Belg. J. Zool. — Volume 121 (1991) — issue 2 — pages 279-294 — Brussels 1991

*(Manuscript received on 7 February 1990)*

# FIBRE COMPOSITION OF THE MASTICATORY MUSCLES OF *PTEROPUS GIGANTEUS* (BRUNNICH, 1782) (MEGACHIROPTERA)

by

GREET DE GUELDRE and FRITS DE VREE
Department of Biology, University of Antwerp (UIA),
2610 Antwerp, Belgium

## SUMMARY

Routine histochemistry was used to study fibre type composition of the masticatory muscles of the frugivorous flying fox, *Pteropus giganteus* (BRUNNICH, 1782). Frozen sections were stained for alkaline- and acid-stable ATPase, NADH-tetrazolium reductase and α-glycerophosphate dehydrogenase, and fibres were subsequently identified as slow-twitch oxidative (SO), fast-twitch oxidative glycolytic (FOG) and fast-twitch glycolytic (FG). Based upon relative proportions of fibre types, muscles and their subdivisions can be classified into three groups : group 1 muscles (superficial and medial temporales), containing less than 10 % of SO fibres, group 2 muscles (superficial and deep masseter, zygomaticomandibularis, deep temporalis, medial pterygoid), containing 20-30 % of SO fibres, and group 3 muscles (anterior and posterior digastrics), containing 30-50 % of SO fibres. Moreover, in group 3 muscles less than 5 % of the fast twitch fibres are fatigue resistant (FOG), whereas in both group 1 and 2 muscles, about 20-30 % of the fast-twitch fibres are FOG.

The histochemical profile of the masticatory muscles is correlated directly with their contraction characteristics and indirectly with their EMG patterns.

*Key words :* histochemistry, contraction characteristics, masticatory muscles

## INTRODUCTION

The wide range of mechanical demands imposed upon the masticatory apparatus of mammals is reflected in its structural and functional diversity. Despite detailed descriptions of the anatomy of skull and mandible, and analyses of patterns of jaw movements and coincident muscle activity in a wide variety of mammals (DE GUELDRE and DE VREE, 1988), relatively few studies consider the histochemical profile of the jaw muscles involved in chewing in their explanation of function. However, studies on limb muscles have indicated that fibre type distribution is related to contractile properties (*e.g.* BURKE, 1978 ; CLOSE, 1972), and hence may

have consequences on attempts to explain the mechanics of movements and their neural control.

The morphology and mechanics of the masticatory apparatus of the flying fox, *Pteropus giganteus* (DE GUELDRE and DE VREE, 1984, 1988, 1990) are adapted to a diet of soft fruit pulp and fruit juices . The aim of the present study was to investigate how this is translated in the fibre type composition of the main masticatory muscles. The histochemical fibre types were obtained by routine staining techniques. Isometric contraction properties of some muscles were also investigated by direct stimulation of whole muscles in vivo.

## MATERIAL AND METHODS

### Histochemistry

Muscle biopsies were obtained from four freshly killed adult *Pteropus giganteus* (BRUNNICH, 1782) (460-500 g), obtained from a commercial dealer from India. Bundles were excised from the middle of the superficial and deep masseter, zygomaticomandibularis, superficial anterior, superficial posterior, medial and deep temporales, medial and lateral pterygoid, and anterior and posterior digastric muscles. Topography and anatomy of these muscles were described in detail previously (DE GUELDRE and DE VREE, 1988). The bundles were rapidly frozen in isopentane, precooled with liquid nitrogen, and subsequently stored at -70° C until required (within 14 days).

From each muscle series of transverse sections (8 μm) were cut in a cryostat (-22° C), mounted on dry slides and air-dried. Subsequent sections of each series were stained for one of the following enzymes (methods see DUBOWITZ, 1985) : alkaline-stable myofibrillar adenosine triphosphatase (mATPase) by preincubation for 15 minutes at pH 9.4 and incubation for 30 min (37° C), acid-stable mATPase by preincubation for 5 minutes at pH 4.6 or 4.35 and incubation for 45 minutes, reduced nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR) with an incubation time of 30 minutes, and menadione linked α-glycerophosphate dehydrogenase (α-GPD) with an incubation time of 60 minutes.

From each series of sections, a representative field of at least 200 fibres was chosen, and the numbers and positions of positively reacting fibers determined for each of the different enzymes. Muscle fibres were classified as slow-twitch oxidative (SO), fast-twitch oxidative glycolytic (FOG), and fast-twitch glycolytic (FG) (PETER *et al.*, 1972). The values of table 1 represent the means (and standard deviation) of the four animals. However, for the posterior part of the superficial temporalis, deep temporalis and lateral pterygoid muscles only one sample was available.

### Contraction characteristics

In vivo stimulation of the medial temporalis, superficial masseter and zygomaticomandibularis muscles was performed on three specimens of *Pteropus giganteus*. The animals were anesthetized with Ketalar (100 mg/kg, IM) and Rom-

pun (0.4 mg/kg, IM). Deep anesthesia was maintained by further administration of Ketalar during the experiment as necessary. The muscles to be studied were exposed bilaterally and unipolar stainless steel electrodes were inserted near their origins and insertions with the aid of 16-gauge hypodermic needles. The free ends of the electrodes were connected to a stimulator (Grass S48). Subsequently, the cranium of each animal was fixed with the occlusal plane of the upper teeth horizontally, and an isometric force-displacement transducer (Grass FT10) was attached under and to the ventral side of the mandible in a horizontal position.

Supramaximal single square wave stimuli (2 ms duration, 5-8 V intensity) or stimulus trains (1-50 Hz) were applied to each muscle bilaterally. The mechanical response (twitch or tetanus) was recorded by means of the isometric force transducer as closing force exerted by the mandible . The signal was passed through a Gould D.C bridge amplifier (Model 13 4312 00), and finally displayed on a Tektronix R 5103 N storage oscilloscope and a Gould (Brush 481) multichannel chart recorder. The output was calibrated by 100-500 g loads. A series of muscle twitches, elicited by stimuli applied at a rate of 1/s, were recorded for each of different jaw positions, ranging from near closure, over wide open, to near closure again. Jaw positions were measured with dividers as the distance between the incisors. Tetanisation was studied in each muscle at the jaw position which gave a maximum twitch.

Each experiment yielded a set of data for each muscle. Twitch duration and time-to-peak-tension of single twitches, as well as peak amplitude at different jaw positions, were determined. Furthermore, tetanic frequency, rate of tension rise and peak tetanic tension were also obtained. Since the transducer measured the closing force of the tip of the mandible (which in fact are measurements of the outforce of the lever system and, hence, have no absolute meaning) tension values were expressed as the percentage of maximum tension, obtained at optimal jaw position (ANAPOL and HERRING, 1989). To allow comparisons among experiments, length measurements were expressed as percentages of optimal jaw position. Finally, initial muscle length as well as the change in muscle length during jaw opening were calculated indirectly for each of the jaw muscles studied from the origin-insertion distance with the jaws closed and the change of this distance at each of different jaw positions.

## RESULTS

### Histochemistry

At least 200 fibres are studied for each muscle. Three distinct fibre types are identified in all muscles on the basis of histochemical staining intensities. One type consists of large fibres, that stain darkly for alkaline-stable mATPase and α-GPD, and lightly for NADH-TR. These histochemical characteristics are consistent with the fast-twitch glycolytic (FG) fibres (PETER *et al.*, 1972). Smaller fibres with a strong NADH-TR and acid-stable mATPase activity, and a weak α-GPD activity correspond with the slow-twitch oxidative (SO) fibres. The remaining intermediate

sized fibres show moderate to strong NADH-TR and α-GPD, and strong alkaline-stable mATPase activity. They conform to the fast-twitch oxydative glycolytic (FOG) fibres. However, as contrasted to limb muscle, the FOG fibres were not readily identifiable after acid preincubation at pH 4.6.

All masticatory muscles of *Pteropus* contain a higher proportion of fast-twitch fibres as compared to the proportion of slow-twitch fibres (Table 1). However, the proportions of each fibre type differ among muscles and muscle subdivisions. Three groups of muscles can be distinguished. The muscles of the first group (group 1) mainly consist of fast-twitch fibres (more than 90 %), more than 60 % of which are of the FG type. Group 1 muscles contain the lowest proportion of slow-twitch (SO) fibres (less than 10 %). To this group belong the superficial and medial temporales. The second group of muscles (group 2) is characterized by 20-30 % of slow-twitch (SO) fibres and 70-80 % of fast-twitch fibres. Of the latter, 20-25 % belong to the FOG type and 45-55 % to the FG type. To this group belong the superficial anterior and deep masseter, zygomaticomandibularis, deep temporalis and medial pterygoid. The muscles of the third group (group 3) contain the highest proportion of slow-twitch (SO) fibres (more than 30 %) and the lowest proportion of fast-twitch fibres, less than 5 % of which belong to the FOG type. The anterior and posterior digastric belong to this group. Two muscles cannot be classified in either of the three groups. The posterior part of the superficial masseter contains a lower proportion of slow-twitch (SO) fibres, and hence, a larger proportion of fast-twitch fibres, as compared to the anterior part of this muscle. On the other hand, the lateral pterygoid exhibits a special fibre composition. This muscle shows similar characteristics as the group three muscles (digastrics) in the division between slow- and fast-twitch fibres. However, all the fast-twitch fibres of this muscle appear to belong to the FOG type.

**Contraction characteristics**

Contraction characteristics are obtained from muscles representative for the histochemically distinct group 1 (medial temporalis) and group 2 (superficial masseter and zygomaticomandibularis). In all muscles studied, the twitches are fast. However, the twitch characteristics differ among muscles. Time-to-peak-tension is significantly different ($P < 0.05$) in all muscles, being longest in the masseter (36.3 ± 3.8 ms) and shortest in the temporalis (21.7 ± 2.4 ms); the zygomatico-mandibularis (28.8 ± 1.25 ms) shows intermediate values. The twitch duration changes similarly (masseter, 68.8 ± 1.25 ms; zygomaticomandibularis, 56.3 ± 3.8 ms; temporalis, 55.0 ± 5.0 ms respectively), but is only significantly longer in the masseter as compared to the other muscles.

The length-tension relationship of single twitches in terms of gape indicates that the different muscles produce their maximum tension with the jaw opened; the associated average muscle lengths at optimum jaw position amount respectively to 1.26 times the initial muscle length in the medial temporalis muscle, 1.22 in the masseter muscle, and 1.29 in the zygomaticomandibularis muscle (Fig. 1). The optimum jaw position coincides with the maximum jaw position observed during

TABEL 1

*Mean percentage distribution* (± SD) *of slow-twitch oxidative* (SO), *fast-twitch oxidative glycolytic* (FOG) *and fast-twitch glycolytic* (FG) *fibres in the main masticatory muscles and their subdivisions of* Pteropus giganteus.

| *Muscle* | SO | FOG | FG | FOG + FG | FG/FOG | FG/FOG + SO |
|---|---|---|---|---|---|---|
| Superficial masseter | | | | | | |
| anterior | 28.3 ± 3.3 | 22.1 ± 2.1 | 49.5 ± 4.8 | 71.5 ± 3.3 | 2.3 ± 0.4 | 1.0 ± 0.2 |
| posterior | 15.5 ± 2.9 | 26.3 ± 0.0 | 58.3 ± 3.0 | 84.6 ± 3.0 | 2.2 ± 0.1 | 1.4 ± 0.2 |
| Deep masseter | 27.4 ± 4.3 | 18.1 ± 2.6 | 54.6 ± 2.3 | 72.6 ± 4.3 | 3.1 ± 0.4 | 1.2 ± 0.1 |
| Zygomaticomandibularis | 22.1 ± 2.4 | 22.3 ± 3.0 | 55.6 ± 7.2 | 77.9 ± 2.4 | 2.8 ± 1.3 | 1.3 ± 0.4 |
| Superficial temporalis | | | | | | |
| anterior | 6.6 ± 0.7 | 28.0 ± 0.3 | 65.4 ± 0.9 | 93.4 ± 0.6 | 2.3 ± 0.1 | 1.9 ± 0.1 |
| posterior | 1.3 | 37.2 | 61.4 | 98.6 | 1.6 | 1.6 |
| Medial temporalis | 8.4 ± 0.0 | 26.8 ± 2.7 | 64.8 ± 2.7 | 91.6 ± 0.0 | 2.5 ± 0.3 | 1.9 ± 0.2 |
| Deep temporalis | 25.4 | 26.8 | 47.9 | 74.7 | 1.8 | 0.9 |
| Medial pterygoid | 27.9 ± 2.3 | 22.5 ± 5.3 | 49.6 ± 4.1 | 72.1 ± 2.3 | 2.4 ± 0.9 | 1.0 ± 0.2 |
| Lateral pterygoid | 48.8 | 51.2 | 0.0 | 51.2 | 0.0 | 0.0 |
| Anterior digastric | 31.1 ± 2.7 | 4.2 ± 3.0 | 64.7 ± 1.2 | 68.9 ± 2.7 | 23.4 ± 9.3 | 1.8 ± 0.1 |
| Posterior digastric | 47.3 ± 1.1 | 3.4 ± 0.6 | 49.4 ± 0.8 | 52.7 ± 1.1 | 15.2 ± 2.5 | 1.0 ± 0.0 |

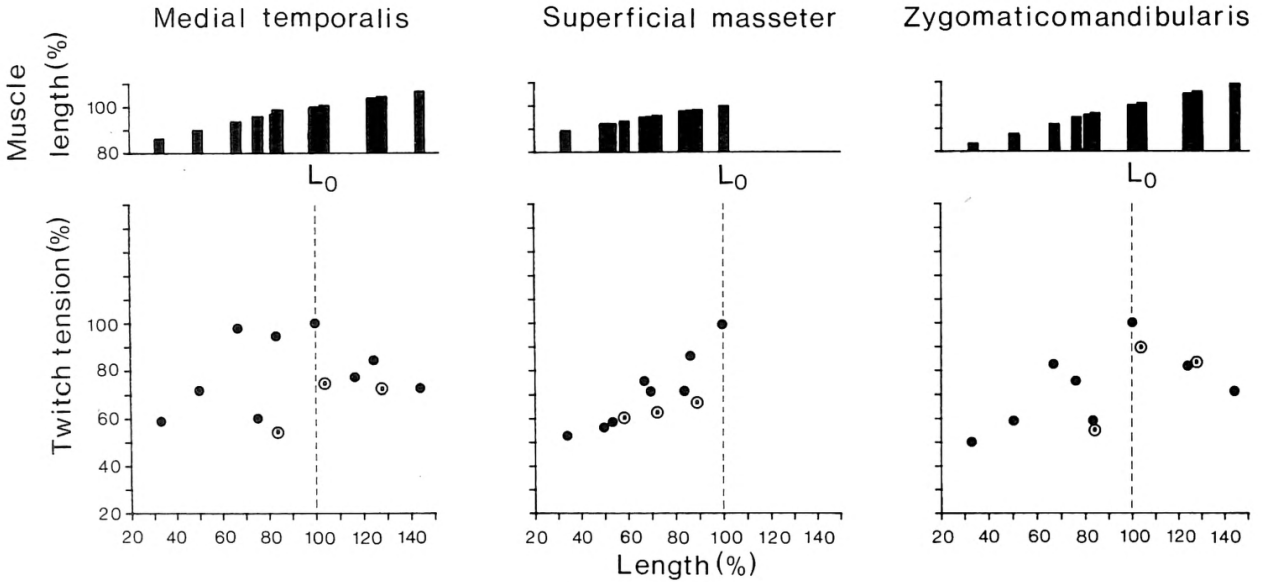


Fig. 1. — Length-tension relation of isometric twitches elicited by stimuli at a rate of 1 Hz at increasing (●) or decreasing (⊙) length (lower trace) and associated mean muscle lengths (upper trace) in medial temporalis, superficial masseter and zygomaticomandibularis muscles. Length is measured as the distance between the incisors and expressed as the percentage of optimum distance. Tension is expressed as the percentage of maximum tension obtained at optimum jaw position. Mean muscle lengths at each of different jaw positions are expressed as percentages of optimum muscle length $L_0$. $L_i$, initial muscle length (with closed jaws).

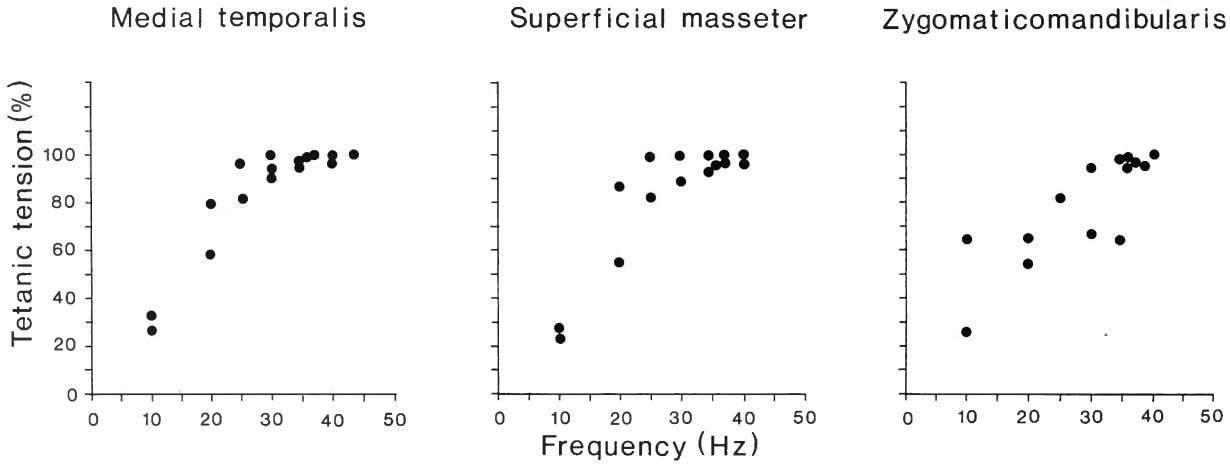


Fig. 2. — Frequency-tension relation of tetanic contractions elicited by stimulus trains of increasing rate at the optimum jaw position in medial temporalis, superficial masseter and zygomaticomandibularis muscles. Tension is expressed as the percentage of maximum tetanic tension.

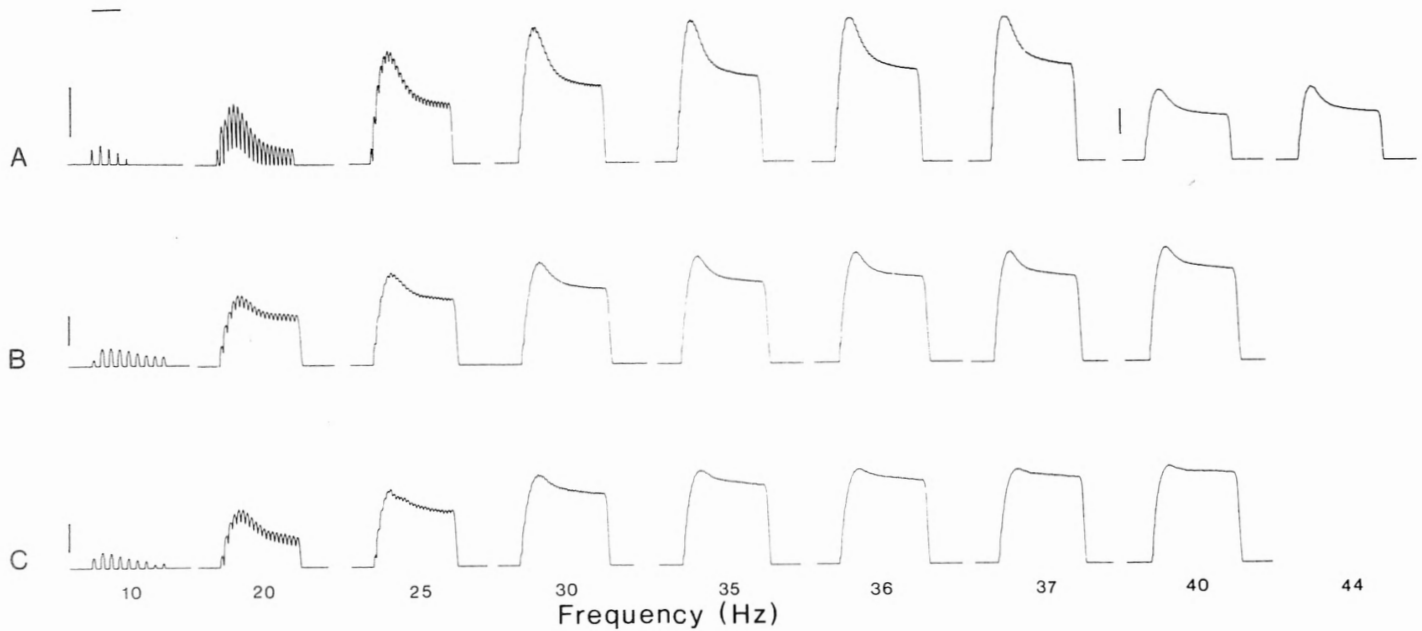


Fig. 3. — Representative isometric responses to stimulus trains of increasing frequency, elicited at optimum muscle length in medial temporalis (A), superficial masseter (B), and zygomaticomandibularis (C). Horizontal line, 200 ms ; vertical line, 100 g.

normal function in the masseter muscle, but is slightly below this maximum in the other two muscles. It is not clear whether this is an artefact of the method used. Furthermore, a slight hysteresis effect is observed in all muscles examined; the measured tension is generally less during stepwise shortening of the muscle after previous lengthening of the muscle, than during lengthening itself.

Maximum tetanic tension, elicited at the optimum muscle length, is reached at a frequency between 37-43 Hz (Fig. 2). This frequency coincides with the threshold frequency for smooth tetanic tension in all three muscles. The rate of tetanic tension rise increases with increasing frequency in the temporalis (time-to-peak tetanic tension decreases from ±240 ms to ± 160 ms in the range from 20-45 Hz); in the masseter and zygomaticomandibularis muscles, the time-to-peak tetanic tension remains ± 220 ms (Fig. 3).

## DISCUSSION

### Fibre types

Most information obtained to date on the histochemical fibre composition of masticatory muscles in mammals indicates their heterogeneous nature, and reveals considerable variation in proportions and cross-sectional areas of each fibre type, both within and among species (Bosley *et al.*, 1972; Clark and Luschei, 1981; Gorniak, 1986; Herring *et al.*, 1979; Hiiemae, 1971; Hiraiwa, 1978; Kita, 1971; Masuda *et al.*, 1974; Maxwell *et al.*, 1979; Ringqvist, 1974; Ringqvist *et al.*, 1977; Schiaffino, 1974; Suzuki, 1971, 1977; Taylor, 1976; Taylor *et al.*, 1973). Intraspecific variation within muscles appears to be correlated with sexual dimorphism in craniofacial skeleton and dentition (Maxwell *et al.*, 1979), with differences in age (Vignon *et al.*, 1980), or with topographical differences within muscles (Maxwell *et al.*, 1979). However, interspecific variation reasonably may be due to differences in feeding specialisations among mammals.

Based on the staining intensitiy for myosin ATPase, NADH-TR and α-GPD, three histochemical fibre types are identified in the masticatory muscles of *Pteropus*. Each of these types exhibit similar characteristics as those reported in other mammalian masticatory and limb muscles. Accordingly, they were here designated as slow-twitch oxidative (SO), fast twitch oxidative glycolytic (FOG) and fast-twitch glycolytic (FG) (Peter *et al.*, 1972). The myosin ATPase activity is proportional to the intrinsic speed of contraction (Barany, 1976). Two types of skeletal myosin have been described, which differ in ATPase activity. Myosin having high ATPase activity is alkaline-stable and acid-labile and dominates in fast muscle fibres. Myosin having low ATPase activity is acid-stable and alkaline-labile and predominates in slow muscle fibers (Burke *et al.*, 1971; Guth and Samaha, 1969; Maxwell *et al.*, 1982; Peter *et al.*, 1972). The staining intensity for NADH-TR is indicative of the oxidative capacity of fibres and correlates with resistance to fatigue (Burke *et al.*, 1971; Peter *et al.*, 1972). The intensity for α-GPD is a measure of the glycolytic capacity of fibres (Pette and Bücher, 1963). In accordance with physiological and histochemical properties of single motor units (Burke

*et al.*, 1973), it appears that FG fibres are designed for rapid, powerful, but unsustained contractions. FOG fibres produce a smaller amount of tension, but are more fatigue resistant. SO fibres are very slow, produce the smallest tension, but are very resistant to fatigue.

Based upon relative proportions of fibre types, three muscle groups could be distinguished among the masticatory muscles of *Pteropus*. However, in each of these groups the fast-twitch muscle fibres dominate ; all adductors contain a high percentage of fast-twitch fibres, ranging between 70-80 % in the masseter, but being greater than 90 % in the largest part of the temporalis. Pteropids are rather voracious animals that feed on soft fruit pulp and fruit juices. In *Pteropus* the chewing rate is 1-2.5 cycles per second (DE GUELDRE and DE VREE, 1984). Comparison with data on the masticatory muscles of other mammals suggests that the proportion of fast fibres in the main adductors is related to chewing rate. The latter is clearly a function of the masticated food, although body size may have some importance too. The masseter muscle of guinea pigs, rats and mice, which is their most important adductor, is composed entirely of fast-twitch fibres (MASUDA *et al.*, 1974 ; SCHIAFFINO, 1974 ; SUZUKI, 1977) ; chewing rates observed are respectively 6/second in guinea pigs (DE VREE, 1977) and 5-7/second in rats (THOMAS and PEYTON, 1983). In cats over 90 % of the fibres of their main adductor, the temporalis, and of the masseter, are fast-twitch (GORNIAK, 1986 ; TAYLOR *et al.*, 1973). As compared to guinea pigs and rats, their chewing rate is slower, being 3-3.5/second (GANS and GORNIAK, 1980). In humans (RINGQVIST, 1974 ; VIGNON *et al.*, 1980), macaques (CLARK and LUSCHEI, 1981), and pigs (HERRING *et al.*, 1979 ; SUZUKI, 1977) the masseter contains 60-80 % of fast-twitch fibres (exception : MAXWELL *et al.*, 1979) ; the available data for the temporalis show somewhat higher values. The chewing rate amounts 2.5-3.3/second in macaques (LUSCHEI and GOODWIN, 1974) and 3/second in pigs (HERRING and SCAPINO, 1973). In contrast, cattle and sheep masseter only contain slow-twitch fibres (SUZUKI, 1971, 1977 ; SUZUKI and TAMATE, 1974). Accordingly, their chewing rate is relativeley slow (40-70/minute, SUZUKI, 1977).

Muscles are not only designed to produce movement ; they are also able to produce large forces, which is especially important in masticating food. Requirements for speed and power do not necessarily exclude each other. Physiological evidence suggests that muscles containing a high proportion of FG fibres mostly combine these functions. In the human masseter muscle a positive correlation is found between the area of FG fibres and maximal isometric bite force (RINGQVIST, 1974). However, FG fibres are not fatigue resistant. A masticatory muscle with a high percentage of SO and FOG fibres is more likely adapted to frequent and long activity. This explains why the masseter of guinea pigs, rats and mice is composed entirely of fast-twitch oxidative glycolytic fibres. A possible loss of tension production is compensated for by an increase of muscle mass. In cattle and sheep, a long and sustained action, but less force, is required for mastication and rumination. Hence, the reported fibre composition (only slow-twitch fibres), as well as mass of their masseter entirely fits these demands.

The masticatory muscles of *Pteropus* appear to be adapted to both speed and power. Speed is important after ingestion, when pieces of fruit are initially punctured by rapid, vigorous orthal movements of the lower jaw. On the other hand, power is required during the last bites of a reduction sequence, when the skin of the fruit pieces is forcefully crushed (DE GUELDRE and DE VREE, 1984). The temporalis of *Pteropus* (histochemical group 1, over 90 % fast-twitch fibres) is the largest adductor, producing most of the bite force (DE GUELDRE and DE VREE, 1988, 1990). Electromyography has revealed that its activity shows little variation in amplitude during the course of a sequence, as well as among food types. Electromyography also indicates that its main part is only active for a short period during the closing phase. Hence, the dominance of FG fibres in the superficial and medial parts of the temporalis may be correlated with requirements for both speed and power. In the cat, a positive correlation between the level and duration of EMG and the percentage of SO + FOG fibres, and a negative correlation with the percentage of FG fibres and the ratio of FG/ FOG + SO fibres has been demonstrated (GORNIAK, 1986). The masseter and medial pterygoid muscles of *Pteropus* (group 2, 20-30 % of slow-twitch fibres) are generally active during diffent phases of the masticatory cycle (DE GUELDRE and DE VREE, 1988). Furthermore, their activity changes considerably as a function of the position of the cycle in the reduction sequence and as a function of food consistency. Probably, their main function is to add chewing force when necessary, especially early and late in the reduction sequence, as well as in the early part of opening. The openers of *Pteropus* (group 3 muscles) contain high proportions of SO fibres (30-50 %). They are active throughout the main part of the chewing cycle, and are probably of little use in the production of force. Furthermore, the inverted position during feeding may account for their fibre composition.

## Contraction characteristics

Some physiological parameters of muscle activity were obtained from muscles representative of two histochemical muscle groups. Since the tension was measured as closing force exerted by the mandible, the technique failed to give reliable results for the group 3 muscles. Hence, only the results from group 1 and group 2 muscles were presented.

The contraction characteristics obtained by in vivo stimulation of whole muscle are supposed to be representative of the behaviour of the muscle as a whole (THEXTON and HIIEMAE, 1975), especially in the range of the intensity of the applied stimuli (NORDSTROM and YEMM, 1974), and to produce the same pattern of length-tension curve as does direct stimulation via the nerve (MACKENNA and TÜRKER, 1978). The first two premises are likely to be valid for *Pteropus,* since electromyography has shown that in this animal all muscle subdivisions considered behave homogeneously during function (DE GUELDRE and DE VREE, 1988). The results thus may be assumed to represent at least those of the predominant fibres.

The contraction properties of the jaw muscles of *Pteropus,* as well as of other mammals for which data are available, appear to correlate with the histochemical

fibre composition. In *Pteropus* the temporalis is faster than the masseter and zygomaticomandibularis, but fatigues more rapidly during tetanic contractions. In opossums (THEXTON and HIIEMAE, 1975), rats (NORDSTROM and YEMM, 1974), guinea pigs (MASUDA *et al.*, 1974), cats (MACKENNA and TÜRKER, 1978 ; TAYLOR *et al.*, 1973), and macaques (MATSUNAMI and KUBOTA, 1972) the masseter, which is shown to contain more fast-twitch fibres, generally shows shorter twitch times as compared to *Pteropus*. In opossums and guinea pigs the temporalis is slower than the masseter, in cats it is faster. Tetanus frequency in the jaw muscles of *Pteropus* appears to be lower than in the rat masseter (NORDSTROM and YEMM, 1974). This is consistent with the fact that fast muscles show higher fusion frequency for tetani.

This study indicates that in the jaw closers of *Pteropus* maximum tension is developed when maximum gape is approached. Hence, our results agree with those of ANAPOL and HERRING (1989), MACKENNA and TÜRKER (1978), NORDSTROM and YEMM (1974), and THEXTON and HIIEMAE (1975). In the mouth openers, which we failed to study, maximum tension is reported to be developed with near-closed mouths (ANAPOL and HERRING, 1989 ; ANAPOL *et al.*, 1987 ; MACKENNA and TÜRKER, 1978). The only exception thus far is the optimum gape of 23° reported for the digastric muscle of the opossum (THEXTON and HIIEMAE, 1975). There are also indications that the tension produced during lengthening is slightly larger, than during subsequent shortening in the muscles studied. This effect was also reported in the opossum (THEXTON and HIIEMAE, 1975).

According to the sliding-filament theory the force-producing capacity of muscles is a function of sarcomere length (GORDON *et al.*, 1966). The effect of jaw opening on tension observed in whole jaw muscles may be related to changes in sarcomere length. Measurements of sarcomere length of masticatory muscles at different jaw positions confirm that in the adductors sarcomere length is significantly larger with the mouth open as compared to the closed position (HERRING *et al.*, 1979, 1984 ; HERZBERG *et al.*, 1980 ; MUHL *et al.*, 1978 ; NORDSTROM and YEMM, 1972 ; NORDSTROM *et al.*, 1974 ; WEIJS and VAN DER WIELEN-DRENT, 1983). In the abductors (digastric), sarcomere length is longer with the mouth closed (HERZBERG *et al.*, 1980). The longer sarcomere lengths are near the top of the ascending limb of the isometric length-tension relation (HERZBERG *et al.*, 1980 ; MUHL *et al.*, 1978). Hence, study of tension production on whole muscles is directly related to changes in sarcomere lengths. However, these studies also reveal regional variation in the effect of jaw position on sarcomere length within certain muscles (masseter) (HERRING *et al.*, 1979 ; HERZBERG *et al.*, 1980 ; NORDSTROM *et al.*, 1974 ; WEIJS and VAN DER WIELEN-DRENT, 1983). The assumption that not all fibres of a muscle reach their optimum at the same gape, concluded from the broad mean length-tension curve in certain masticatory muscles of the opossum (THEXTON and HIIEMAE, 1975), is consistent with these findings. However, this effect is probably only slightly present in the muscle subdivisions of *Pteropus*.

A functional interpretation of the length-tension relationships in the adductors of *Pteropus* is difficult. The superficial masseter and medial temporalis muscles are both effective in the nearly orthal closure of the mouth during mastication ; the zygomaticomandibularis muscle aids in the slight lateral deviation of the mandible

early in closure (DE GUELDRE and DE VREE, 1988). Electromyograms obtained during normal feeding indicate that the muscles studied are not (superficial masseter muscle) or only slightly (medial temporalis and zygomaticomandibularis muscles) active at the optimum length measured in this study ; on the contrary, in all muscles maximum activity is produced with the jaws nearly closed (DE GUELDRE and DE VREE, 1988). Only during biting are the muscles active with the jaws open. On the other hand, moments around the bicondylar or vertical (the latter mainly for the zygomaticomandibularis muscle) axis increase during closure in all three muscles (DE GUELDRE and DE VREE, 1990). So it is possible that the optimum muscle lengths near maximum gape compensate for a decreased mechanical efficiency. In the digastric muscle moments around the bicondylar axis are indeed largest with the jaws open.

**Conclusions**

It appears that jaw muscles are able to regulate their fibre composition in response to the need for a certain chewing rate and force. Basic chewing rates observed among mammals are correlated with fibre distribution. Coincidently, variation in amplitude of EMG may represent a form of load compensation. In *Pteropus* this regulation seems to be muscle dependent (DE GUELDRE and DE VREE, 1988, 1990). Jaw muscles with a higher proportion of oxidative fibres seem to be capable to regulate their muscular activity to a larger extent. Furthermore, sarcomere number in jaw muscles is adapted to allow the optimum sarcomere length to be reached at a certain gape (WEIJS and VAN DER WIELEN-DRENT, 1983). Possibly, this sarcomere number is regulated by active tensions at jaw positions where the muscle is highly active (Active Position Hypothesis, HERRING *et al.*, 1984), but muscle mechanics are probably involved also. Since it has been demonstrated that muscle fibre types reflect functional differences of the innervating motoneuron (HENNEMAN and OLSON, 1965 ; YELLIN, 1967), it may be suggested that the structural and functional heterogeneity of the masticatory apparatus among mammals reflects differences in neural control.

## ACKNOWLEDGMENTS

We wish to thank Prof. Dr. J. J. Martin for his permission to use his laboratory and material for histochemistry (supported by FGWO 3.0019.86 to Laboratory of Neurobiology Born Bunge Stichting), Mrs. J. Fret for her assistance during the experiments, and Prof. Dr. W. Verraes and two anonymous reviewers for comments on the manuscript.

## REFERENCES

ANAPOL, F. C. and S. W. HERRING (1989) — Length-tension relationships of masseter and digastric muscles of miniature swine during ontogeny. *J. exp. Biol.*, **143** : 1-16.

ANAPOL, F. C., Z. F. MUHL and J. H. FULLER (1987) — The force-velocity relation of the rabbit digastric muscle. *Archs oral Biol.*, **32** : 93-97.

BARANY, M. (1976) — ATPase activity of myosin correlated with speed of muscle shortening. *J. General Physiol.*, **50** : 197-218.

BOSLEY, M. A., F. W. J. CODY and A. TAYLOR (1972) — The correlation of histochemistry and speed of contraction in cat jaw muscles. *J. Physiol.*, **224** : 92-94.

BURKE, R. E. (1978) — Motor units : physiological/histochemical profiles, neural connectivity and functional specialisation. *Amer. Zool.*, **18** : 127-134.

BURKE, R. E., D. N. LEVINE, P. TSAIRIS and F. E. ZAJAC (1973) — Physiological types and histochemical profiles in motor units of the cat gastrocnemius. *J. Physiol.*, **234** : 723-748.

BURKE, R. E., D. N. LEVINE, F. E. ZAJAC, P. TSAIRIS and W. K. ENGEL (1971) — Mammalian motor units :physiological-histochemical correlation in three types in cat gastrocnemius. *Science*, **174** : 709-712.

CLARK, R. W. and E. S. LUSCHEI (1981) — Histochemical characteristics of mandibular muscles of monkeys. *Exp. Neurol.*, **74** : 654-672.

CLOSE, R. I. (1972) — Dynamic properties of mammalian skeletal muscles. *Phys. Rev.*, **52** : 129-197.

DE GUELDRE, G. and F. DE VREE (1984) — Movements of the mandibles and tongue during mastication and swallowing in *Pteropus giganteus* (Megachiroptera) : A cineradiographical study. *J. Morphol.*, **179** : 95-114.

DE GUELDRE, G. and F. DE VREE (1988) — Quantitative electromyography of the masticatory muscles of *Pteropus giganteus* (Megachiroptera). *J. Morphol.*, **196** : 73-106.

DE GUELDRE, G. and F. DE VREE (1990) — Biomechanics of the masticatory apparatus of *Pteropus giganteus* (Megachiroptera). *J. Zool., London*, **220** : 311-332.

DE VREE, F. (1977) — Mastication in guinea pigs, *Cavia porcellus*. *Amer. Zool.*, **17** : 886A.

DUBOWITZ, V. (1985) — *Muscle biopsy, a practical approach.* Baillière Tindall, London, Philadelphia, Toronto.

GANS, C. and G. C. GORNIAK (1980) — Electromyograms are repeatable : Precautions and limitations. *Science*, **210** : 795-797.

GORDON, A. M., A. F. HUXLEY and F. J. JULIAN (1966) — The variation in isometric tension with sarcomere length in vertebrate muscle fibres. *J. Physiol.*, **184** : 170-192.

GORNIAK, G. C. (1986) — Correlation between histochemistry and muscle activity of jaw muscles in cats. *J. Appl. Physiol.*, **60** : 1393-1400.

GUTH, L. and F.J. SAMAHA (1969) — Qualitative differences between actomyosin ATPase of slow and fast mammalian muscle. *Exp. Neurol.*, **25** : 138-152.

HENNEMAN, E. and C. B. OLSON (1965) — Relations between structure and function in the design of skeletal muscles. *J. Neurophysiol.*, **28** : 581-598.

HERRING, S. W. and R. P. SCAPINO (1973) — Physiology of feeding in miniature pigs. *J. Morphol.*, **141** : 427-460.

HERRING, S. W., A. F. GRIMM and B. R. GRIMM (1979) — Functional heterogeneity in a multipinnate muscle. *Am. J. Anat.*, **154** : 563-576.

HERRING, S. W., A. F. GRIMM and B. R. GRIMM (1984) — Regulation of sarcomere numbers in skeletal muscle : A comparison of hypotheses. *Muscle and Nerve*, **7** : 161-173.

HERTZBERG, S. R., Z. F. MUHL and E. A. BEGALE (1980) — Muscle sarcomere length following passive jaw opening in the rabbit. *Anat. Rec.*, **197** : 435-440.

HIIEMAE, K. M. (1971) — The structure and function of the jaw muscles in the rat (*Rattus norvegicus* L.). II. Their fibre type and composition. *Zool. J. Linn. Soc.*, **50** : 101-109.

HIRAIWA, T. (1978) — Histochemical properties of masticatory muscles of growing rat and of matured mammals. *Comp. Biochem. Physiol.*, **59A** : 231-238.

KITA, S. (1971) — Histochemical observation on tongue and masseter muscles of dog. *Sapporo Med. J.*, **39** : 225-234.

LUSCHEI, E. S. and G. M. GOODWIN (1974) — Patterns of mandibular movement and jaw muscle activity during mastication in the monkey. *J. Neurophysiol.*, **37** : 954-966.

MACKENNA, B. R. and K. S. TÜRKER (1978) — Twitch tension in the jaw muscles of the cat at various degrees of mouth opening. *Archs. oral Biol.*, **23** : 917-920.

MASUDA, K., S. TAKAHASHI and H. KURIYAMA (1974) — Studies on the fibre types of the guinea pig masticatory muscles. *Comp. Biochem. Physiol.*, **47** : 1171-1184.

MATSUNAMI, K. and K. KUBOTA (1972) — Muscle afferents of trigeminal mesencephalic tract nucleus and mastication in chronic monkeys. *Japan J. Physiol.*, **22** : 545-555.

MAXWELL, L. C., J. A. FAULKNER and R. A. MURPHY (1982) — Relationship among fibre type, myosin ATPase activity and contractile properties. *Hist. Jour.*, **14** : 981-997.

MAXWELL, L. C., D. S. CARLSON, D.A. MCNAMARA Jr. and J. A. FAULKNER (1979) — Histochemical characteristics of the masseter and temporalis muscles of the rhesus monkey (*Macaca mulatta*). *Anat. Rec.*, **193** : 389-402.

MUHL, Z. F., A. F. GRIMM and P. L. GLICK (1978) — Physiologic and histologic measurements of the rabbit digastric muscle. *Archs. oral Biol.*, **23** : 1051-1059.

NORDSTROM, S. H. and R. YEMM (1972) — Sarcomere length in the masseter muscle of the rat. *Archs. oral Biol.*, **19** : 895-902.

NORDSTROM, S. H. and R. YEMM (1974) — The relationship between jaw position and isometric active tension produced by direct stimulation of the rat masseter muscle. *Archs. oral Biol.*, **19** : 353-359.

NORDSTROM, S. H., M. BISHOP and R. YEMM (1974) — The effect of jaw opening on sarcomere length of the masseter and temporalis muscles of the rat. *Archs. oral Biol.*, **19** : 151-155.

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

PETTE, D. and T. BÜCHER (1963) — Proportionskonstante Gruppen in Beziehung zur Differenzierung der Enzymaktivitätsmuster von Skeletmuskeln des Kaninchens. *Hoppe-Seylers Z. Physiol. Chem.*, **331** : 180-195.

RINGQVIST, M. (1974) — Fiber types in human masticatory muscles. Relation to function. *Scand. J. Dent. Res.*, **82** : 333-355.

RINGQVIST, M., I. RINGQVIST and L. A. THORNELL (1977) — Differentiation of fibers in human masseter, temporalis and biceps brachii muscles. *J. Neurol. Sci.*, **32** : 265-273.

SCHIAFFINO, S. (1974) — Histochemical enzyme profile of the masseter muscle in different mammalian species. *Anat. Rec.*, **180** : 53-62.

SUZUKI, A. (1971) — Histochemical classification of individual skeletal muscle fibers in the sheep. III. On the M. masseter. *Jap. J. Zootech. Sci.*, **43** : 161-166.

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.

SUZUKI, A. and H. TAMATE (1974) — Histochemical classification of skeletal muscle fibers in the cattle. *Acta Histochem. Cytochem.*, **7** : 319-327.

TAYLOR, A. (1976) — Fibre types in the muscles of mastication. In : *Mastication* (ed. D. J. Anderson and B. Mathews), pp. 16-24. John Wright, Bristol.

TAYLOR, A., F. W. J. CODY and M. A. BOSLEY (1973) — Histochemical and mechanical properties of the jaw muscles of the cat. *Exp. Neurol.*, **38** : 99-109.

THEXTON, A. J. and K. M. HIIEMAE (1975) — The twitch-contraction characteristics of opossum jaw musculature. *Archs. oral Biol.*, **20** : 743-748.

THOMAS, N. R. and S. C. PEYTON (1983) — An electromyographic study of mastication in the freely-moving rat. *Archs. oral Biol.*, **28** : 939-945.

VIGNON, C., J . F. PELLISSIER and G. SERRATRICE (1980) — Further studies on masticatory muscles. *J. Neurol. Sci.*, **45** : 157-176.

WEIJS, W. A. and T. K. VAN DER WIELEN-DRENT (1983) — The relationship between sarcomere length and activation pattern in the rabbit masseter muscle. *Archs. oral Biol.*, **28** : 307-315.

YELLIN, H. (1967) — Neural regulation of enzymes in muscle fibers of red and white fibers. *Exp. Neurol.*, **19** : 92-103.