called 'fiber types' of mammalian skeletal muscle. *Experimental Neurology* **31**, 277–300.

HERRING, S. W., GRIMM, A. F. & GRIMM, B. R. (1979). Functional heterogeneity in a multipinnate muscle. *American Journal of Anatomy* **154**, 563–576.

HIIEMAE, K. (1971). The structure and function of the jaw muscles in the rat (*Rattus norvegicus L.*). II. Their fibre type and composition. *Zoological Journal of the Linnean Society* **50**, 101–109.

HIRAIWA, T. (1978). Histochemical properties of masticatory muscle of growing rat and matured mammals. *Comparative Biochemistry and Physiology* **59A**, 231–238.

LOEB, G. E. (1984). The control and responses of mammalian muscle spindles during normally executed motor tasks. *Exercise and Sport Sciences Reviews* **12**, 157–204.

MABUCHI, K., PINTER, K., MABUCHI, Y., SRETER, F. & GERGELY, J. (1984). Characterization of rabbit masseter muscle fibers. *Muscle and Nerve* **7**, 431–438.

MAXWELL, L. C., CARLSON, D. S., MCNAMARA, J. A., FAULKNER, J. A. & FAULKNER, J. R. (1979). Histochemical characteristics of the masseter and temporalis muscles of the rhesus monkey (*Macaca mulatta*). *Anatomical Record* **193**, 389–401.

MEADE, J. O. (1987). Improved rapid freezing using cooled isopentane. *Journal of Histotechnology* **10**, 189.

NOORDEN, C. J. F. VAN, BHATTACHARYA, R. D. & VOGELS, I. M. C. (1983). Enzyme cytochemical staining of individual cells with the use of a polyacrylamide carrier. *Acta histochemica* **73**, 71–78.

PETER, J. B., BARNARD, R. J., EDGERTON, V. R., GILLESPIE, C. A. & STEMPEL, K. E. (1972). Metabolic profiles of three fiber types of skeletal muscle in guinea pigs and rabbits. *Biochemistry* **11**, 2627–2633.

RINGQVIST, M. (1973). Histochemical enzyme profiles of fibres in human masseter muscles with special regard to fibres with intermediate myofibrillar ATPase reaction. *Journal of the Neurological Sciences* **18**, 133–141.

ROKX, J. T. M., WILLIGEN, J. D. VAN & JANSEN, H. W. B. (1984). Muscle fibre types and muscle spindles in the jaw musculature of the rat. *Archives of Oral Biology* **29**, 25–40.

SCHIAFFINO, S. (1974). Histochemical enzyme profile of the masseter muscle in different mammalian species. *Anatomical Record* **180**, 53–62.

SERRATRICE, G., PELLISSIER, J. F., VIGNON, C. & BARET, J. (1976). The histochemical profile of the human masseter. *Journal of the Neurological Sciences* **30**, 189–200.

STÅLBERG, E., ERIKSSON, P. O., ANTONI, L. & THORNELL, L. E. (1986). Electrophysiological study of size and fibre distribution of motor units in the human masseter and temporal muscles. *Archives of Oral Biology* **31**, 521–527.

STARON, R. S., HIKIDA, R. S. & HAGERMAN, F. C. (1983). Reevaluation of human muscle fast-twitch subtypes: evidence for a continuum. *Histochemistry* **78**, 33–39.

STARON, R. S. & PETTE, D. (1986). Correlation between myofibrillar ATPase activity and myosin heavy chain composition in rabbit muscle fibers. *Histochemistry* **86**, 19–23.

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

TAMARI, J. W., TOMEY, G. F., IBRAHIM, M. Z. M., BARAKA, A., JABBUR, S. J. & BAHUTH, N. (1973). Correlative study of the physiologic and morphologic characteristics of the temporal and masseter muscles of the cat. *Journal of Dental Research* **52**, 538–554.

TAYLOR, A. (1976). Fiber types in the muscles of mastication. In *Anderson and Matthew's Mastication*, pp. 16–24. Bristol: John Wright & Sons Ltd.

TAYLOR, A., CODY, F. J. W. & BOSLEY, M. A. (1973). Histochemical and mechanical properties of the jaw muscles of the cat. *Experimental Neurology* **38**, 99–109.

WALMSLEY, B., HODGSON, J. A. & BURKE, R. E. (1978). Forces produced by medial gastrocnemius and soleus muscles during locomotion in freely moving cats. *Journal of Neurophysiology* **41**, 1203–1216.

WEIJS, W. A. & DANTUMA, R. (1981). Functional anatomy of the masticatory apparatus in the rabbit. *Netherlands Journal of Zoology* **31**, 99–147.

WEIJS, W. A., BRUGMAN, P. & GRIMBERGEN, C. A. (1989). Jaw movements and muscle activity during mastication in growing rabbits. *Anatomical Record* (in Press).

WEIJS, W. A., BRUGMAN, P. & KLOK, E. M. (1987). The growth of the skull and jaw muscles of the rabbit and its functional consequences in the New Zealand rabbit (*Oryctolagus cuniculus*). *Journal of Morphology* **194**, 143–161.

WEIJS, W. A. & VAN DER WIELEN-DRENT, T. K. (1983). The relationship between sarcomere length and activation pattern in the rabbit masseter muscle. *Archives of Oral Biology* **28**, 307–315.

WINESKI, L. E. & HERRING, S. W. (1983). Innervation and activity patterns in the masseter and zygomatico-mandibularis muscles of the pig. *Anatomical Record* **205**, 217A.