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# A comparative histochemical study of fibre types in middle ear muscles

A. VEGGETTI,* F. MASCARELLO§ AND E. CARPENÈ‡

*Istituti di Anatomia degli Animali Domestici con Istologia ed Embriologia delle Università di Bologna* e di Milano§ ed Istituto di Biochimica dell'Università di Bologna‡, Italy*

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## INTRODUCTION

Previous work on the middle ear muscles in mammals and, in particular, of tensor tympani, shows considerable interspecies differences not only in structure, but also in the degree and time of onset of the processes of involution it undergoes with age (Romano, 1926; Malan, 1934; Filogamo, 1940; Getty, Foust, Presley & Miller, 1956; Kobayashi, 1956*a*, *b*; Malmfors & Wersäll, 1960*a*, *b*; Blevins, 1963, 1964, 1967, 1968; Candiollo, 1964*a*, *b*, 1965; Gerhardt, David & Marx, 1966; David, Gerhardt & Uerlings, 1966; Candiollo & Guglielmone, 1969; Fernand & Hess, 1969; Seiden, 1971; Hirayama & Daly, 1974; Hirayama, Davidowitz & Daly, 1974; Giannotti & Veggetti, 1975). The functional hypotheses suggested on the basis of such morphological differences have not been experimentally confirmed because of the paucity of comparative studies not only of the physiological characteristics (Wersäll, 1958; Eccles, Eccles & Lundberg, 1958; Erulkar, Shelanski, Whitsel & Ogle, 1964; Teig, 1972*a*, *b*; Kevanishvili & Gvacharia, 1972), but also of the histochemical (Hoshino, 1967; Asmussen & Wohlrab, 1971; Teig & Dahl, 1972; Anderson, 1975; Burgener & Mayr, 1980).

We therefore decided to determine the histochemical fibre type composition of these muscles in different mammalian species so as to gain some clues to function. It is known that skeletal muscle fibres can be assigned to specific motor units on the basis of their histochemical oxido-reductive activities and myosin ATPase activity, which can be correlated with fatiguability (Edström & Kugelberg, 1968) and contraction time (Barany, 1967; Guth & Samaha, 1969; Barnard, Edgerton, Furukawa & Peter, 1971), respectively. Without entering into a discussion of the different classification criteria (for which see our previous papers: Mascarello, 1975; Mascarello, Aureli & Veggetti, 1979; Mascarello & Veggetti, 1979) we would mention that on the basis of these reactions one can identify at least three main fibre types belonging to fast-twitch, fast-fatiguing (FF), fast-twitch, fatigue-resistant (FR), and slow-twitch (S) types (Burke *et al.* 1971; Burke, Levine, Tsairis & Zajac, 1973). These three main types represent, however, only the extremes between which there are some intermediate types, as can be demonstrated histochemically and immunohistochemically (Askanas & Engel, 1975; Jansson, Sjödin & Tesch, 1978; Nemeth, Hofer & Pette, 1979; Billeter *et al.* 1980; Pierobon Bormioli, Sartore, Vitadello & Schiaffino, 1980), as well as physiologically (Garnett, O'Donovan, Stephens & Taylor, 1978; Buchthal & Schmalbruch, 1980).

## MATERIALS AND METHODS

The stapedius and tensor tympani muscles were removed from six horses (three mares, one stallion, and two geldings between 6–15 months of age), eight cows (four males and four females between 6 months and 3 years), eight pigs (five females and three castrated males between 6 and 8 months), five sheep (four females and one male between 1 and 3 years), four dogs (two males and two females between 7 months and 3 years), four cats (two males and two females between 3 and 6 months), and four rabbits (two males and two females between 4 and 5 months). Muscles from the ungulates were dissected out immediately after slaughter in the public slaughterhouse. Muscles from the carnivores and rabbits were removed from animals killed with sodium pentobarbital. Degenerative signs were absent or negligible in all the muscles taken.

Because these muscles are very small, each muscle was wrapped in a piece of another skeletal muscle immediately after its removal and these were frozen together in isopentane cooled by liquid nitrogen or dry ice. The muscles were sectioned together for the whole length of the middle ear muscles and serial sections 10 $\mu$m thick were tested for the following reactions: (*a*) myosin adenosinetriphosphatase (myosin ATPase), according to Padykula & Herman (1955) at pH 9·4, preceded by pre-incubation for 10 minutes in 0·1 M acetate buffer at pH 4·0, 4·2, 4·35, 4·6 and in 0·1 M 2-amino-2-methyl-1-propanol adjusted to pH 10·2 or 10·35 with NaOH, according to Guth & Samaha (1969). The alkaline pre-incubation was preceded by fixation at room temperature in the solution described by Guth (1973), but diluted 1:5 and for only 1–2 minutes. In the case of the tensor tympani and stapedius muscles the fixation concentrations and times proposed by Guth cause all myosin ATPase activity to disappear. For the same reason, the acid pre-incubation times for the pig tensor tympani were reduced to 3–4 minutes. In addition, to section the tensor tympani from the pig it was necessary to lower the cryostat temperature considerably, because of the much higher adipose tissue content. (*b*) Succinate dehydrogenase (SDH) according to Dubowitz & Brooke (1973). (*c*) Menadione $\alpha$-glycerophosphate dehydrogenase (M $\alpha$-GPDH) according to Dubowitz & Brooke (1973).

### *Size and frequency of fibre types*

The diameters of the various fibre types were measured with an eyepiece micrometer on the transverse sections treated for myosin ATPase activity after acid pre-incubation. For each species we measured the diameter of 50 fibres in one stapedius and one tensor tympani muscle in each sex. The fibres were chosen in random fields of the stapedius and in the tympanic and tubal portions of the tensor tympani. The mean diameter for each fibre was taken as the mean of its largest and smallest diameters. The frequencies of the fibre types were also counted on the whole cross sectioned area of sections treated for myosin ATPase activity after acid pre-incubation. At least one male and one female of each species was used. The sections used were those taken at the maximum thickness of the muscle; in this manner the tensor tympani sections were certain to include both tympanic and tubal portions. In Table 1 and in the histograms of Figure 3 the data given are the means of values from one male and one female, since there were no significant sexual differences in fibre size.

Table 1. *Mean diameters ($\Phi$) of fibre types in middle ear muscles*

| | Stapedius | | Tensor tympani | |
|---|---|---|---|---|
| | Type I | Type II | Type I | Type II |
| | $\Phi$ ($\mu$m) $\sigma$ | $\Phi$ ($\mu$m) $\sigma$ | $\Phi$ ($\mu$m) $\sigma$ | $\Phi$ ($\mu$m) $\sigma$ |
| Sheep | 16±2·8 | 20±3·0 | 13±2·2 | 20±2·5 |
| Cat | 20±2·4 | 32±6·5 | 17±2·3 | 25*±4·4 |
| Dog | 20±3·1 | 30±5·2 | 14±2·5 | 28*±3·8 |
| Rabbit | 10±1·1 | 25±3·5 | 10†±0·8 | 20†±1·6 |
| | | | 20‡±2·4 | 60‡±12·7 |
| Horse | 26±3·6 | 40±5·9 | 21±5·3 | 30±5·7 |
| Cow | 14±2·7 | 27±4·6 | 9±1·6 | 12±2·3 |
| Pig | 12±2·4 | 18±2·0 | 10§±1·6 | |

* Type IIM; † radial portion; ‡ tubal portion; § unclassifiable fibres.

## RESULTS

### *Stapedius muscle*

In all the species studied the stapedius was a cone-shaped muscle of extremely small size, and was wrapped in a capsule of connective tissue which adhered tightly to the perineurium of the facial nerve. It was composed of short muscle fascicles that left the capsule and converged on the terminal tendon which contained a small bony or cartilaginous nodule, known as Sylvius' nodule. The connective tissue component was particularly abundant in the rabbit and cat. The muscle component was only moderately well developed in all the species and was formed of fibres of different diameters.

The fibres with the greater diameter showed an acid-labile, intensely alkaline-stable myosin ATPase reaction, and were positive for SDH and M $\alpha$-GPDH. The fibres of smaller diameter showed an acid-resistant, weakly alkaline-stable myosin ATPase reaction, were positive for SDH, and were negative for M $\alpha$-GPDH. On the basis of their overall histochemical reactions, the fibres with the larger diameters can be classified as fast-twitch red fibres and those with smaller diameters as the slow-twitch type (Barnard *et al.* 1971). These fibres, as is known, correspond to Types IIA and I (Engel, 1962), alternatively known as $\alpha$R and $\beta$R (Ashmore & Doerr, 1971), or FOG and SO (Peter *et al.* 1972), respectively (Fig. 1).

In the sheep and pig (Fig. 2), the fast-twitch fibres could be divided into two subgroups, one having negative ATPase reaction after both pH 4·35 and 4·2 pre-incubation (classical IIA) and the other with a negative ATPase reaction after pH 4·2 only, but which stained less intensely than classical IIB fibres after pH 4·35 pre-incubation. The former also had less intense SDH and stronger M $\alpha$-GPDH activities than the latter groups.

In the dog and cat, however, two subgroups of the fast-twitch fibres could also be demonstrated, but only by the myosin ATPase reaction after pH 4·35 pre-incubation.

In the horse, some slow-twitch fibres showed a stronger M $\alpha$-GPDH activity than the others (Fig. 1).

The diameters (Table 1), which were always much less than those of the corresponding fibre types of the surrounding skeletal muscle, showed, nevertheless, inter-species variation (Figs. 1, 2). We found smaller and very uniform diameters in the pig (10 $\mu$m for the Type I fibres and 13–15 $\mu$m for Type IIA), whereas the horse

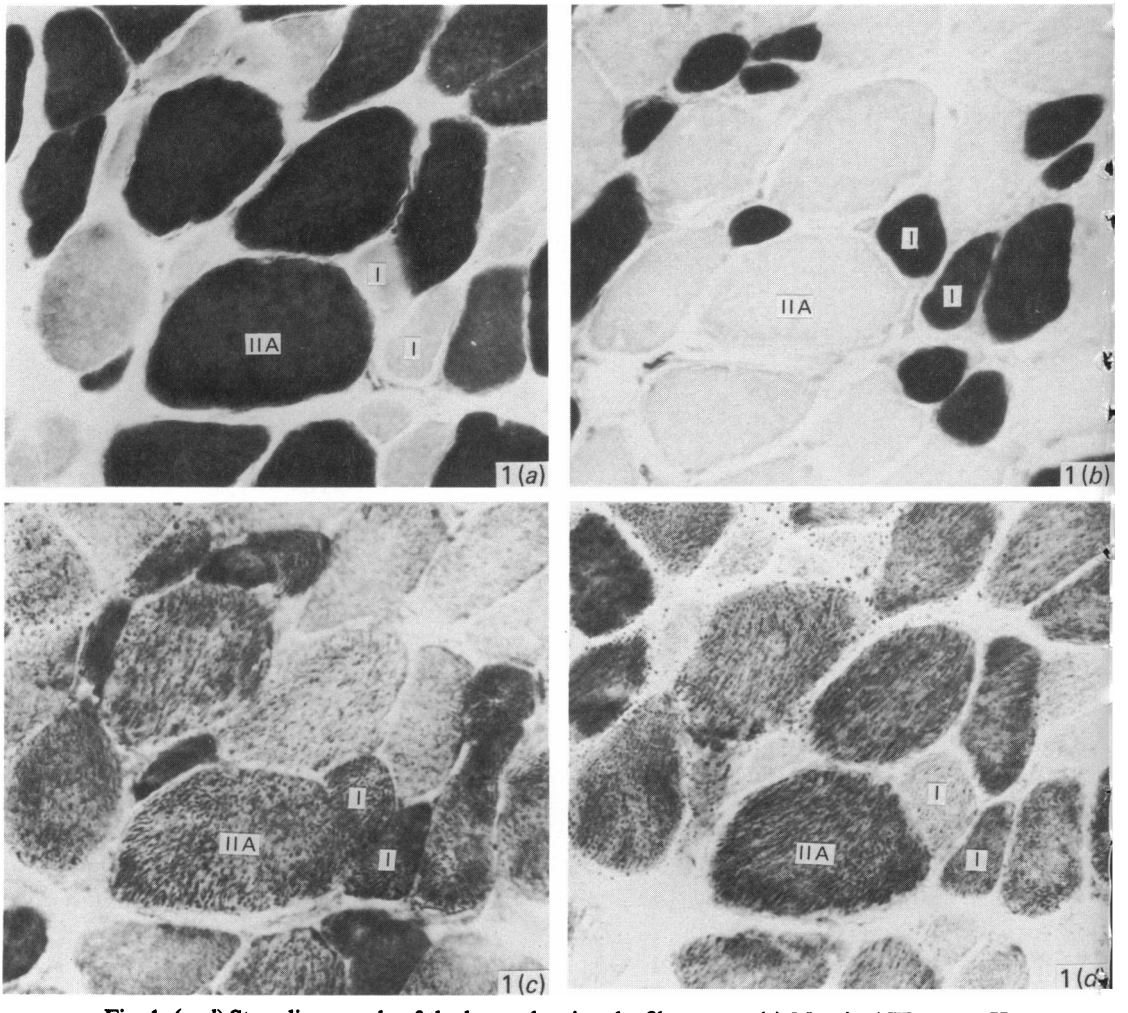


Fig. 1. (*a–d*) Stapedius muscle of the horse showing the fibre types. (*a*) Myosin ATPase at pH 9·4. (*b*) After pre-incubation at pH 4·2. (*c*) SDH. (*d*) M α-GPDH. × 380.

stapedius showed a wider range (15–35 μm for Type I fibres and 28–50 μm for Type IIA).

The frequencies of the various fibre types also varied in the different species. In the horse, cow, and pig, the numbers of the Type I and IIA fibres tended to be equal, whereas, in the other species, Type IIA predominated, showing a maximum frequency (*c.* 80% of the total fibres) in the cat and sheep (Fig. 3).

In all species, the myosin ATPase reaction with acid pre-incubation demonstrated a rich capillary network between the muscle fibres. In the dog, in particular, the capillaries had diameters quite similar to those of Type I fibres.

Muscle spindles were not found in these species.

### *Tensor tympani muscle*

The tensor tympani consisted of two portions, the tubal and the tympanic, having different insertions; however, these were not equally well developed in all the species.

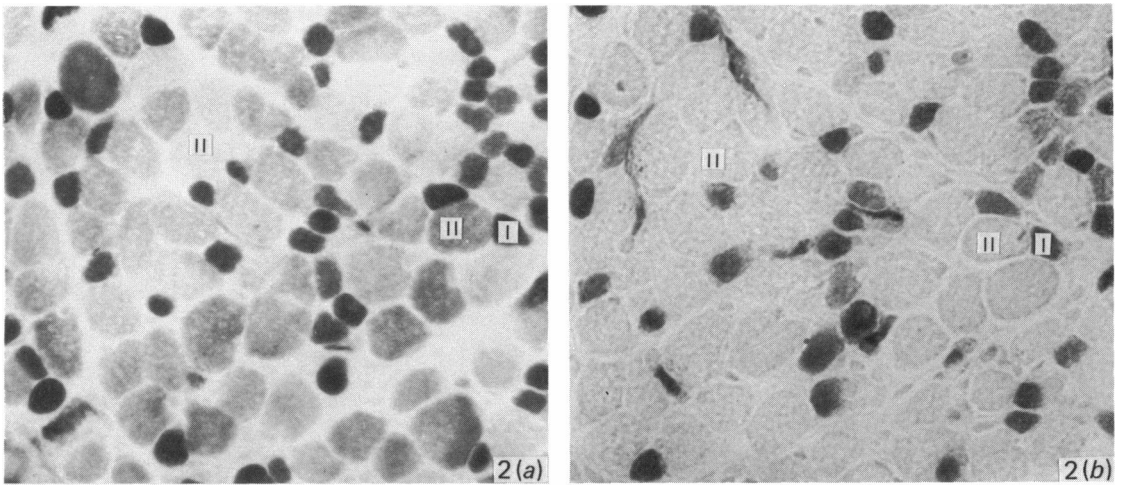


**Fig. 2. (*a–b*) Stapedius muscle of the pig showing the fibre types. (*a*) Myosin ATPase after pre-incubation at pH 4·35. (*b*) At pH 4·2. In some Type II fibres the myosin ATPase activity is negative after pH 4·35, in others it becomes negative only after pH 4·2. × 380.**

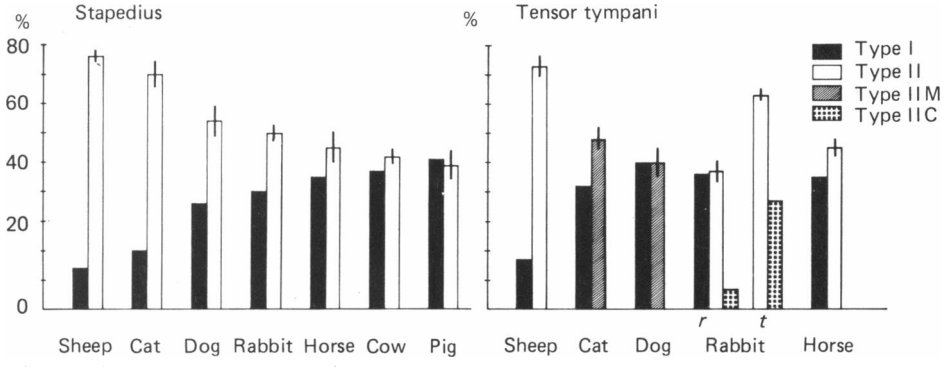


**Fig. 3. Histograms showing the fibre types frequencies in middle ear muscles. In the tensor tympani of the cow and pig it was not possible to estimate the frequencies because it was not possible to classify the fibres with certainty. Radial (*r*) and tubal (*t*) portions.**

If the tympanic portion predominated, the muscle had a spheroidal shape, whereas, if the tubal portion predominated, it was spindle-shaped (Fig. 10).

In the tubal portion, the muscle fibres ran parallel and formed a longitudinal bundle which went from the tendon of origin, to the tensor tendon. In the tympanic portion, on the other hand, they formed two bundles, one central (tubal bundle) and one peripheral (tympanic bundle). The tubal bundle consisted of parallel fibres which continued into the longitudinal bundle of the tubal portion, when the latter was present. The tympanic portion consisted of a system of radial fascicles which went from the inner surface of the thick connective tissue capsule to the tendon cone which formed a conspicuous part of the tympanic portion. The tensor tendon penetrated into the tympanic portion with a cone that was more or less developed, and which, at the centre of the muscle, radiated towards the capsule in numerous septa, between which were the muscle fascicles of the tympanic bundle. Depending on the species, either the muscle or the connective tissue component predominated in the

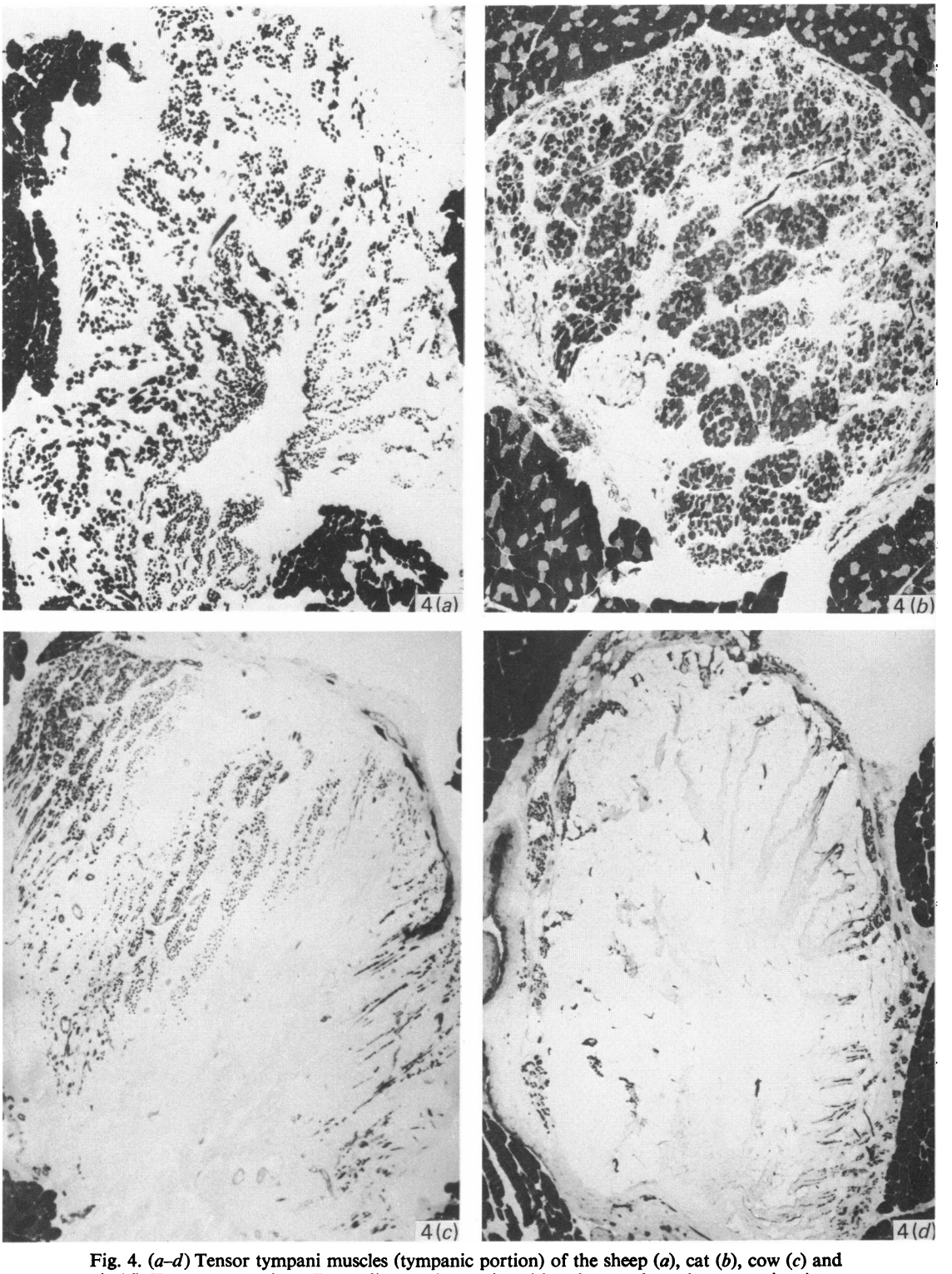


Fig. 4. (*a–d*) Tensor tympani muscles (tympanic portion) of the sheep (*a*), cat (*b*), cow (*c*) and pig (*d*). Transverse sections. Depending on the species, either the muscle or the connective tissue component predominates. Myosin ATPase at pH 9·4 (*a*, *c*, *d*) and after pre-incubation at pH 10·2 (*b*). ×34.

tympanic portion (Figs. 4, 10). As in the stapedius, muscle spindles were not found in the tensor tympani of any of the species examined. (For more structural details in domestic mammals see Malan, 1934.)

*Sheep*

This was the only species studied in which the tensor tympani, which was club-shaped (Fig. 10), had a well developed tympanic and tubal portion. In the tympanic portion, the muscle component clearly predominated over the connective one. The tendon cone, in fact, was quite narrow; the short, thin septa radiated out from it and became lost among the radial muscle bundles. The latter were well developed, as was the central bundle, whose parallel fibres continued into the tubal portion (Fig. 4*a*).

Both the tubal and tympanic portions contained slow-twitch fibres (Type I) with weak, acid-resistant, alkaline-labile myosin ATPase activity, positive SDH and negative M $\alpha$-GPDH reactions, and fast-twitch red fibres (Type IIA) with acid-labile, alkaline-stable myosin ATPase activity, which were more positive both for SDH and M $\alpha$-GPDH.

The Type I fibres varied in diameter from 6 to 20 $\mu$m, whereas the Type IIA fibres varied between 6 and 28 $\mu$m. In the tubal portion, the average diameter of both types was about 8–10 $\mu$m, whereas, in the tympanic portion, the fibres had a larger diameter (12–14 $\mu$m for Type I and 16–20 $\mu$m for Type IIA). The Type I fibres represented about 17% of the total measured on sections that included both tubal and tympanic portions. This percentage increased to 28% in the radial tympanic portion.

*Horse*

The tensor tympani had the shape of a slightly curved spindle, with a thickened tympanic end (Fig. 10). The tubal portion predominated over the tympanic one. The latter consisted mainly of connective tissue rich in fat cells, with a very small muscle component of thin radial fascicles and a short, thin tubal bundle. Type I and IIA fibres were present. Whereas in the tubal portion and tubal bundle the two types were equal in number, the thin radial fascicles of the tympanic portion were composed almost exclusively of Type I fibres.

The diameters of the Type I fibres of the radial bundle were much smaller (8–10 $\mu$m) compared to those of the fibres of the same type in the tubal portion, whose diameters were generally about 20 $\mu$m. The IIA fibres had a diameter of about 30 $\mu$m.

*Rabbit*

The tensor tympani had the same shape as that described for the horse (Fig. 10), with a tubal portion which predominated over the tympanic one. The muscle component was, however, more developed than in the horse, although connective tissue was still abundant. Adipose tissue, however, was almost absent. The muscle was longitudinally intersected by a tendon bundle on which both the radial and longitudinal fibres were attached (Fig. 5). There was a characteristic, thin bundle of very long fibres which ran from the convex side of the muscle (which corresponded to the tympanic portion) to the tubal end. In the radial tympanic portion the fibres had a considerably smaller diameter than the majority of fibres in the tubal portion (Fig. 5).

Three fibre types, I, IIA and IIC, could be identified in the radial portion. The first (10 $\mu$m in diameter, 52% of the total fibres) showed a weakly positive myosin ATPase reaction which was acid-resistant and alkaline-labile. The SDH reaction was

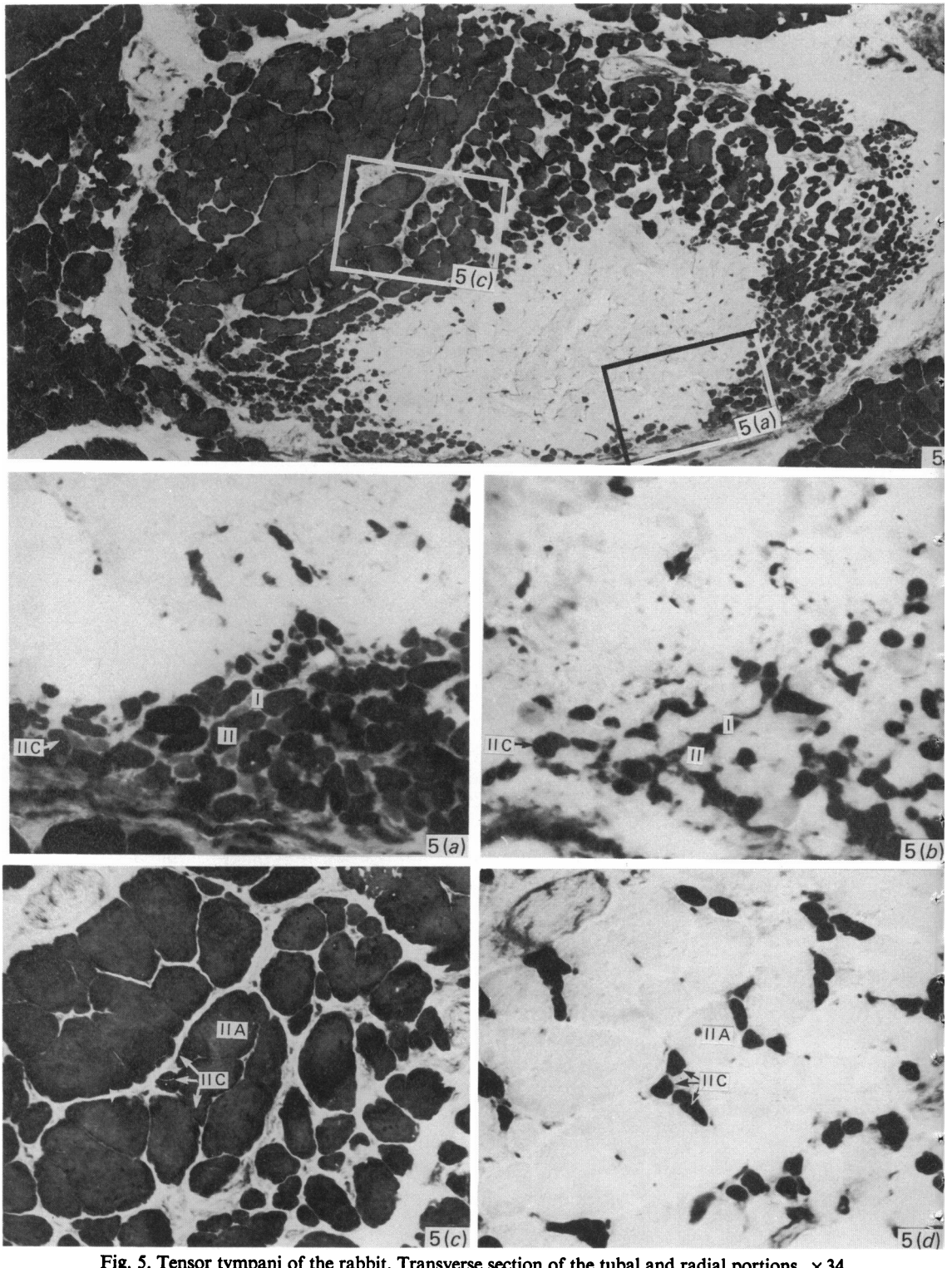


Fig. 5. Tensor tympani of the rabbit. Transverse section of the tubal and radial portions. × 34. (*a–b*) Detail of radial portion. (*c–d*) Detail of tubal portion. × 232. Myosin ATPase after pre-incubation at pH 10·2 (5, 5*a*, *c*) and 4·2 (5*b*, *d*).

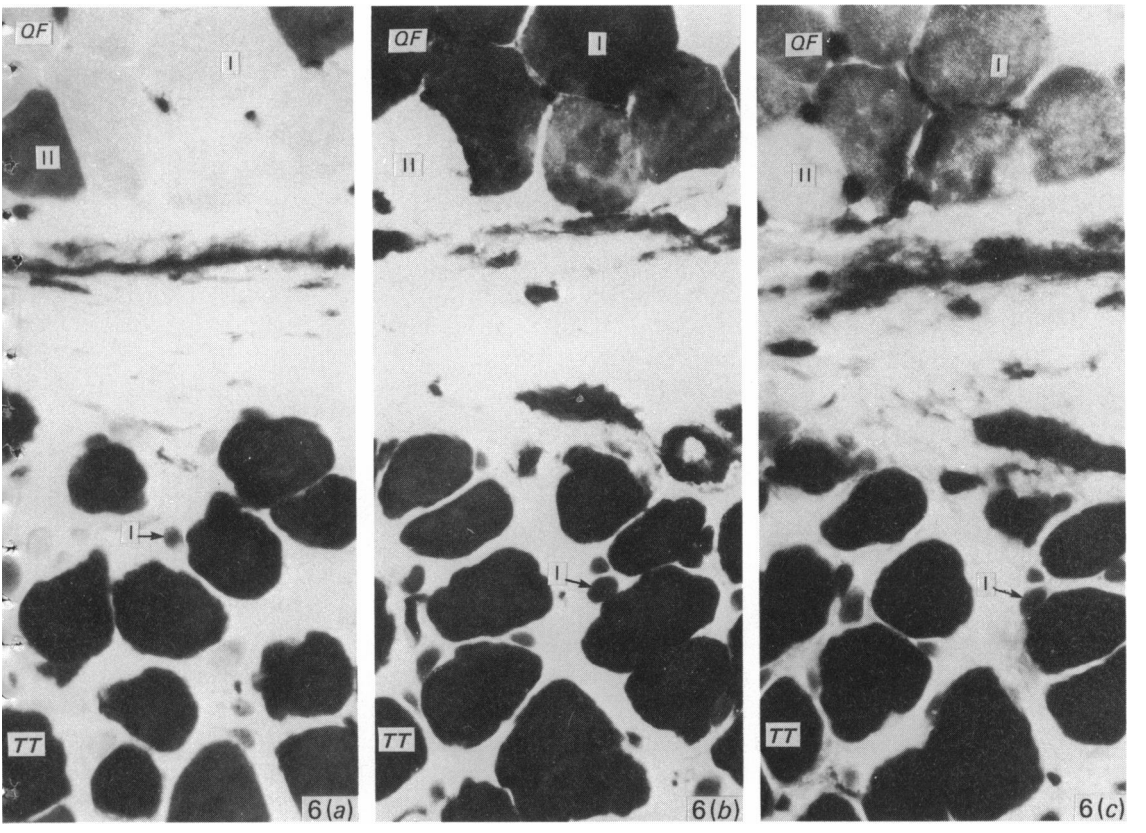


**Fig. 6. (*a–c*) Dog. This composite block shows the different behaviour of the myosin ATPase activity of tensor tympani (*TT*) fibres after both alkaline and acid pre-incubation, compared to quadriceps femoris muscle (*QF*). Myosin ATPase after pre-incubation at pH 10·2 (*a*), 4·2 (*b*) and 4·0 (*c*). × 380.**

intense, whereas the M α-GPDH reaction was almost negative. The second type (diameter slightly larger than 20 μm, 46%) showed an acid-labile, alkaline-resistant myosin ATPase reaction and were SDH and M α-GPDH-positive (Fig. 5*a*, *b*). The third had a myosin ATPase activity which was both acid and alkaline-stable.

In the tubal portion there were two types of fibres, which had, however, considerably different diameters (Fig. 5*c*, *d*). Whereas both types were positive to the myosin ATPase reaction preceded by alkaline pre-incubation, this reaction remained positive after acid pre-incubation for the fibres with smaller diameter (20 μm, 37%) only. The large diameter fibres (60 μm), which had weak SDH activity and strong M α-GPDH activity, could be classified as Type IIA or as a transition form between Types IIA and IIB. The smaller diameter fibres, which were also positive or intensely positive for SDH and negative for M α-GPDH, were probably IIC fibres.

*Dog and cat*

Here the tensor tympani had a tympanic portion only and was of a spheroidal shape (Fig. 10). The tubal portion was quite short and exclusively tendinous. The muscle component of the tympanic portion was well developed, and fatty tissue, which was abundant between the tendon bundles, was nearly absent between the

muscle bundles (Fig. 4*b*). In the cat, the connective tissue interposed between the muscle fascicles was more abundant than it was in the dog.

The fibres with the larger diameters (20–35 $\mu$m) showed an intense myosin ATPase activity which remained unchanged after both alkaline and acid pre-incubation (Figs. 6, 7*a–d*). The intensity of the reaction remained strong even at pH 4·0, at which point activity in the fast-twitch skeletal muscle fibres used as a control was almost absent (Figs. 6*c*, 7*d*). These fibres were SDH-positive and strongly positive to M $\alpha$-GPDH (Fig. 7*e*, *f*).

The small diameter fibres (10–20 $\mu$m) had myosin ATPase activity which was alkali-labile and acid-stable at pH 4·35 and 4·2, but which was much reduced or abolished at pH 4·0 (Fig. 7*d*). SDH reaction varied between strong and moderate, whereas the M $\alpha$-GPDH reaction was negative (Fig. 7*e*, *f*). The two types of fibres were numerically equal in the dog; in the cat those of larger diameter predominated slightly (58 %).

*Cow*

The tensor tympani consisted of a tympanic portion only and was of a spheroidal shape (Fig. 10). The connective tissue component clearly predominated over the muscle one and fat tissue was sparse (Fig. 4*c*). The muscle fibres were very small ($\Phi$ 5–15 $\mu$m), most having a diameter about 9 $\mu$m. Differentiation of the different fibre types with the myosin ATPase reaction after acid and alkaline pre-incubation was obtained by reducing the optimal pre-incubation times for the stapedius and the muscles of the limbs in the same species. With the usual pre-incubation times, activity was completely abolished in all fibres of the tensor tympani.

In general, the fibres of greater diameter showed an alkaline-labile, acid-stable myosin ATPase activity, were SDH-positive and weakly M $\alpha$-GPDH-positive, and could therefore be classified as slow-twitch, or Type I fibres (Fig. 8). The other fibres, of small or intermediate diameter, were SDH-positive and strongly M $\alpha$-GPDH-positive; some could be clearly classified as Type IIA in that they gave a positive myosin ATPase reaction after alkaline pre-incubation which was abolished after acid pre-incubation (Fig. 8).

It was difficult to classify a large number of smaller diameter fibres because of their wide range of staining intensities with the myosin ATPase reaction (Fig. 8, arrow). In many of the very small fibres a crescent-shape area was found that proved to be negative for all the histochemical reactions and which corresponded to the nuclear area.

*Pig*

Only the tympanic portion was present in the tensor tympani and the muscle was of spherical shape (Fig. 10). About four fifths was connective tissue and very abundant fat tissue. The muscle component was almost insignificant and was located only along the radial connective tissue septa (Fig. 4*d*). Not all the short muscle fibres made contact with the capsule and the conspicuous central tendon cone (Fig. 4*d*). They were, in fact, arranged step-wise to each other so that it was extremely difficult to identify the same fibre in the serial sections subjected to the different histochemical methods.

All the short, very thin fibres had diameters between 5–10 $\mu$m; only a few fibres reached diameters of about 12 $\mu$m. They gave a heterogeneous, generally intense response to the myosin ATPase reaction at pH 9·4 (Fig. 9*a*), independently of their

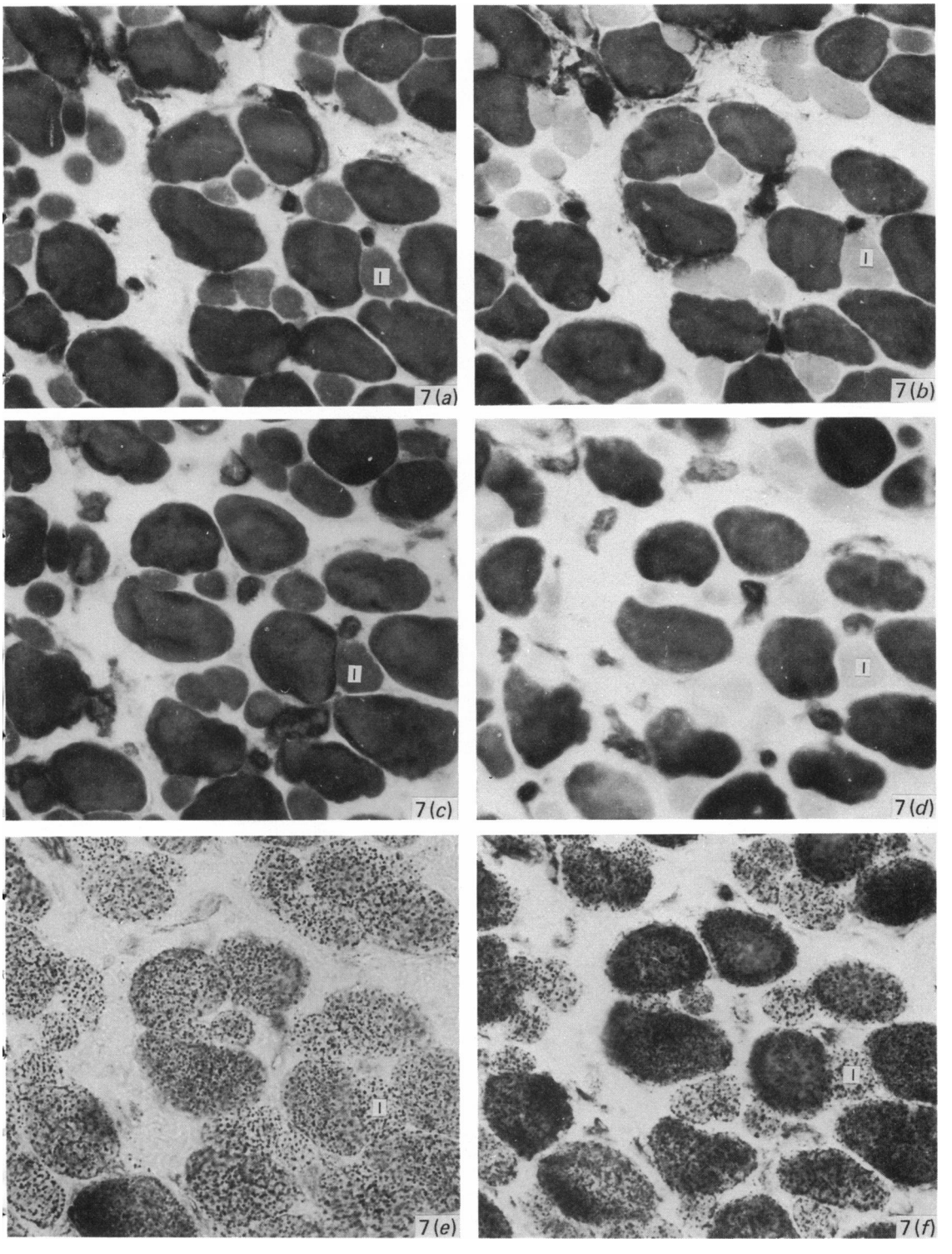


Fig. 7. (*a–f*) Tensor tympani muscle of the cat showing the fibre types. Myosin ATPase at pH 9·4 (*a*) and after pre-incubation at pH 10·2 (*b*), 4·2 (*c*), 4·0 (*d*); SDH (*e*); M α-GPDH (*f*). × 380.

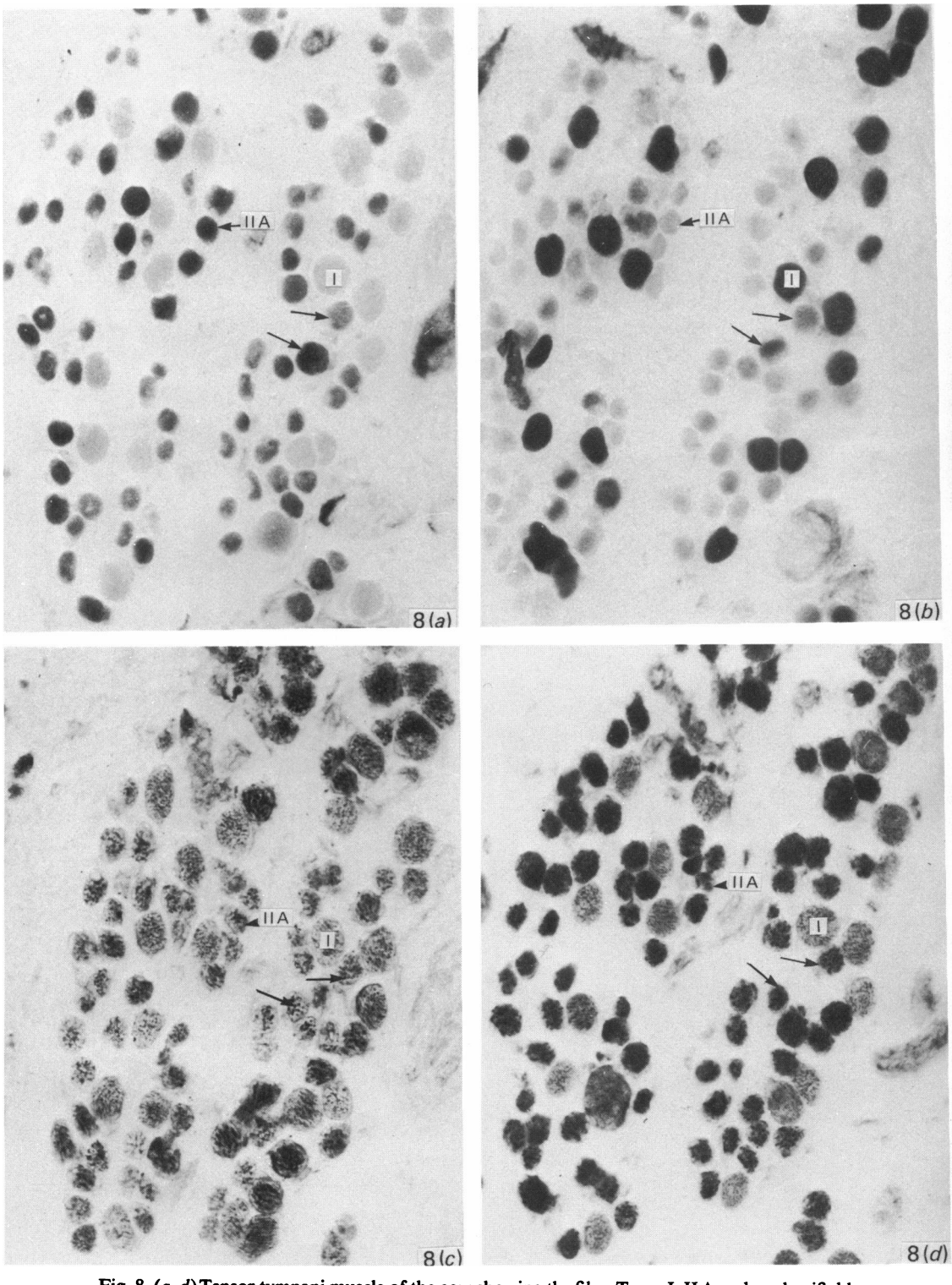


Fig. 8. (*a–d*) Tensor tympani muscle of the cow showing the fibre Types I, II A and unclassifiable type (arrows). Myosin ATPase after pre-incubation at pH 10·2 (*a*) and 4·2 (*b*); SDH (*c*); M α-GPDH (*d*). × 380.

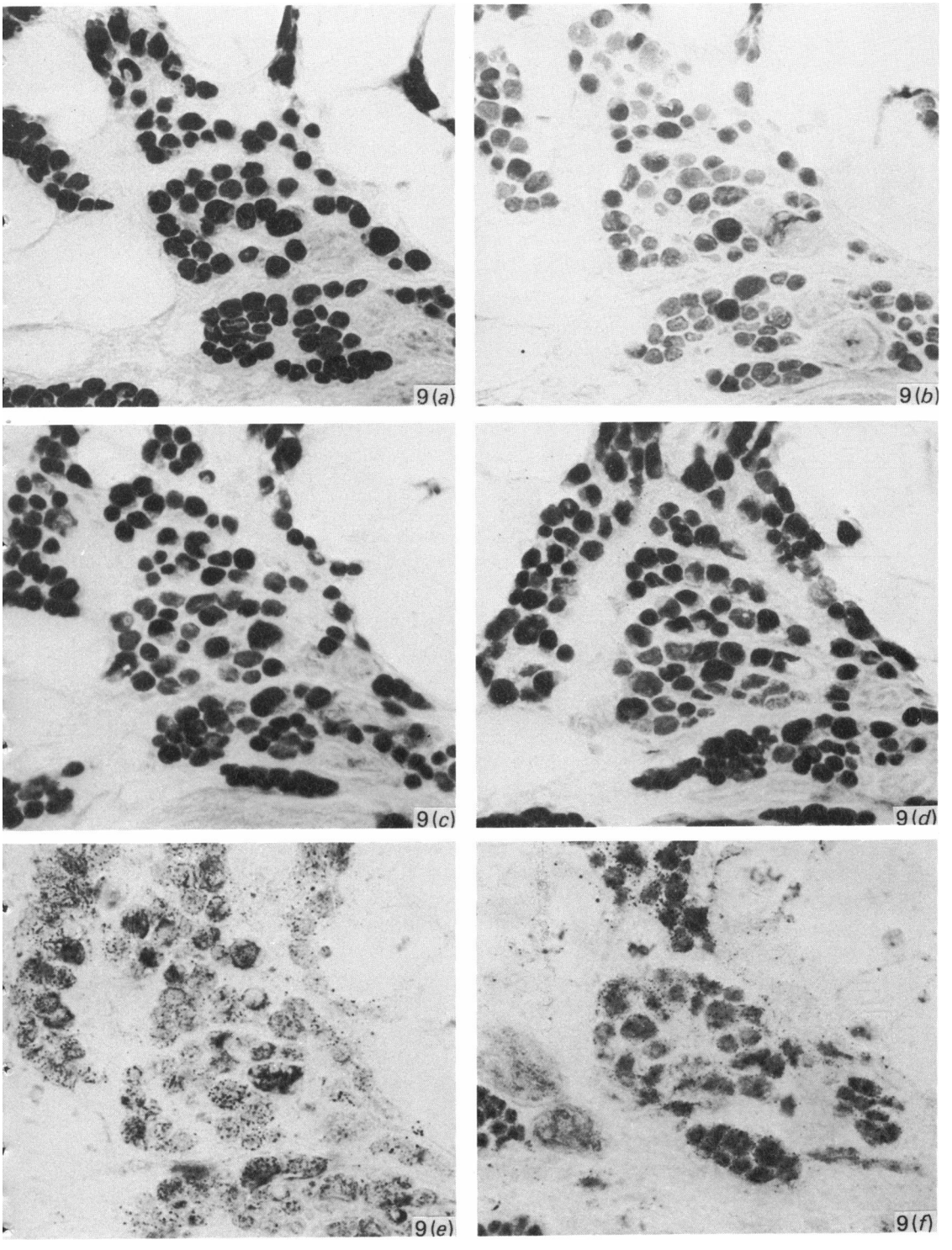


Fig. 9. (*a–f*) Tensor tympani muscle of the pig showing fibre types. Myosin ATPase at pH 9·4 (*a*) and after pre-incubation at pH 10·2 (*b*), 4·35 (*c*), 4·2 (*d*); SDH (*e*); M α-GPDH (*f*). × 380.

diameter. In all cases, longer and more alkaline pre-incubations tended to abolish the myosin ATPase reaction. A shorter incubation time in pH 10·2 buffer resulted in weak staining of some fibres, and after acid pre-incubation some fibres were still strongly positive, others slightly so, and others almost negative (Fig. 9*b–d*). Scarce, irregular precipitates were noted in all muscle fibres of the sections subjected to the SDH and M $\alpha$-GPDH (Fig. 9*e*, *f*). It was therefore not possible to classify these fibres into the three types on the basis of their diameters and histochemical reactions.

## DISCUSSION

The stapedius and tensor tympani are very small muscles and are made up of extremely thin fibres which can usually be well characterised histochemically. In all of the species examined, the stapedius showed quite similar morphological features and was made up of a discrete, well developed muscle component. The tensor tympani, on the other hand, showed marked differences, both in its shape and its muscle component, which in some species was negligible as compared to the connective tissue component. Although both muscles consisted of small or very small diameter fibres, in the same species it was always the stapedius that showed slightly larger diameters.

### *Stapedius muscle*

In all the species examined, the stapedius muscle consisted of slow-twitch fibres (Type I) and fast-twitch red fibres (Type IIA), which were positive to the SDH reaction to different degrees. SDH-negative, fast-twitch white fibres (Type IIB) were never found.

In the cat, dog, sheep, and, more rarely, pig it was possible to identify at least two subtypes of fast-twitch fibres on the basis of their different degree of resistance to pH 4·35 pre-incubation in the myosin ATPase reaction. These subtypes, which are SDH-positive, are probably subgroups of Type IIA, rather than Type IIA and IIB (Brooke & Kaiser, 1970). Indeed, some authors (Hennemann & Olson, 1965; Close, 1972; Luff & Atwood, 1972; Kugelberg, 1973) have been able to identify the presence of several types of fast motor units on the basis of different degrees of fatigue resistance.

With regard to the carnivores, a greater abundance of fast fibre subtypes has also been encountered in the intrinsic muscles of the larynx (Mascarello & Veggetti, 1979).

In the stapedius muscle, as in the intrinsic muscles of the larynx, especially in the carnivores, there is an abundance of capillaries, which is a measure of the predominantly oxidative type of metabolism typical of muscles formed of slow and fast fatigue resistant motor units (Hennemann & Olson, 1965; Andersen & Henriksson, 1977).

It is difficult to make a direct comparison of the present results with previously published data for other species because the methods and the criteria of classification are not the same. On the whole, the fibre type composition of stapedius of the guinea-pig, fruit bat, and rabbit seems to be quite similar to the present findings, but with the exception of the cotton rat, where Type I fibres are absent, and of the fruit bat where the classical white fibres are present (Anderson, 1975). Those results obtained in the guinea-pig could not be confirmed by Burgener & Mayr (1980) who

described the presence of 'myotube-like' fibres of the histochemical IIC type, and adult-like 'atypical' Type I fibres as elsewhere found in developing muscle.

In the horse, cow, and pig, the two types of fibres tended to be numerically equal, whereas, in the dog, cat, rabbit and sheep, the fast-twitch fibres clearly outnumbered the slow-twitch ones, suggesting that, in the latter group of species, stapedius is a faster muscle.

The quantitativa data calculated for the cat are in accord with those found by Teig & Dahl (1972) who, on the other hand, did not find subtypes among the fast-twitch fibres. The fast contraction time suggested by the histochemistry of stapedius in the cat and rabbit is confirmed by physiological studies that report contraction times of 21 ms and 22 ms, respectively (Teig, 1972*a*, *b*). In the cat, this contraction time is slightly greater than that for the extensor digitorum longus (19·5 ms), but less than the average contraction time of the flexor muscles of the leg. Teig also reported that the tetanic contraction of the stapedius, which has an average value of 13·9 g in the cat and 15·4 g in the rabbit, is much more critically dependent upon initial length of the muscle than is the case for other skeletal muscles.

The stapedius is, therefore, able to produce sufficient tension to reduce the cochlear amplification potential from 30 to 1·5 $\mu$V (Teig, 1972*a*) causing a reduction in sound transmission of about 26 dB in the cat.

Since the fibre type percentages in the dog and, even more so, in the sheep, are very similar to those in the cat, one can hypothesise that in these species the stapedius muscle has dynamic characteristics that are very much like those demonstrated in the cat. The physiological properties demonstrated in the cat cannot, however, be extended to the stapedius muscles of other species where there are almost as many slow fibres as fast ones.

### *Tensor tympani*

With regard to the tensor tympani, the different histochemical behaviours correspond to the fundamental morphological differences in the muscle fibres seen in different species. In the sheep, where the tubal and tympanic portions are equally well developed, and in the horse, where the tubal portion predominates, it is possible to distinguish the slow-twitch fibres of Type I and the fast-twitch, red fibres of Type II. In the sheep, in both muscle portions, the fast-twitch fibres predominate; in the tubal portion of the horse, the two fibre types also tend to be numerically equal, whereas, in the tympanic portion, the slow-twitch fibres predominate. Moreover, in the last mentioned species, the composition of the tubal portion is without doubt that which determines the functional characteristics of the muscle, for it is the more developed part of the muscle with large diameter fibres.

The tympanic bundle of the rabbit, formed of fibres of a smaller diameter than those of the tubal portion, has fast-twitch red fibres and slow-twitch fibres. The tubal bundle shows, in addition to the larger diameter fast-twitch fibres, others with a considerably smaller diameter and which are probably IIC, since they show a myosin ATPase activity which is alkaline-stable like that of fast-twitch fibres, but also acid-resistant. Asmussen & Wohlrab (1971) found that in the tubal portion all the fibres showing positive myosin ATPase activity could be subdivided into three types on the basis of their diameters and their different intensities with SDH reaction. They described large diameter fibres with little activity, small diameter fibres with an intense reaction, and medium diameter fibres with a medium-to-strong activity. The last two types correspond to those which were found to be positive to the myosin

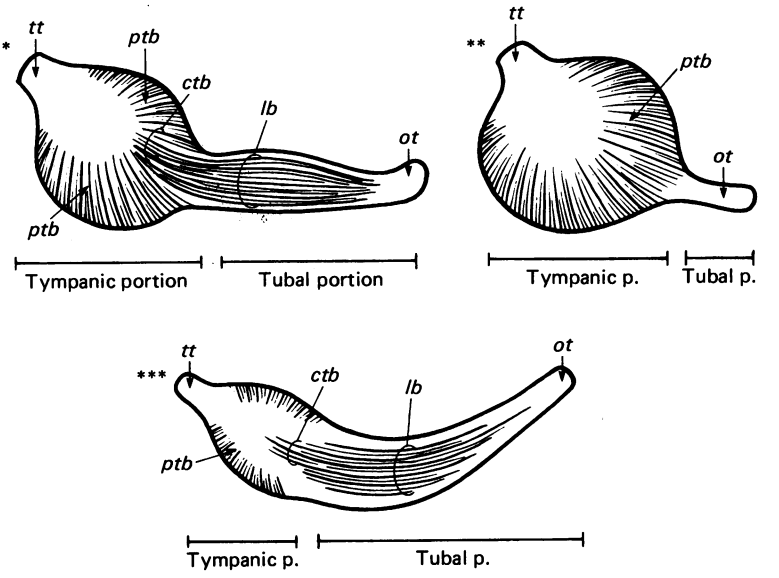


Fig. 10. Diagrammatic representation of longitudinal sections of the tensor tympani muscles showing the different development of the tympanic and tubal portions and the particular arrangement of the fibres and the fibre bundles. Muscle club-shaped (*) (sheep); muscle spheroidal-shaped (**) (dog, cat, cow, pig); muscle spindle-shaped (***) (horse, rabbit). Tensor tendon (*tt*); origin tendon (*ot*); peripheral tympanic bundle (radial fibres) (*ptb*); central tubal bundle (parallel fibres) of the tympanic portion (*ctb*); longitudinal bundle of the tubal portion (*lb*).

ATPase reaction after acid pre-incubation. Such myosin ATPase activity has also been described in IIC fibres which, because of the coexistence of fast and slow myosin (Gauthier, Lowey & Hobbs, 1978; Gauthier & Lowey, 1979; Billeter *et al.* 1980), and because of the high glycolytic and oxidative activity (Jansson, 1975), are today interpreted as transitional fibres between the slow-twitch and fast-twitch red types (Jansson *et al.* 1978).

In the dog and cat, the two fibre types of the tympanic portion can be classified as slow-twitch, or Type I, fibres and another particular type of fibre, IIM, which is easily characterised histochemically, but cannot be classified with any of the fibre types of hind limb muscles of the same species. The larger diameter fibres, similar in size to other skeletal muscles fibres and also described by Teig & Dahl (1972) in the tensor tympani of the cat, show a myosin ATPase reaction which is identical to that of a fibre type found in the masticatory muscles of the same species (Mascarello *et al.* 1979; Bosley & Rowlerson, 1980) and the monkey (Maxwell, Carlson, McNamara & Faulkner, 1979; Maxwell, Carlson & Brangwyn, 1980). Teig & Dahl (1972) hold that, because of the alkaline-stable myosin ATPase activity, these fibres correspond to the rapid motor unit of this muscle, which has a contraction time of 29·2 ms. Since they found that the fast motor units of the tensor tympani have contraction times between 23 and 40 ms, which is close to the 16–30 ms for the fast motor units of the cat gastrocnemius (Burke, 1967), Teig & Dahl (1972) assert that the particular myosin ATPase activity seen in the tensor tympani fibres does not reflect important physiological differences. If, instead, these fibres are classified with the characteristic ones of the masseter and temporalis muscles of the cat, the interpretation is different. In fact, Taylor, Cody & Bosley (1973) have found that these muscles have clearly

shorter contraction times (11 ms) than those found for the gastrocnemius (25 ms). It has been suggested that these fibres, called 'super-fast', could be characterised by a particular type of myosin responsible for the myosin ATPase response (Bosley & Rowlerson, 1980). The existence of this isomyosin has been confirmed by both biochemical and immunohistochemical studies (Rowlerson *et al.* 1981). Only through analogous research will it be possible to determine if these tensor tympani fibres also possess this characteristic myosin.

The slow component (42 %) of the cat tensor tympani, which is greater than that of the stapedius causes a longer contraction time and a greater tetanic tension (about 54·3 g), which is sufficient to reduce the sound intensity by 20 dB. This could be valid for the dog also, which similarly has a slow component in the tensor tympani which is greater than that in the stapedius.

From the histochemical point of view, as Teig & Dahl (1972) and Burgener & Mayr (1980) also observed, it is not possible in the present material to distinguish a type of fibre classifiable with the slow-tonic fibres characterised by multiple innervation, found by some authors in the middle ear muscles of the cat (Erulkar *et al.* 1964; Fernand & Hess, 1969), of the guinea-pig (Seiden, 1971), of the rabbit (Hirayama & Daly, 1973; Hirayama *et al.* 1974). On the other hand, some authors have failed to demonstrate multiply innervated slow-tonic fibres (Anderson, 1975; Burgener & Mayr, 1980). Because the fibres of the slow-tonic type can be clearly distinguished from the slow-twitch type by immunohistochemical methods, as demonstrated by Pierobon Bormioli *et al.* (1979), an immunohistochemical study has been undertaken to resolve this matter.

In the cow, the tensor tympani is notable for its considerably reduced muscle component, formed, in the main, by fibres with an extremely small diameter. In spite of this fact, many fibres can clearly be classified as Type I, or the slow-twitch type, and the larger diameter fibres as Type IIA, or fast-twitch, red, fibres. It is not possible to classify by the myosin ATPase reaction fibres that make up almost all of the even more sparse muscle component of the pig tensor tympani, and thus, similarly, it is not possible to interpret the significance of the myosin ATPase reaction of these fibres. However, because of the considerable reduction of the muscle component, the extreme thinness of the fibres, and the poorly characterised histochemical picture, it can be concluded that this muscle would have poor dynamic characteristics in the cow and even more so in the pig. This fact confirms the hypothesis already put forward that, in these animals, the tensor tympani serves more as a ligament than a muscle. Since this sparse muscle component undergoes precocious degeneration and since the same histochemical characteristics have been found in the full term cow fetus (work in progress), it is believed that this is a muscle which undergoes regression even before reaching the stage of histochemical differentiation found in other species.

In conclusion, in the sound-transmission mechanism, the function of protecting the endolabyrinth from high pressures is taken by the stapedius muscle, which, in all the species examined, is well differentiated both morphologically and histochemically, although it performs this function with different dynamic properties in the horse, cow, and pig on the one hand and the dog, cat, rabbit and sheep on the other.

The different histochemical properties in the tensor tympani could point to a different degree of fidelity, rather than a different mechanism, of sound transmission, especially for the louder sounds and higher frequencies.

SUMMARY

The stapedius and tensor tympani muscles of young horse, sheep, cow, pig, dog, cat, and rabbit were examined to compare the histochemical and morphological differences, which are marked in the tensor tympani muscle.

The stapedius muscle, though small in size, has a well developed muscle component; by staining for myosin ATPase, following alkali and acid pre-incubation, and SDH and M $\alpha$-GPDH activities, Type I and IIA fibres, presumably belonging to slow and fast contracting fatigue-resistant motor units (S and FR types), were both present in different proportions in all species.

The tensor tympani muscle presented a different histochemical profile. In the sheep, where both tubal and tympanic portions were present, and in the horse, where the tubal portion was predominant, the muscle tissue component was well developed and formed by Type I and IIA fibres. In the rabbit, Type IIC fibres were also present. In the dog and cat, where only the tympanic portion was present, this muscle was well developed and two fibre types were present, Type I, and a type with an atypical myosin ATPase (IIM) which had a higher activity than the smaller ones (Type I) after both alkali and acid pre-incubation. In the cow and pig, the muscle component was only in the tympanic portion, was small compared to the connective tissue present and consisted of a small number of very small fibres. These fibres showed such a different and varied histochemical profile that it was very difficult to identify the different fibre types. This fact confirms the opinion that the tensor tympani of the cow and pig performs more the function of a ligament than that of a muscle.

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