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EFFECTS OF AGE ON CONTRACTILE AND ENZYME-HISTOCHEMICAL PROPERTIES OF FAST- AND SLOW-TWITCH SINGLE MOTOR UNITS IN THE RAT

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SUMMARY

1. Contractile, enzyme-histochemical and morphometrical properties of muscle fibres were studied in single motor units of tibialis anterior (t.a.) and soleus muscles in young (3–6 months) and old (20–24 months) male albino rats. The technique of measuring glycogen depletion as a marker of previous muscle contraction was used for direct correlation of enzyme-histochemical and contractile parameters within single motor units of the fast- and slow-twitch type.

2. In t.a., the fast-twitch motor units covered 18 ± 9 and 22 ± 16 % (P = not significant, n.s.) of t.a. cross-sections, included 148 ± 65 and 162 ± 63 muscle fibres per unit (P = n.s.) and had a cross-sectional area of $0{\cdot}50 \pm 0{\cdot}32$ and $0{\cdot}44 \pm 0{\cdot}22$ mm^2 (P = n.s.) in the young and old animals, respectively (means ± S.D.).

3. In soleus, the slow-twitch motor units covered 53 ± 11 and 71 ± 12 % (P = n.s.), included 55 ± 10 and 83 ± 13 muscle fibres per unit ($P < 0{\cdot}01$) and had a total cross-sectional area of $0{\cdot}14 \pm 0{\cdot}02$ and $0{\cdot}22 \pm 0{\cdot}06$ mm^2 ($P < 0{\cdot}01$) in the young and the old animals, respectively. The calculated number of motor units in soleus accordingly decreased ($P < 0{\cdot}01$) from 49 ± 10 in the young to 29 ± 10 in the old animals resulting in a loss of muscle fibres and an increased innervation ratio in old age (mean ± S.D.).

4. Clusters of more than three muscle fibres were rarely seen in any of the glycogen-depleted motor units in either the young or the old animals. However, in the slow-twitch motor units of old animals the muscle fibres were less randomly distributed within the motor unit territory ($P < 0{\cdot}05$), indicating a denervation–reinnervation process.

5. The contraction and half-relaxation times of the isometric twitch were significantly prolonged in old age. In 274 randomly isolated single motor units of t.a. the contraction time increased from 13 ± 1 in young animals to 17 ± 3 ms in old ones and the half-relaxation time from 12 ± 2 to 16 ± 5 ms ($P < 0{\cdot}01$ in both cases). In 236 randomly isolated soleus single motor units, the contraction and half-relaxation times increased ($P < 0{\cdot}001$) from 24 ± 5 to 31 ± 7 ms and from 26 ± 8 to 35 ± 9 ms, respectively (means ± S.D.).

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6. The results show that the age-related decrease in the number of muscle fibres is due to a loss of whole motor units and that in old age reinnervation of previously denervated muscle fibres is incomplete.

7. It is concluded that the reduced speed of isometric contraction during ageing is primarily related to changes in the contractile properties of both fast- and slow-twitch motor units, and that the age-related decrease in the number of type II muscle fibres observed in soleus is of less importance in this respect.

INTRODUCTION

During ageing, profound impairment of different neuromuscular functions takes place and senile muscle atrophy is by far the most commonly encountered type of muscle atrophy in man. In spite of this, the effects of ageing on striated muscles, especially at the single motor unit level, have received little attention and many of the results of those studies published have been divergent and conflicting (for references see Larsson, 1982). However, there has been agreement that the amount of contractile material and the speed of contraction decrease in the process of ageing in both fast- and slow-twitch muscles in many mammals, including man (Gutmann, Hanzlikova & Vyskocil, 1971; Campbell, McComas & Petito, 1973; Caccia, Harris & Johnson, 1979; Belanger, McComas & Elder, 1983; Davies, White & Young, 1983; Larsson & Edström, 1986*a*). In histopathological studies of striated muscle in old age, alterations indicating a process of deterioration of both myogenic and neurogenic origin have been observed (e.g. Serratrice, Roux & Aquaron, 1968; Tomlinson, Walton & Rebeiz, 1969; Tomonaga, 1977). The mechanism underlying the changes in morphological and contractile properties of ageing skeletal muscle remains obscure, however, partly on account of the paucity of correlative morphological and physiological studies of ageing skeletal muscle. In a previous series of studies in humans we attempted to shed some light on the factors underlying the impaired muscle function in old age and the senile muscle atrophy (for references see Larsson, 1978, 1982). Observations were made of a number of significant age-related trends in enzyme-histochemical, biochemical and ultrastructural properties of skeletal muscles that co-varied with alterations in maximum voluntary strength and speed of movement. It cannot be determined, however, whether the findings in these *in vivo* studies represent causal relationships or not.

In a recent study the effects of ageing on fast- and slow-twitch muscles of young adult and old rats were investigated (Larsson & Edström, 1986*a*). In the present study those experiments have been extended to include the final functional unit in the motor system, i.e. the motor unit. The technique of measuring glycogen depletion as a marker of previous muscle contraction was used in order to permit direct evaluation of the correlations between enzyme-histochemical and physiological factors within single motor units of different types (Edström & Kugelberg, 1968; Kugelberg & Edström, 1968; Kugelberg & Lindegren, 1979). A preliminary account of this work has been presented elsewhere (Larsson & Edström, 1986*b*, *c*).

METHODS

The study was carried out on male albino rats of the same strain (Wistar), fed *ad libitum* with standard laboratory food and tap water. Animals that were sick or moribund or which displayed gross pathological organ changes were excluded from the study. The animals were anaesthetized with pentobarbitone (30 mg/kg) administered intraperitoneally. Rats of the Wistar strain have a mean life duration of 24 months and may live for approximately 36 months (ILAR, 1981). In order to avoid unpredictable effects on skeletal muscle in very old age, such as those of extreme obesity, disease and disuse, we chose to study old rats that had not yet reached an advanced age. The rats were divided into a young (3–6 months) and an old (20–24 months) group.

Physiological technique. With the animal under pentobarbitone anaesthesia, the ventral roots, L4 to the fast-twitch tibialis anterior (t.a.) or L5 to the slow-twitch soleus muscle, were exposed by laminectomy, transected proximally and maintained in a mineral oil pool formed by the skin around the incision over the vertebral column. The skin over the lower part of the left hindlimb was removed. When t.a. was to be studied, the fascia overlying the muscle was removed, the distal part of the muscle was freed and the plantar flexor muscles were denervated by transecting the motor nerves (Edström & Kugelberg, 1968; Kugelberg & Lindegren, 1979). The innervation of t.a. and the other plantar extensors was kept intact, since denervation of these muscles is apt to impair the blood supply to t.a. (Edström & Kugelberg, 1968). For studies of soleus, the distal half of this muscle was exposed, the plantar extensors were denervated and the tendons of the gastrocnemius and plantaris muscles were cut (Kugelberg, 1973). The left popliteal artery was exposed and a fine thread was placed loosely around it to make it more easily accessible.

The animal was placed in the prone position on a steel plate which was heated to maintain the body temperature. The dissected limb was put into a bath through a hole in the wall of the bath chamber. The skin of the thigh was stretched over a flange around the hole to make the bath leak-proof. The limb was fixed rigidly in the bath by means of a steel rod drilled through the tibia close to the knee joint, and a clamp on the foot. Mineral oil was circulated through the bath. The bath temperature was thermostatically controlled at 36 °C (Kugelberg & Lindegren, 1979).

Single motor units in t.a. or soleus were functionally isolated by microdissection of the L4 or L5 ventral roots, respectively. The criterion was an all-or-none twitch response to finely graded current pulses 0·2 ms in duration. Supramaximal stimulation was used in all experiments.

The tendon of the t.a. or soleus muscle was oriented along the natural pull of the muscle and attached to a strain gauge (UC 2, Statham Instr., Inc., Oxnard, CA, U.S.A.). A load cell accessory (UL 4–10, Statham Instr.) was attached to the strain gauge when the muscle twitch response was recorded during whole ventral root stimulation in order to increase the load range of the strain gauge. The strain gauge was calibrated after each experiment. The mechanical responses were amplified (AD 6, Medelec Ltd, Old Woking, Surrey, U.K.), displayed on an oscilloscope (M-scope, Medelec, or a dual-beam storage oscilloscope, Tektronix 5113) and recorded on Kodak Linagraph direct print paper (Eastman Kodak, Rochester, NY, U.S.A.). Contractions were recorded with the muscle set at an optimum length as determined from the maximum isometric twitch force. The isometric twitch contraction time was measured from the beginning of contraction to the peak force, and the half-relaxation time from the peak force to the time at which the force had decreased by 50%. Mechanical properties of single motor units were recorded during repetitive stimulation. Trains of 4 s duration and with stimulation frequencies of 10, 46 and 196 Hz were used. The motor unit was allowed to recover for 3 min between each train in order to avoid potentiation and fatigue effects.

The muscle fibres of the unit were depleted of glycogen by stimulation with trains of twenty impulses with a frequency of 100 Hz repeated once a second until the tetanic force had decreased to near zero. The stimulation was then terminated and the unit was stimulated with one impulse train every 10 s until the force had almost recovered. This sequence was repeated 3–5 times. Fatigue-resistant units in the t.a. muscle and all units in the soleus muscle were stimulated during ischaemia, which was produced by clamping of the popliteal artery with a small clip. The clip was removed during the recovery periods (Kugelberg, 1973; Kugelberg & Lindegren, 1979).

Histological technique. After each experiment the muscle was gently dissected free from surrounding tissue and clamped at approximately the *in situ* length. The muscle was then weighed, frozen in Freon chilled with liquid nitrogen and stored at −80 °C until processed further. The muscle was then cut perpendicular to its longitudinal axis into serial 10 μm thick cross-sections

with a cryotome (−20 °C) at the motor point (soleus) or at the greatest girth (t.a.). For comparison, another fast-twitch muscle, the extensor digitorum longus (e.d.l.), was also analysed histochemically, since it has an identical enzyme-histochemical fibre composition to that of t.a. but has a less complicated fibre arrangement than t.a. and is similar in size to soleus. Unfortunately, this muscle

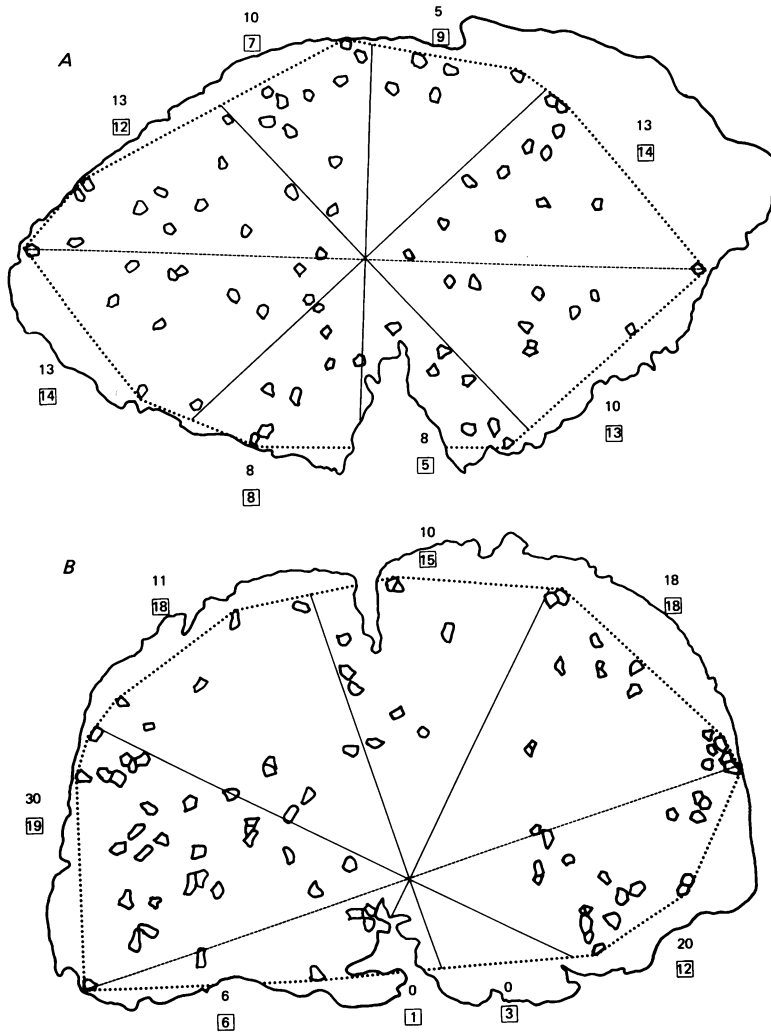

Fig. 1. Glycogen-depleted fibres in two slow-twitch motor units in the soleus muscle from a young (*A*) and an old (*B*) animal. The randomness of the muscle fibre arrangement was assessed as the mean difference between the measured and predicted (within squares) numbers of muscle fibres in eight sectors of the motor unit territory. The differences were corrected for inter-individual variations in the number of depleted fibres. The average of the corrected differences was 2·4 in the young (*A*) and 4·8 in the old (*B*) animal.

has not been found suitable for studies of single motor units with the present experimental set-up. This is probably associated with the low innervation ratio in this muscle, which makes it unfeasible to determine whether the microdissected, stimulated nerve root filaments contain single or multiple motoneurones to e.d.l. (Larsson & Edström, 1986*a*).

The muscle fibres in the motor units were mapped as unstained fibres in PAS (periodic acid–Schiff reagent) -stained sections and identified in the subsequent sections stained for myofibrillar ATPase.

Cross-sectional areas of the individual glycogen-depleted muscle fibres were measured semiautomatically from magnified black and white photographic prints of PAS-stained muscle cross-sections with the aid of a digitizer (Apple Graphic tablet) connected to an Apple II microcomputer (Apple Computer Inc., CA 95014, U.S.A.). The t.a. and e.d.l. cross-sections were stained for myofibrillar ATPase (Padykula & Herman, 1955) after alkaline and acid pre-incubations (Brooke & Kaiser, 1969, 1970; Dubowitz & Brooke, 1973). All fibres in the cross-sections were classified according to the pH sensitivity of the myofibrillar ATPase, type I fibres being those with acid-stable ATPase (after pre-incubation at pH 4·35) and alkali-labile ATPase (after pre-incubation at pH 9·4), and type II showing the reverse pH sensitivity. Type II fibres were further subdivided into types II A and II B, the former of which were inhibited at pH 4·5. The soleus cross-sections were stained for myofibrillar ATPase (1) at pH 9·4 (Brooke & Kaiser, 1969), (2) after 55 min of formaldehyde fixation at 4 °C (Hayashi & Freiman, 1966) and (3) after acid pre-incubation at pH 4·35 (Brooke & Kaiser, 1969). The fibres were classified into types I and II according to the pH sensitivity of the myofibrillar ATPase in the same way as for the fast-twitch muscles. The modification devised by Hayashi & Freiman (1966) involves fixation in methanol-free formaldehyde before the routine enzyme-histochemical procedure. This causes almost complete inhibition of type I fibres and the type II fibres are presented in many grades of activity from intermediate to strong (i.e. from light grey to black). Type II fibres were then subdivided into types II A and II C, the former being completely inhibited at pH 4·35 (for detailed presentation of the fibre classification, see Kugelberg, 1975, 1976; Kugelberg & Lindegren, 1979). The total number of fibres of each type was counted on magnified photomicrographs of whole e.d.l. and soleus muscle cross-sections and the relative number of each type was calculated.

A model was designed to assess the randomness of the muscle fibre arrangement in the motor unit. The difference between the predicted and measured numbers of glycogen-depleted fibres in PAS-stained sections in eight different sectors of the motor unit territory was calculated. The angle between the two radii of the sectors was 45 deg, the centre of the sectors was at the mid-point of the axis between the two most distant glycogen-depleted fibres in the motor unit, and the motor unit territory border was the base of each of the eight different sectors (Fig. 1). Assuming that the muscle fibres were randomly arranged within the motor unit territory, the number of muscle fibres in each sector could be predicted on the basis of the size of the sector in relation to the size of the unit territory and the 'total' number of muscle fibres in the unit. The 'total' number of muscle fibres was larger than the innervation ratio, since the glycogen-depleted fibres that were divided by a sector radius were counted in both the adjacent sectors. The difference between the measured and predicted number of muscle fibres was calculated in the different sectors. In order to avoid a systematic overestimation of these differences in motor units including a large number of muscle fibres, the differences were corrected for variations in 'total' number of fibres, i.e. each difference was multiplied by the factor 100/'total' number of fibres. The average of the corrected differences was used as an index of the randomness of the fibre arrangement in the motor unit.

Statistics. All the values reported in the following are means and standard deviations (S.D.), which were calculated from individual values by standard procedures. The two-tailed independent t test was used for inter-group comparisons. Differences were considered significant at $P < 0{\cdot}05$.

RESULTS

Animal and muscle weights

In conformity with results of studies of this and other albino rat strains (e.g. ILAR, 1981; Larsson & Edström, 1986*a*) the body weight increased from 452 ± 72 g in young animals (3–6 months, $n = 60$) to 586 ± 82 g in old ones (20–24 months, $n = 41$). The weights of the fast-twitch t.a. (932 ± 147 *vs.* 976 ± 252 mg) and e.d.l. muscles (243 ± 59 *vs.* 248 ± 103 mg) and the weight of the slow-twitch soleus muscle (241 ± 56 *vs.* 247 ± 90 mg) did not differ significantly between young and old rats.

TABLE 1. Comparison of the numbers and proportions of different types and subtypes of fibres in the extensor digitorum longus (e.d.l.) and soleus muscles of young and old animals. Values are expressed as means ± S.D. and ranges are given within parentheses

Muscle	Age (months)	n	Type I		Type IIA		Type IIB		Type IIC		Total number of fibres
			n	%	n	%	n	%	n	%	
e.d.l.	3–6	40	120 ± 46 (23–201)	3 ± 1 (1–5)	719 ± 252 (393–1917)	19 ± 5 (11–37)	2838 ± 442 (1910–3689)	77 ± 5 (59–85)	—	—	3687 ± 573 (2500–5197)
e.d.l.	20–24	13	114 ± 61 (26–267)	3 ± 2* (1–7)	682 ± 183 (431–1057)	17 ± 4* (11–23)	3246 ± 522 (1980–4024)	80 ± 4* (74–86)	—	—	4042 ± 579 (2503–4850)
Soleus	3–6	32	2527 ± 208 (1974–2755)	91 ± 5 (81–100)	205 ± 140 (4–456)	7 ± 5 (0–17)	—	—	51 ± 49 (0–170)	2 ± 2 (0–6)	2752 ± 243 (2213–3204)
Soleus	20–24	27	2409 ± 303* (1796–2852)	99 ± 3‡ (87–100)	9 ± 18‡ (0–71)	0 ± 1‡ (0–3)	—	—	20 ± 47† (1–236)	1 ± 2* (0–10)	2450 ± 297‡ (1804–2798)

* No statistically significant difference. † $P < 0.05$. ‡ $P < 0.001$.

TABLE 2. Comparison of territory, number of muscle fibres (innervation ratio) and average cross-sectional areas of muscle fibres in single motor units in young and old animals. One unit is studied in each animal and the fast- and slow-twitch units are from t.a. and soleus, respectively. Motor unit number is not calculated in t.a. (see Results). Values are expressed as individual values and means ± S.D.

			Young (3–6 months)				Old (20–24 months)			
Motor unit	Animal number	Fibre type	Territory (%)	Innervation ratio	Average fibre area (μm^2)	No. of motor units	Territory (%)	Innervation ratio	Average fibre area (μm^2)	No. of motor units
Fast-twitch	1	IIA	3	45	1300	—	14	75	3300	—
	2	IIB	16	126	2420	—	11	134	6220	—
	3	IIB	17	118	3320	—	12	104	4810	—
	4	IIB	18	186	2510	—	17	169	2270	—
	5	IIB	27	223	4580	—	17	190	1680	—
	6	IIB	28	192	4150	—	30	197	1960	—
	7	IIB	—	—	—	—	55	263	2810	—
			18 ± 9	148 ± 65	3050 ± 1210		22 ± 16*	162 ± 63*	3290 ± 1660	
Slow-twitch	1	I	41	57	1910	46	49	87	3570	29
	2	I	47	65	2130	41	67	63	2140	44
	3	I	47	58	2570	51	74	96	2150	17
	4	I	52	49	3640	50	76	91	2820	30
	5	I	65	39	3110	66	78	71	3340	37
	6	I	68	63	2500	39	81	90	2300	20
			53 ± 11	55 ± 10	2640 ± 640	49 ± 10	71 ± 12*	83 ± 13‡	2720 ± 630*	29 ± 10

* No statistically significant difference. † $P < 0.05$. ‡ $P < 0.01$.

Enzyme-histochemical fibre spectra of fast- and slow-twitch muscles

The relative numbers of muscle fibre types and subtypes were identical in the young and old fast-twitch e.d.l. muscles (Table 1). In this study, detailed enzyme-histochemical measurements were only performed in the e.d.l., because of the large size of the t.a. and the identical proportions of fibre types in t.a. and e.d.l. in both young and old rats (Larsson & Edström, 1986*a*). Total fibre counts from single cross-sections of the e.d.l. and t.a. have to be considered with reservation, since all muscle fibres do not pass through the mid-portion or the greatest girth of the muscle (for references see Larsson & Edström, 1986*a*).

In the slow-twitch soleus muscle, all muscle fibres pass through the mid-portion (motor point) of the muscle and accurate total fibre counts can accordingly be made from single cross-sections of this muscle (for references see Larsson & Edström, 1986*a*). The total number of muscle fibres was 11% lower ($P < 0{\cdot}001$) in the old rats than in the young ones. The decrease in the number of fibres was mainly referable to fibres of type II, in particular type II A, while no significant difference was found in the number of type I fibres. The proportions of different types of fibres accordingly changed in the old animals. Thus, the proportions of fibres of types I and II A were higher and lower ($P < 0{\cdot}001$), respectively, in the old than in the young animals, while there was no significant difference in the percentage number of type II C fibres (Table 1). The absolute and relative numbers of fibres of type II A were small, or such fibres were absent in many old animals, whereas type II A fibres were never lacking in the young animals (Table 1). Similar results were recently obtained in young adult (6 months) and old (20–24 months) male albino rats (Larsson & Edström, 1986*a*).

Motor unit territory, innervation ratio and motor unit cross-sectional area

In the fast-twitch motor units in t.a., the average motor unit territory, innervation ratio and motor unit cross-sectional area did not differ between young and old animals. The fast-twitch units covered 18 and 22% of t.a. cross-sections, included 148 and 162 muscle fibres per unit and had a total cross-sectional muscle fibre area of 0·50 and 0·44 mm^2 in the young and old animals, respectively (Table 2). These values conform with the motor unit territory (17%) and innervation ratio (132) found by Edström & Kugelberg (1968) in fast-twitch units in the t.a. of young animals. The fast-twitch motor units containing type II A fibres covered a smaller proportion of the muscle, were seen in the deep part of the muscle and had muscle fibres of small or intermediate size irrespective of the animal's age. The motor units containing type II B fibres covered on average 21 and 27% of the cross-sections in the young and old animals, respectively, were seen in the superficial part of the muscle and contained muscle fibres of large size (Plate 1).

In the slow-twitch motor units in soleus, the average motor unit territory did not differ significantly between young and old animals; it ranged from 41 to 68% (53 ± 11) in the young animals and from 49 to 81% (71 ± 12%) in the old ones (Table 2). The innervation ratio, on the other hand, was significantly higher ($P < 0{\cdot}01$) in the old animals, the number of muscle fibres per motor unit ranging from 39 to 65 (55 ± 10) in the young and from 63 to 96 (83 ± 13) in the old animals (Table 2). The cross-sectional area of the slow-twitch motor units was increased

($P < 0{\cdot}01$) by 37% in the old animals compared with that in young ones ($0{\cdot}22 \pm 0{\cdot}06$ and $0{\cdot}14 \pm 0{\cdot}02$ mm^2, respectively), since there was no significant change in the area of type I muscle fibres (Plate 2).

The motor unit territory and innervation ratio of the slow-twitch units of young animals in this study conform with those originally reported by Kugelberg (1973). In a later study, Kugelberg (1976) found a higher innervation ratio in slow-twitch units of young rats, i.e. 83 ± 16 (range 47–120), which is identical to that in the old animals in this study. The reason for the discrepancy in innervation ratio between the slow-twitch units in the soleus of young animals is not fully clear. As discussed by Kugelberg (1973, 1976), the innervation ratio may be underestimated as a result of incomplete glycogen depletion, which will presumably be due to a conduction block in some terminal nerve branches or to a block in neuromuscular transmission during the repetitive nerve stimulation under ischaemia. It seems unlikely, however, that a systematic error in the completeness of the glycogen depletion was the reason for the discrepancy in innervation ratio between the young animals, since the experimental conditions were almost identical in the two studies, except for a difference in the male albino rat strains used (Sprague–Dawley rats in Kugelberg's studies and Wistar in the present one). Kugelberg (1973, 1976) reported innervation ratios as high as 120 in the slow-twitch motor units of young rats. An innervation ratio as high as this was never found in the present study, either in young or in old animals. Motor units with similar twitch forces and with almost identical thresholds for activation were often found to be closely packed in the same part of the ventral root. Microdissected root filaments can thus be mistakenly believed to include one single motor axon to soleus instead of two. Most of the slow-twitch units in this study had an innervation ratio that was approximately 50% of that of the largest motor units studied by Kugelberg (1976) and the possibility cannot be ruled out that some of the largest slow-twitch motor units reported by Kugelberg (1973, 1976) were in fact two single motor units with very close thresholds for activation and highly intermingled territories. A systematic error due to incomplete glycogen depletion in young but not in old animals is an unlikely reason for the finding of an age-related increase in innervation ratio. If anything, the reverse would be expected, since the number of complex terminal nerve branches has been reported to be increased in old age (Harriman, Taverner & Woolf, 1980), indicating a reinnervation process. These reinnervating sprouts would in fact be expected to be especially vulnerable to ischaemia and conduction block and it cannot be excluded that the age-related increase in innervation ratio observed in this study is underestimated.

Randomness of the muscle fibre arrangement in the motor unit

Clustering of muscle fibres of the same enzyme-histochemical type is a common histopathological finding, which indicates a rearrangement of the muscle fibres in the motor unit. This is due to reinnervation of denervated individual muscle fibres due to collateral sprouting from nearby axons (Kugelberg, Edström & Abbruzzese, 1970). In this study, clusters of more than three muscle fibres were rarely seen in single motor units either in young or in old animals, irrespective of the motor unit type. However, in a slow on-going denervation–reinnervation process it is assumed that the distribution of muscle fibres in the motor unit becomes rearranged before the clus-

tering becomes manifest. A special model was therefore designed to test whether there were any signs of muscle fibre rearrangement in the old motor units. With this model the randomness of the muscle fibre arrangement was assessed as the mean difference between the measured and predicted number of muscle fibres in eight sectors of the motor unit territory. These differences were corrected for individual variations in innervation ratios and motor unit sector areas (see Methods). The average absolute differences between predicted and measured numbers of fibres in the eight sectors were not affected by age in the fast-twitch units; they ranged from 2·0 to 5·2 (3·5 ± 1·1) in the young and from 1·7 to 5·9 (3·0 ± 1·3) in the old animals. In the slow-twitch motor units, on the other hand, the absolute differences were increased ($P < 0·05$) in old age; ranging from 1·6 to 4·1 (2·9 ± 0·8) in the young animals and from 2·9 to 4·8 (4·1 ± 0·7) in the old ones, indicating a rearrangement of the muscle fibres in the old slow-twitch motor units (Fig. 1).

Number of motor units

The total number of motor units can be calculated assuming that (1) the mapped motor units are representative of all the units in the muscle, (2) all fibres in the unit are depleted of glycogen and (3) the total number of muscle fibres is known. As discussed above, all fibres in t.a. do not pass through the greatest girth of the muscle, and the total number of muscle fibres cannot be accurately determined from a single cross-section of this muscle (for references see Larsson & Edström, 1986*a*); the number of motor units was therefore not calculated in t.a.

In the slow-twitch soleus, the total number of fibres can be accurately determined from a single cross-section and the number of muscle fibres in fast- and slow-twitch units is similar in this muscle (Kugelberg, 1976). The number of motor units was calculated to range from 41 to 66 (49 ± 9) in the young and from 17 to 44 (29 ± 9) in the old animals; thus there was a 40% smaller ($P < 0·01$) number of motor units in old age. These results indicate that the muscle fibre loss in old soleus was due to a loss of whole motor units and that the higher innervation ratio in old age was a result of reinnervation of previously denervated fibres. Kugelberg (1976) calculated the number of motor units to be approximately 34. This value conforms with the findings in some morphological studies, where 30–32 α-motoneurones have been counted in the motor nerve to soleus in young rats (see Kugelberg, 1976). However, as discussed by McComas (1977), calculations of innervation ratios based on studies on peripheral nerves, as opposed to ventral roots, are unlikely to prove helpful, since α-motoneurones cannot be separated from other myelinated neurones and the relative number of α-motoneurones among the total number of myelinated neurones in the nerve supplying a muscle is not known.

The number of motor units was also estimated in soleus by whole ventral root microdissection and also by comparison of L5 and single motor unit twitch forces. When the whole L5 ventral root was microdissected, the numbers of motor units in a young and an old animal were found to be 42 and 24, respectively. When, on the other hand, the twitch force evoked by L5 ventral root stimulation was divided by the average twitch force of the single motor units in the same animal, the number of motor units was found to range from 24 to 44 (35 ± 7, $n = 6$) in the young animals and from 13 to 33 (25 ± 5, $n = 12$) in the old ones, which meant that the number was

29% smaller ($P < 0{\cdot}01$) in old age. Thus, an age-related decrease in the number of motor units was found in soleus irrespective of the method of evaluation used. However, the total number of soleus motor units is probably underestimated by both the above methods, since all motoneurones probably do not survive the dissection and the large majority, but not all, motoneurones to soleus are included in the L5 root.

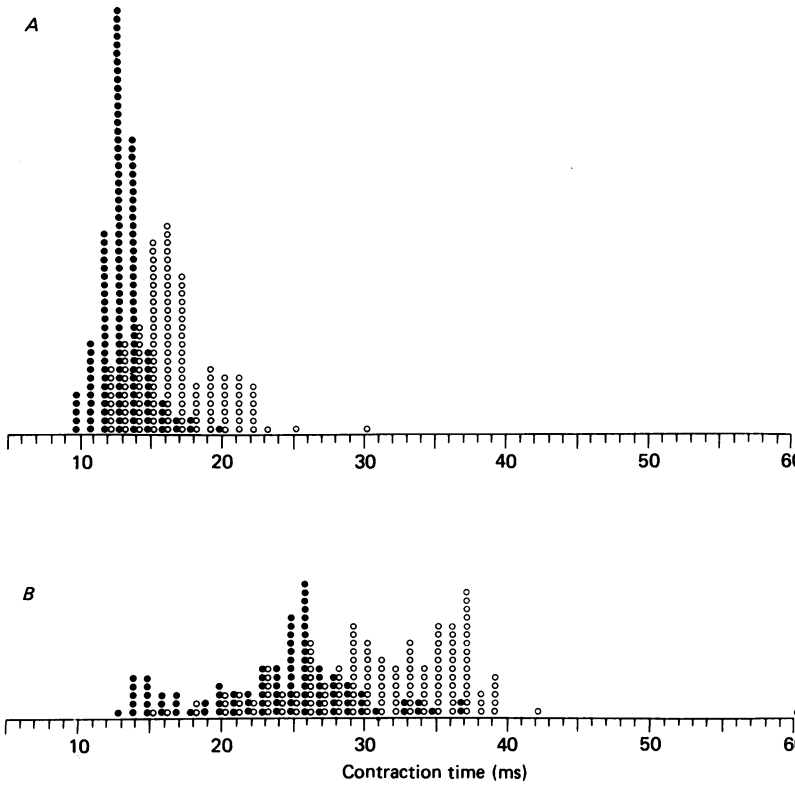

Fig. 2. The contraction times of single motor units in the tibialis anterior (*A*) and soleus (*B*) muscles in young (3–6 months, ●) and old rats (20–24 months, ○).

Contractile properties

In randomly isolated single motor units, the average twitch duration in t.a. and soleus was significantly longer in the old (20–24 months) than in the young animals (3–6 months), as a result of an increased contraction and half-relaxation time. In t.a., the contraction and half-relaxation times increased ($P < 0{\cdot}001$) from 13 ± 1 to 17 ± 3 ms (Fig. 2) and from 12 ± 2 to 16 ± 5 ms, respectively (a total of 138 motor units were studied in the young animals and 136 in the old). The twitch force was significantly ($P < 0{\cdot}01$) higher in the old (61 ± 42 mN) than in the young (38 ± 35 mN) animals. In soleus, the duration of contraction and half-relaxation increased ($P < 0{\cdot}001$) from 24 ± 5 to 31 ± 7 ms (Fig. 2) and from 26 ± 8 to 35 ± 9 ms, respectively (a total of 94 motor units were studied in the young animals and 142 in the old). In this muscle the twitch force did not differ significantly between young ($6{\cdot}9 \pm 2{\cdot}9$) and

old (8·8±4·9 mN) animals. Thirty single motor units in t.a. and seventeen in soleus were marked through glycogen depletion and subsequently identified enzyme-histochemically. (Morphometrical properties were presented in twenty-five of these motor units in Table 2. The remaining five units were identified enzyme-histo-

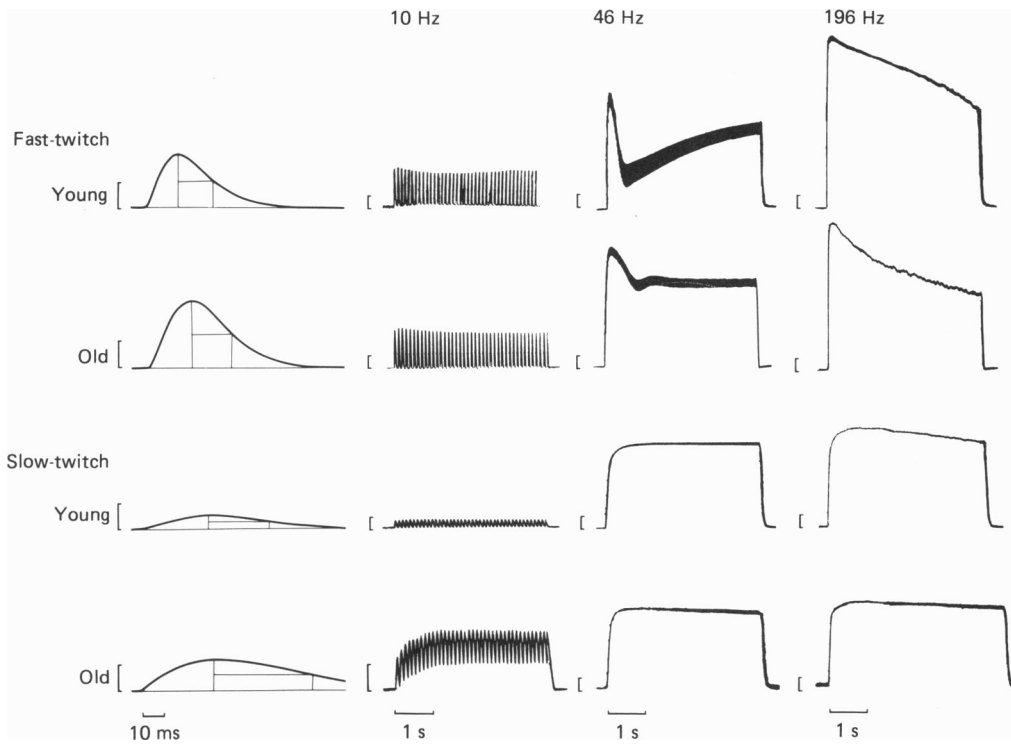

Fig. 3. Isometric twitch and force responses to stimulation at 10, 46 and 196 Hz in typical fast- and slow-twitch single motor units in young (3–6 months) and old (20–24 months) rats. The contraction and half-relaxation times are indicated in the isometric twitches. The vertical bars denote 0·1 N.

chemically, but it was not possible to make an accurate estimation of the total number of muscle fibres in these units. Since, a diffuse regional glycogen depletion was noted just beneath the muscle fascia in these units. This regional glycogen depletion is probably related to a local trauma when the muscle is dissected free from surrounding tissue prior to the freezing.) All the glycogen-depleted muscle fibres in t.a. were classified according to their enzyme-histochemical features as type II fibres and those in soleus as type I fibres. In the fast-twitch motor units contraction time increased from 12±2 to 15±4 ms ($P < 0·05$) and half-relaxation time from 13±3 to 15±4 ms ($P < 0·05$). In the slow-twitch motor units corresponding increases from 26±4 to 31±3 ms ($P < 0·05$) and from 28±7 to 36±6 ms ($P < 0·01$), respectively, were found. In the soleus of old animals, in which the relative number of type II fibres was 1% (Table 1), the contraction and half-relaxation times were almost identical in the randomly isolated and the enzyme-histochemically classified motor units. In the soleus of young animals the relative number of type II fibres was 9% (Table 1), and

on average the duration of both contraction and half-relaxation times was 2 ms shorter in the randomly isolated than in the glycogen-depleted and enzyme-histochemically classified motor units. However, this latter difference was much smaller than the age-related change in contraction and half-relaxation times, indicating that the age-related change in the proportions of different types of fibres was of minor importance for the reduced speed of contraction observed in old age.

Age had no significant effect on the force in a completely fused tetanic recording in either fast- or slow-twitch motor units. However, in the fast-twitch units, the twitch:tetanus ratio increased ($P < 0{\cdot}01$) from $0{\cdot}20 \pm 0{\cdot}08$ ($n = 16$) in the young animals to $0{\cdot}30 \pm 0{\cdot}08$ ($n = 14$) in the old ones as a result of an increase in twitch force. Since there was no change in the staircase potentiation with age, the force on stimulation at 10 Hz in relation to the tetanus force was also increased ($P < 0{\cdot}1$), from $0{\cdot}26 \pm 0{\cdot}09$ in the young to $0{\cdot}35 \pm 0{\cdot}11$ in the old animals. A more complete fusion and a less prominent sag were observed on stimulation at 46 Hz in the old fast-twitch units and the force at 46 Hz in relation to the tetanus force was higher ($P < 0{\cdot}05$) in the old ($0{\cdot}87 \pm 0{\cdot}09$) than in the young ($0{\cdot}68 \pm 0{\cdot}17$) animals (Fig. 3). In the slow-twitch motor units there was no significant difference in the twitch:tetanus ratio between young ($0{\cdot}07 \pm 0{\cdot}04$, $n = 10$) and old animals ($0{\cdot}12 \pm 0{\cdot}07$, $n = 7$). The force at 10 Hz in relation to the tetanus force increased ($P < 0{\cdot}05$) from $0{\cdot}13 \pm 0{\cdot}10$ ($n = 10$) in the young animals to $0{\cdot}30 \pm 0{\cdot}18$ ($n = 7$) in the old ones, since summation was more pronounced in the old animals on account of the longer twitch duration. The force at 46 Hz in relation to the tetanus force, on the other hand, was not influenced by age in the slow-twitch units, this value being $0{\cdot}92 \pm 0{\cdot}07$ in the young animals and $0{\cdot}93 \pm 0{\cdot}03$ in the old ones.

The tetanus tension, i.e. tetanus force per motor unit cross-sectional area, was calculated in twenty-four fast- and slow-twitch motor units. Very high tensions were found in both young and old animals irrespective of the motor unit type. The average tetanus tensions ranged between 43·1 and 57·8 N/cm^2 in the two age groups. Especially high values were noted in some old fast- and slow-twitch units. The reason for these erroneously high tensions (cf. Barany & Close, 1971) is not known, but they may be due to incomplete glycogen depletion of some fibres in the unit, or to an underestimation of the motor unit cross-sectional area as a result of overstretching of the muscle when clamped or shrinkage of the fibres when frozen or during the histochemical staining procedure. Further work is in progress in an attempt to clarify this point.

DISCUSSION

The decreased speed of contraction in old age, which is observed before senile muscle wasting becomes manifest (Larsson & Edström, 1986*a*), has been proposed to be secondary either to a loss of fast-twitch motor units or to changes in the properties of the contractile material during ageing, or both (Campbell *et al.* 1973). An age-related decrease in the proportion of fast-twitch (type II) muscle fibres has been reported in various mammals (for references see Larsson, 1982) and a loss of type II fibres, especially of type II A, was seen in the slow-twitch soleus muscle in this study. However, it was found that the contraction speed, determined from the isometric

twitch, decreased to a similar extent in old age in both fast- and slow-twitch motor units. This reduced speed of contraction at the single motor unit level parallels the age-related changes in the speed of contraction observed previously in both fast-twitch t.a. and slow-twitch soleus muscles (Larsson & Edström, 1986*a*). The proportion of different types of fibres only changed in soleus, however, during this age span (Larsson & Edström, 1986*a*). It is therefore concluded that the reduced speed of contraction in old age is primarily due to alterations in contractile properties in remaining motor units in both fast- and slow-twitch muscles and that the age-related decrease in the number of type II fibres in soleus is of considerably less importance.

A series of events in the excitation–contraction coupling determines the time course of the contractile response. The capacity of the sarcoplasmic reticulum for calcium release and recapture and the composition of fast and slow isoforms of the myofibrillar proteins are the two key factors that determine the contraction time of the muscle twitch (e.g. Brody, 1976; Dulhunty & Valois, 1983; Kugelberg & Thornell, 1983). In skeletal muscle of young adults, the sarcoplasmic reticulum proteins and myofibrillar proteins appear in a very co-ordinated way in the different muscle fibre types (Salviati, Betto, Danielo Betto & Zeviani, 1983), presumably triggered by motoneurone discharge properties. In old age, however, this co-ordination is partially lost in fast- and slow-twitch skeletal muscle fibres (Salviati *et al.* 1983) and the surface and volume densities of the T-tubules and of the sarcoplasmic reticulum are reported to be decreased (DeCoster, DeReuck, Sieben & Vander Eecken, 1981). Thus, the complex age-related changes reported in T-tubules, sarcoplasmic reticulum proteins and myofibrillar proteins, have obvious effects on the excitation–contraction coupling and probably underlie the reduced speed of contraction observed in both fast- and slow-twitch motor units in this study. The mechanisms underlying these changes in the properties of the sarcoplasmic and myofibrillar proteins are not known, but they may be related to an alteration in muscle protein synthesis or catabolism induced either by a primary myogenic age-related mechanism or by a change in motoneurone properties. The close connection between motoneurone discharge properties and the synthesis and accumulation of calcium ATPase in the sarcoplasmic reticulum (Jolesz & Sreter, 1981), together with the changes in motoneurone discharge properties in old age (Borg, 1981; Nelson, Soderberg & Urbscheit, 1984), lend some support to the latter alternative.

The age-related alterations in fibre-type proportions and the number of fibres in the soleus conform with those recently presented in young adult (6 months) and old (20–24 months) male albino rats (Larsson & Edström, 1986*a*). It was suggested that the most probable mechanisms underlying these alterations were a continuation of the transformation process that occurs during development and a subsequent fibre loss irrespective of the enzyme-histochemical type, or alternatively, a selective degeneration of fast-twitch motor units during ageing (Larsson & Edström, 1986*a*). It was found that severe muscle atrophy (cross-sectional fibre areas less than 1000 μm^2) occurred predominantly in type II fibres and these atrophic fibres often had an angular appearance, indicating previous denervation (Larsson & Edström, 1986*a*). These findings, together with the selective degeneration of the largest and most rapidly conducting motoneurones reported in old age (for references see Larsson, 1982), lend some support to the hypothesis that the altered fibre-type proportions

and the loss of muscle fibres in ageing soleus muscle are related to a selective loss of fast-twitch motor units. However, preliminary results from rats of ages 6–20 months show that the fibre-type proportions are altered prior to the muscle fibre loss (T. Ansved, L. Larsson & L. Edström, 1986, unpublished observations), suggesting a transformation of type II muscle fibres to type I followed by an unselected loss of motor units beginning in middle age. The atrophic angular type II fibres observed in old rats (Larsson & Edström, 1986*a*) may in fact be type I fibres which through loss of neural control have been transformed to type II. In support of this idea it has been reported that fast myosin is preferentially synthesized in type I muscle fibres after denervation (for references see Jenny, Weber, Lutz & Billeter, 1980). Further studies of the whole spectrum of ages between 6 and 20 months are presently being undertaken to clarify this point.

Histopathological studies of aged skeletal muscle have shown that both neurogenic (e.g. grouped atrophy and fibre-type grouping) and myogenic changes (e.g. central nuclei, necrosis, phagocytosis and interstitial fibrosis) occur, the neurogenic type predominating (e.g. Tomonaga, 1977). Interestingly, myogenic changes are also seen in long-standing neurogenic disorders and may thus reflect a disturbance of the motoneurone (Mumenthaler, 1970). The redistribution of the muscle fibres and the increased innervation ratio indicate a slow on-going denervation–reinnervation process in the ageing soleus. The site of the age-associated denervation process is, however, controversial. Both a slow loss of neuromuscular contact due to degeneration at the end-plate region and a loss of entire motoneurones have been suggested. Gutmann & Hanzlikova (1966) concluded that the age-related fibre loss in rat soleus muscle, between adult (4 months) and old (24 months) animals, was secondary to a degeneration of motor end-plates, which was found to cause a 33% decrease in motor unit innervation ratio but no change in the number of motor units. This conclusion was based on measurements of the number of myelinated nerve fibres in the motor nerve to soleus and the total number of muscle fibres in soleus together with morphological studies of end-plate structures. However, in the present study an increased innervation ratio, a redistribution of muscle fibres in the unit and a decreased number of motor units were observed in the soleus of old rats, indicating that the muscle fibre loss in old rat soleus muscle is due to a loss of entire motoneurones and incomplete reinnervation of denervated muscle fibres by the remaining motoneurones. These findings are supported by the results of various studies in which the number of functioning motor units has been estimated by the method of McComas, Sica & Campbell (1971), where an age-related decrease in the number of motor units both in man (Campbell *et al.* 1973) and in the mouse and rat (Caccia *et al.* 1979) has been found. As discussed by McComas (1977), calculations of innervation ratios based on studies on peripheral nerves are unlikely to prove helpful, since α-motoneurones cannot be separated from other myelinated nerves and the relative contribution of α-motoneurones to the total number of myelinated neurones in the nerve to a muscle is not known. Furthermore, electrophysiological studies have shown that the profound ultrastructural alterations in the neuromuscular junction in old age (Gutmann *et al.* 1971) do not impair neuromuscular transmission and that they are either not rate limiting or are well compensated for (Banker, Kelly & Robbins, 1983).

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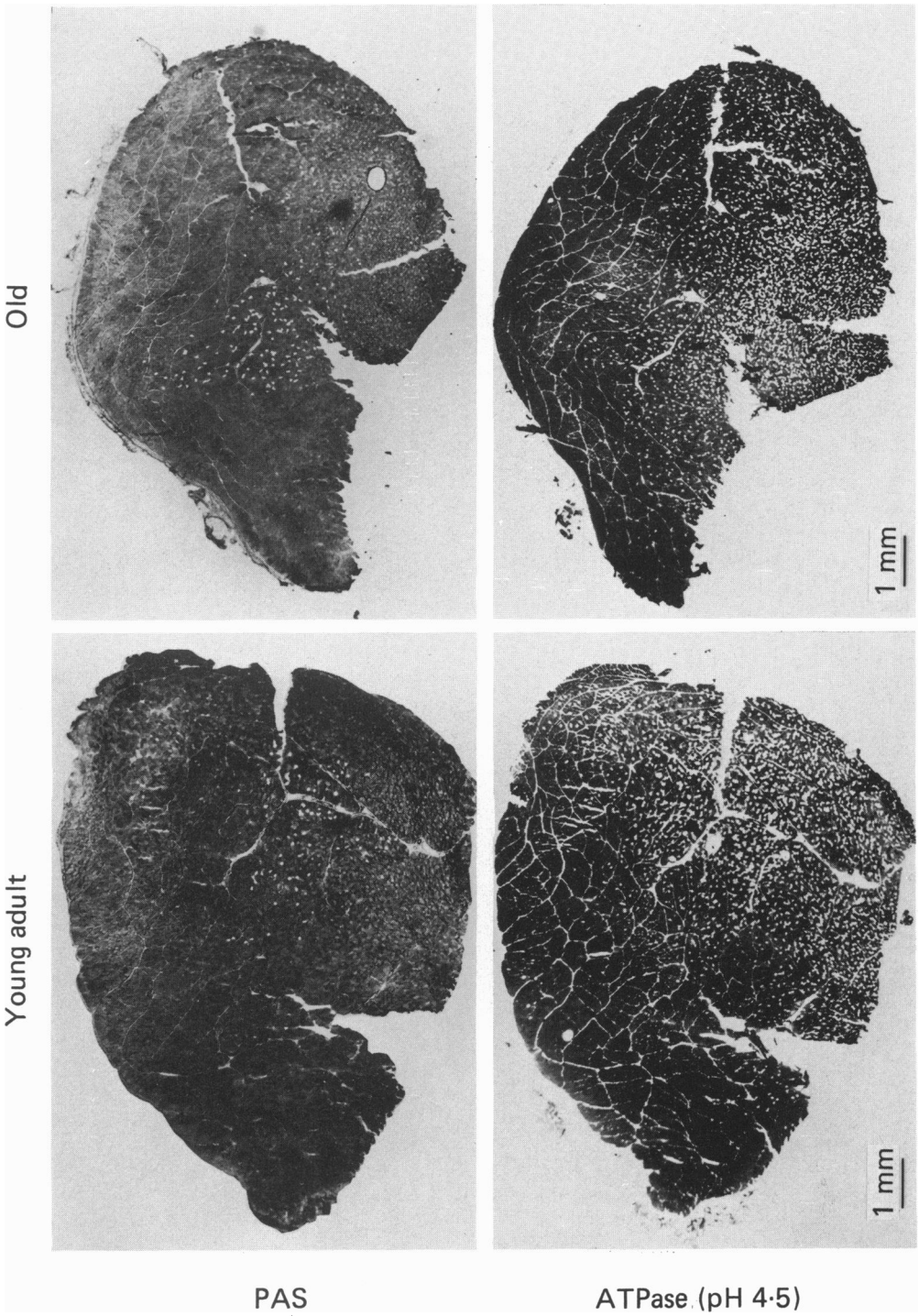
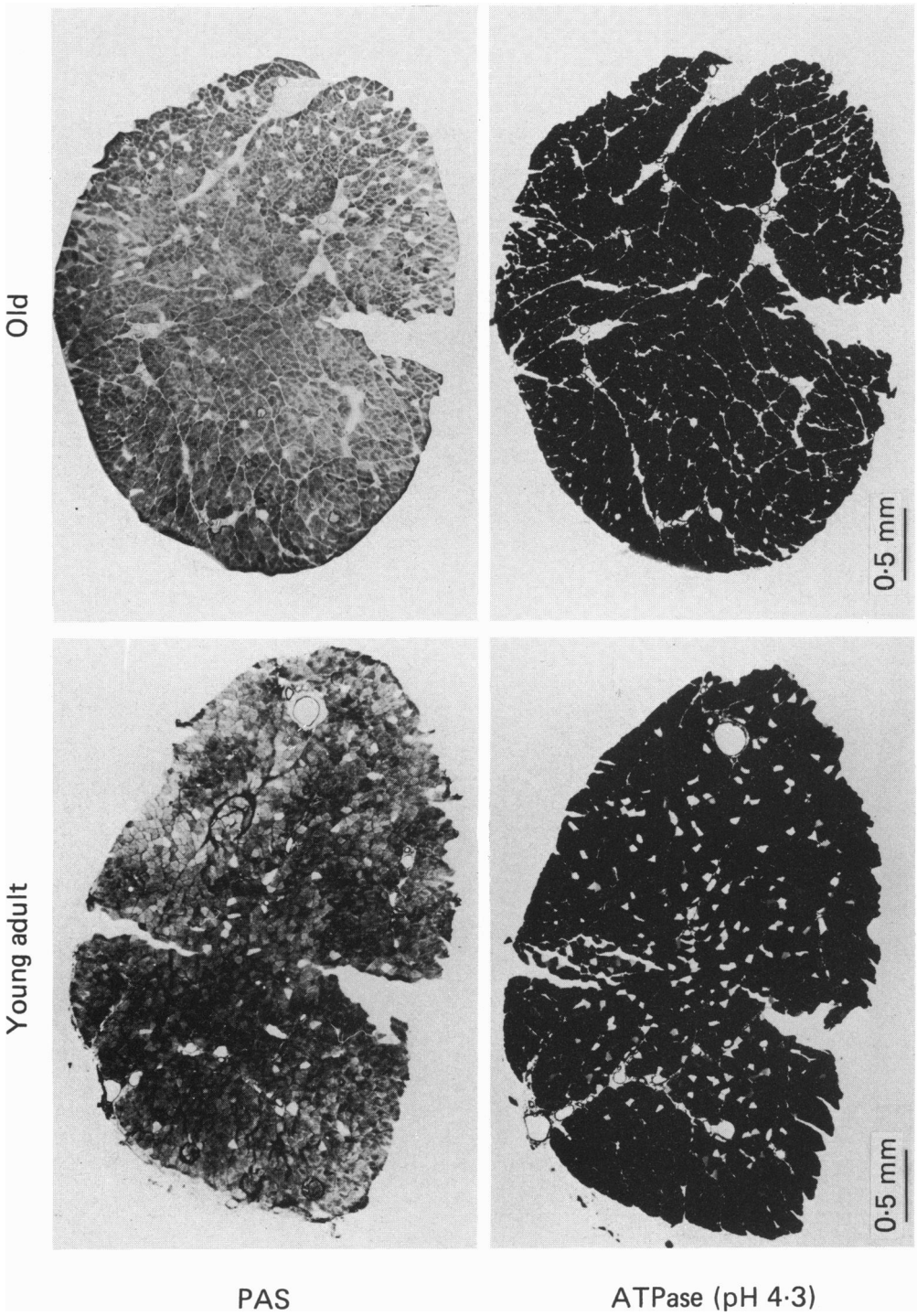

Old
Young adult
1 mm
1 mm
PAS
ATPase (pH 4·5)


Old
Young adult
0·5 mm
0·5 mm
PAS
ATPase (pH 4·3)

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EXPLANATION OF PLATES

PLATE 1

Photomicrographs of serial transverse sections of the tibialis anterior muscle of a young and an old rat. The upper section, stained for PAS, demonstrates the distribution of glycogen-depleted fibres in a fast-twitch motor unit. The small, lightly stained fibres in the deep central part of the muscle are slow-twitch type I fibres, which stain less intensely for glycogen in the rat. The lower section is stained for myofibrillar ATPase after pre-incubation at pH 4·35.

PLATE 2

Photomicrographs of serial transverse sections of the soleus muscle of a young and an old rat. The upper section, stained for PAS, demonstrates the distribution of glycogen-depleted fibres in a slow-twitch motor unit. The lower section is stained for myofibrillar ATPase after pre-incubation at pH 4·35.