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CONTROL OF JUNCTIONAL ACETYLCHOLINESTERASE BY NEURAL AND MUSCULAR INFLUENCES IN THE RAT

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SUMMARY

1. The development of AChE at ectopic neuromuscular junctions forming between a transplanted foreign nerve (the superficial fibular nerve) and the denervated soleus muscle has been studied in adult rats.

2. Junctional AChE activity began to appear in the vicinity of the fibular nerve sprouts 6–7 days after section of the soleus nerve and 3–4 days after the onset of transmission.

3. No histochemically detectable AChE appeared when the fibular nerve was cut 0–4 days after the soleus nerve had been cut.

4. Direct electrical stimulation of the denervated soleus muscle caused plaques of true AChE, as determined by inhibitor studies, to appear in muscles where the fibular nerve had been cut 2–4 days after the soleus nerve but not in muscles where the two nerves had been cut at the same time. The plaques appeared only in the vicinity of fibular nerve sprouts and coincided with newly formed but stable peaks of ACh sensitivity. Local application of Neostigmine prolonged and increased the depolarising responses evoked by pulses of ACh at these sites.

5. In muscles where the fibular nerve was intact the AChE plaques changed gradually over a few weeks from an immature appearance to a mature appearance characteristic of normal end-plates. In stimulated muscles where the fibular nerve had been cut the plaques stained intensely but remained morphologically immature.

6. We conclude (1) that muscle activity is important for the appearance of AChE at developing neuromuscular junctions and (2) that AChE accumulates only at sites on the muscle surface where the nerve fibres have left a 'trace' upon contact with the muscle fibres. These traces form quickly and persist after nerve–muscle interaction of as little as 2 days. The muscle appears as a major source of junctional AChE since stimulation of the muscle induces intense AChE activity in muscles where the nerve has degenerated.

INTRODUCTION

The function of acetylcholinesterase (AChE) at neuromuscular junctions is relatively well established. By hydrolysing acetylcholine (ACh) AChE brings about a rapid termination of the action of transmitter released by the nerve (Katz & Miledi, 1973). The hydrolysis has the additional function of providing choline molecules for

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resynthesis of ACh after uptake into nerve terminals (Birks & MacIntosh, 1961; Collier & MacIntosh, 1969). On the other hand, it is not known which cells produce the junctional AChE nor how its synthesis and accumulation at the end-plate are controlled. These questions are the subject of this paper.

We have taken advantage of a preparation in which a transplanted nerve makes new ectopic synapses with a denervated adult muscle (Lømo & Slater, 1978). Transmission and nerve evoked muscle activity start at these new synapses about 3 days after cutting the normal innervation and junctional AChE begins to appear after a further 3–4 days. We have found that if the transplanted nerve is cut before AChE appears and the denervated muscle is then stimulated through chronically implanted electrodes, AChE accumulates at sites where, prior to its degeneration, the transplanted nerve had interacted with the surface of the muscle fibre. This suggests that muscle activity plays an important role in the control of junctional AChE.

A brief account of some of this work has already appeared (Lømo & Slater, 1976).

METHODS

All experiments were performed on male Wistar rats weighing about 200 g at the time of the initial operation. The formation of ectopic synapses was induced by transplanting the fibular nerve onto the surface of the soleus muscle and then, after 2–6 weeks, cutting the soleus nerve. Details of the surgical procedures are given in Lømo & Slater (1980). In some experiments, the soleus muscle was kept active after complete denervation by direct stimulation through chronically implanted electrodes, as described elsewhere (Lømo & Westgaard, 1975).

Acute experiments. Intracellular recordings and maps of ACh sensitivity of individual muscle fibres were made as previously described (Lømo & Slater, 1980). At sites of high ACh sensitivity Neostigmine was sometimes ejected by pressure (2 kg/cm^2) from a third immediately adjacent pipette (containing 0·5 g/ml. Neostigmine) and the effect on the ACh response noted. The recording pipettes filled with 0·5 M-KCl also contained 2% Pontamine Sky Blue (Gurr) which allowed the dye to be injected by pressure into the fibre to mark the sites of high ACh sensitivity.

Histology and histochemistry. After the acute experiment some of the muscles were fixed for 1 hr in 4% formaldehyde pre-incubated for 10 min at room temperature in Sørensens phosphate buffer (pH 6·4) containing either the specific AChE inhibitor BW 284c51 (10^{-4} M) or the non-specific ChE inhibitor ethopropazine (10^{-4} M), and then stained for ChE for 30–60 min according to Buckley & Heaton (1968) in the presence of the same inhibitors. Inhibitors were not always used. The muscles were then fixed overnight at 4 °C in 4% formaldehyde, washed in water, dehydrated in alcohol, cleared in benzyl benzoate and mounted whole in benzyl benzoate for photography. On some occasions single muscle fibres were teased from the muscle after staining and fixation, placed in a drop of water on slides and photographed. Other muscles were stained with Methylene Blue (Waerhaug & Korneliussen, 1974) before ChE staining using the following procedure: (1) 15 min in a mixture of one part saline solution (see above) and one part Sørensen phosphate buffer (pH 6·4) containing 0·01% Methylene Blue, bubbled with O_2 (95%) and CO_2 (5%) at 37 °C; (2) 10 min rinse in saline; (3) overnight fixation in 8% ammonium molybdate; (4) ½ hr wash in water; (5) 1–2 hr staining for ChE (Buckley & Heaton, 1968); (6) 15 min wash in water; (7) dehydration in alcohol and clearing in benzyl benzoate.

RESULTS

Development of junctional AChE

The formation of ectopic synapses between the transplanted fibular nerve and the denervated soleus muscle has been described previously (Lømo & Slater, 1978). Transmission starts about 3 days after the nerve to the soleus is cut. At this time, there was no histochemical evidence of post-synaptic ChE activity at the new neuro-

muscular junctions (n.m.j.s). After a further 3–4 days, when many fibres on the surface of the soleus muscle have become innervated by the fibular nerve, plaques of ChE reaction product were found in the vicinity of the fibular nerve sprouts (Pl. 1*A*, *B*, *C*). This staining was virtually completely inhibited by a specific inhibitor of AChE (BW 284c51, 10^{-4} M) and was unaffected by an inhibitor of pseudo-ChE (ethopropazine, 10^{-4} M) indicating that the induced ChE was largely AChE.

TABLE 1. Appearance of AChE plaques during development of ectopic innervation

		Acetylcholinesterase plaques							
		Number			Intensity		Maturity		
Time after section of soleus nerve (days)	No. of muscles	0	< 30	> 30	+	+ +	Immature	Interm. mixed	Mature
0	6	4	1	1	—	2	—	2	—
2–4	5	5	—	—	—	—	—	—	—
6–8	10	6	3	1	3	1	4	—	—
9–22	13	3	10	—	—	10	1	7	2
70	2	—	—	2	—	2	—	—	2

The plaques of ChE product coincided with peaks of high ACh sensitivity which develop in association with sites of neurally released transmitter at an early stage in the development of ectopic n.m.j.s (Lømo & Slater, 1980). This was shown by locating the sensitive spots with ACh ejected electrophoretically from an external micropipette and then marking the spots by passing a dye (Pontamine Sky Blue) from the recording electrode into the muscle fibre at that spot. Subsequent staining for ChE revealed characteristic ChE plaques at each of twelve spots in nine fibres studied (Pl. 1*D*). Staining of muscles with Methylene Blue showed further that axons terminated at ChE plaques in a manner characteristic of motor end-plates (Pl. 1*B*, *C*).

For all these reasons it seems clear that the plaques of ChE reaction product represent junctional AChE activity induced at the new ectopic synapses. We shall refer to them as 'AChE plaques' in the remainder of the paper.

After their initial appearance, the AChE plaques went through a process of maturation which lasted at least several weeks (Pl. 2). 'Immature' plaques were usually long, diffusely outlined and oriented along the muscle fibres. 'Mature' plaques consisted of distinctly outlined clusters of deposit similar in appearance to those at normal soleus end-plates but commonly of larger size. This is consistent with a recent report that the superficial fibular nerve makes larger end-plates than the soleus nerve, both with its own muscle (fibular brevis) and, after transplantation, with the soleus (Waerhaug, Korneliussen & Sommerschild, 1977). At intermediate times, 2–3 weeks after section of the soleus nerve, some plaques were immature or intermediate in their state of development.

An estimate was made of the number of plaques, their staining intensity and degree of maturation at different stages in the development of ectopic innervation. Table 1 shows that the ectopic AChE plaques changed progressively during the first 2–4 weeks after cutting the soleus nerve and became more numerous, more intensely stained and more mature.

Development of AChE after cutting the fibular nerve

To learn whether the presence of the fibular nerve is required during the period of 3–5 days between the onset of transmission and the appearance of AChE, we cut the fibular nerve 2–7 days after cutting the soleus nerve. In twenty-one of twenty-five such muscles, AChE plaques failed to appear in the region underneath the degenerated fibular nerve (Lømo & Slater, 1980) (Pl. 3*B*, *D*, *F*; Table 2, upper part) or elsewhere in the muscle. The significance of the staining that did occur is uncertain. In a few cases AChE deposits also appeared in the region of the transplanted nerve in muscles where the soleus nerve had never been cut (Table 1) or where it was cut at the same time as the transplanted nerve (Table 2). Some of these deposits were intensely stained, fully mature looking plaques which probably represented ectopic synapses formed soon after the transplantation, perhaps as a result of damage to the muscle surface during the operation (Lømo & Slater, 1978). ChE activity has also been shown to appear to a varying degree along growing nerve fibres (Duchen, 1970) and some of this may remain after the nerve has degenerated. Nevertheless, the results show that a continuing influence of the nerve is essential for the normal formation of junctional AChE.

Chronic stimulation causes appearance of AChE plaques. To see if this influence of the nerve might be related to the muscle activity it normally evokes, we subjected muscles in which the fibular nerve had been cut 2–7 days after cutting the soleus nerve to chronic stimulation, using implanted platinum electrodes. A pattern of stimulation was used which effectively abolishes extrajunctional ACh sensitivity in denervated muscles (Lømo & Westgaard, 1975) and prevents their ectopic innervation by a foreign nerve (Lømo & Slater, 1978). In each of seventeen such stimulated muscles, numerous intense ChE plaques appeared in the region where the fibular nerve sprouts had been (Pl. 3*A*, *C*, *E*; Pl. 4; Table 2, lower part).

To determine the specificity of the ChE activity induced by stimulation, many stimulated muscles were stained in the presence of and after pre-incubation with ethopropazine (10^{-4} M), a non-specific ChE inhibitor. There was no obvious effect on the intensity of the staining. In one experiment the stimulated muscle was cut longitudinally into two parts with each part containing a substantial number of surface fibres covered by the degenerated fibular nerve. One part was pre-incubated with ethopropazine and then stained for ChE in the presence of the same inhibitor. Numerous strongly stained plaques appeared which were indistinguishable from those seen in the absence of the inhibitor. In the other part, pre-incubated and then stained in the presence of BW 284c51 (10^{-4} M), while no obvious plaques appeared in the region of the old end-plate band or underneath the degenerated fibular nerve, detailed examination at high magnification revealed granular spots in both regions of the muscle which had the same shape and distribution as the plaques seen in the absence of inhibitor. These findings indicate that the ChE staining is due largely to the presence of AChE.

AChE plaques coincide with peaks of ACh sensitivity. If the AChE plaques induced by stimulation represent synaptic specializations, they should coincide with spots of high ACh sensitivity which would resist the effects of muscle stimulation (Lømo & Slater, 1980). To test this prediction, we determine the position of peaks of ACh

TABLE 2. Appearance of AChE plaques after cutting the fibular nerve

Time between section of soleus nerve and acute experiment (days)	Time between section of soleus and fibular nerve (days)	Time between section of soleus nerve and onset of stimulation (days)	No. of muscles	Acetylcholinesterase plaques							
				Number			Intensity		Maturity		
				0	< 30	> 30	+	++	Immature	Interm. mixed	Mature
9–11	0	—	5	4	1	—	1	—	—	1	—
11	2	—	1	1	—	—	—	—	—	—	—
8–17	3–4	—	19	16	3	—	2	1	2	—	1
15–23	7	—	5	4	1	—	—	1	1	—	—
9–11	0	3–4	5	4	1	—	—	1	—	1	—
9–11	2	3–4	2	—	1	1	—	2	2	—	—
8–35	3–4	4	8	—	—	8	—	8	8	—	—
15–21	7	7	4	—	—	4	—	4	1	2	1
22–23	7	16	3	—	—	3	—	3	3	—	—

sensitivity (Lømo & Slater, 1980) on five individual muscle fibres from muscles in which, 2–4 days after cutting the soleus nerve, the fibular nerve had been cut and at the same time stimulation had been started and then continued for 8–9 days. The sites of high ACh sensitivity were marked by injecting dye into the muscle fibre and after fixing and staining, corresponding AChE plaques were found in each case.

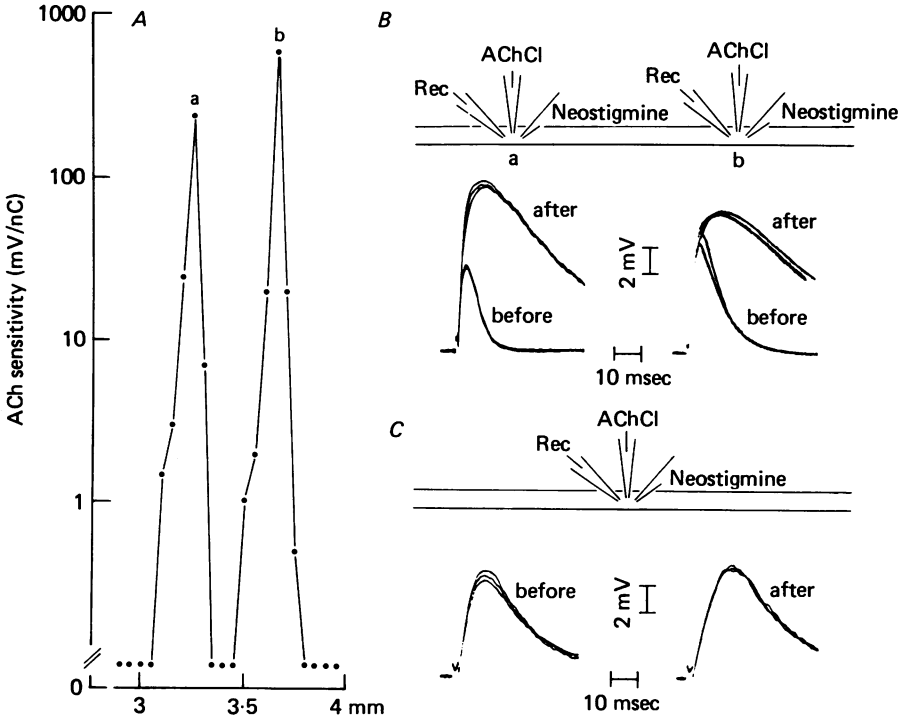

Fig. 1. Two spots of high ACh sensitivity (a and b in *A*) along single muscle fibre in region of fibular nerve growth 13, 9 and 9 days after cutting soleus nerve, fibular nerve and starting stimulation of muscle respectively. *B* and *C*, responses to pulses of ACh before and after local application of Neostigmine (0·5 g/ml.) at spots a and b (*B*) and at a random spot in a fibre in the contralateral non-stimulated but otherwise similarly treated soleus muscle (*C*).

Following on from the previous result, it was possible to obtain evidence for the presence of AChE activity at peaks of ACh sensitivity which was independent of the histochemical method. In Fig. 1, the distribution of ACh sensitivity along a single muscle fibre is shown from a muscle treated as described in the previous paragraph. Two peaks of ACh sensitivity were present (Fig. 1*A*) and at each site, the response to ACh (Fig. 1*B*) was faster than that at any randomly chosen spot on the contralateral non-stimulated (and hence uniformly hypersensitive muscle (Fig. 1*C*)). This difference in time course was related to the presence of AChE activity in the stimulated muscle for when Neostigmine was applied locally (see Methods) the duration of the ACh response in the stimulated, but not the unstimulated, muscle was markedly prolonged. These sites of combined high ACh sensitivity and pharmacologically defined AChE activity were then marked with dye and found to correspond to histochemically demonstrable AChE activity.

The nerve determines the position of AChE plaques early in synaptogenesis. Our results show that the distribution of AChE on the muscle fibre surface is determined by an interaction with the axon terminal. To see when this interaction occurs, we cut the fibular nerve, and at the same time started stimulation, at different stages of synaptic development. If the fibular nerve was cut 7 days after cutting the soleus nerve, when weak, sparse AChE activity may already have appeared in some of the muscles (Table 1), numerous intense AChE plaques were present after 8–14 days of stimulation. If the fibular nerve was cut 3–4 days after the soleus nerve, still prior to the normal appearance of AChE, numerous strongly stained AChE plaques were present after periods of stimulation as brief as 4 days (Pl. 4*C*) (shorter durations were not tried) or as long as 31 days (Pl. 4*D*). Even if the fibular nerve was cut on the second day after cutting the soleus nerve, before the earliest signs of neuromuscular transmission can be found (Lømo & Slater, 1978), well stained AChE plaques could be found on the stimulated muscle (Pl. 4*A*).

In contrast, if the fibular nerve was cut at the same time as the soleus nerve, so that there was no opportunity for ectopic synapse formation to begin, no AChE plaques were found in four out of five muscles studied (Table 2). This suggests that the appearance and localization of AChE plaques is not determined by some unspecific effect of the transplanted nerve or of chronic stimulation on the muscle. Rather, it seems that as an early step in synaptogenesis, the nerve leaves 'traces' on the muscle fibre surface which determine where AChE will subsequently appear. To see if these traces persist after degeneration of the fibular nerve we waited until 9 days after cutting the transplanted nerve, well after the disappearance of the axon terminals which degenerate in less than a day (Lømo & Slater, 1980), before starting the stimulation. In each of three muscles studied, numerous AChE plaques were present after 6–7 days of stimulation (Pl. 4*G*; Table 2).

The structure of the AChE plaques induced by stimulation remains immature. The structure of the ectopic AChE plaques that appeared after cutting the fibular nerve and subsequently stimulating the muscle remained immature (Table 2) even 19–35 days after denervation (Pl. 4*D*, *G*) when many of the plaques underneath an intact fibular nerve are well circumscribed with clusters of deposits characteristic of mature end-plates (Pl. 4*H*). Most of the plaques in the stimulated muscles continued to look like the immature plaques made at an earlier stage by an intact fibular nerve (Pl. 2*A*). They became intensely stained but kept their appearance of long streaks along the fibres without any distinctive clusters. This indicates that in the absence of continued nerve–muscle interaction a gradual maturation and restructuring of the ChE plaques does not take place.

DISCUSSION

Our experiments suggest that the development of AChE at ectopic nerve–muscle junctions is influenced by the nerve in two distinct ways. First, at an early stage in synaptogenesis, the nerve induces a persistent local alteration of the muscle fibre surface ('trace') which makes it a preferred site for the subsequent appearance of AChE. Secondly, by generating muscle activity, the nerve causes the appearance of AChE at that site.

Possible nature of the 'trace'. It is now clear that AChE is bound to the basal lamina of mature n.m.j.s (McMahan, Sanes & Marshall, 1978). It is possible that during

synaptogenesis the nerve induces binding sites for AChE in the basal lamina and that these constitute the persistent 'trace'. Biochemical studies have led to a similar suggestion based on the finding that certain species of AChE molecules have a non-catalytic tail unit which may serve to bind the catalytic unit to the basal lamina (Lwebuga-Mukasa, Lappi & Taylor, 1976).

Another possibility is that the nerve induces an alteration in the muscle fibre itself which permits local synthesis and secretion of AChE once the muscle becomes active.

Whatever the detailed nature of the trace may be, our results show that it can be formed at an early stage in synaptogenesis and in the absence of effective neuromuscular transmission and the muscle activity it evokes. Once formed, the trace can persist for at least a week.

Effects of muscle activity. The local deposits of AChE activity which appear as a result of direct muscle stimulation resemble those at immature ectopic n.m.j.s both in the form and distribution of the histochemical reaction product and in the occurrence of the enzyme activity exactly at sites of high and stable ACh sensitivity. It thus seems likely that muscle activity *per se* is an important factor in the control of AChE activity at the n.m.j.

A number of other experiments provide further support for this view. In the adult rat, AChE is restored at denervated end-plates if the muscle fibres become functionally innervated elsewhere (Guth, Zalewski & Brown, 1966; Weinberg & Hall, 1979). In contrast, AChE does not appear at the developing n.m.j.s of chick embryos which have been paralysed by α-neurotoxin (Giacobini, Filogamo, Weber, Boquet & Changeux, 1973). Moreover, curare largely prevents the appearance of AChE at synapses forming in co-cultures of muscle with spinal cord explants. But if the same innervated myotubes are stimulated directly in the continued presence of curare, AChE appears (Rubin, Schuetze, Weill & Fischbach, 1978). These findings suggest that both the initial formation and the subsequent maintenance of junctional AChE require muscle activity.

How muscle activity influences junctional AChE is unclear. There is evidence that AChE is released from muscle fibres (Wilson, Nieberg, Walker, Linkhart & Fry, 1973) as well as from other cells (Chubb & Smith, 1975) and that this release is influenced by impulse activity. Morphological work suggests that AChE is synthesized within the muscle fibre, transported as AChE-rich granules to the post-junctional membrane and released there by exocytosis (Wake, 1976). That impulse activity may increase the activity of enzymes related to synaptic transmission is also indicated by studies on the nor-adrenergic system (Zigmond & Ben-Ari, 1977).

The origin of junctional AChE. In our experimental situation in which AChE is induced to appear after the fibular nerve terminals have degenerated, the muscle fibre is the only likely cell of origin of the enzyme. When the nerve is intact, however, the situation may be more complicated. Salpeter, Rogers, Kasprzak & McHenry (1978) suggest the presence of two layers of AChE in the synaptic cleft, one distributed along the prejunctional membrane and the other along the post-junctional membrane. Recent results indicate that nerve impulse activity causes axon terminals to release the 4S and 10S forms of AChE but not the 16S form (Skau & Brimijoin, 1978) although this form is present in nerves (Di Giamberardino & Couraud, 1978).

The 16S form which is found primarily in the end-plate region of many muscles, perhaps on the post-synaptic membrane (Vigny, Koenig & Rieger, 1976) reappears at denervated end-plates in ectopically innervated muscle fibres (Weinberg & Hall, 1979). It is thus possible that the two layers of AChE in the synaptic cleft represent different forms of the enzyme, one released from the nerve and one from the muscle. Nerve impulse activity may also indirectly give rise to junctional AChE by causing the release of unidentified substances which increase muscle AChE activity (Younkin, Brett, Davey & Younkin, 1978).

While AChE may be released from cells by electrical activity, other mechanisms of control, independent of electrical activity, may also exist. Thus, junctional AChE activity is reduced by colchicine applied locally to the nerve to block axonal transport (Fernandez & Inestrosa, 1976). Furthermore, a reduction in junctional AChE following nerve section occurs earlier with a short than with a long nerve stump (Davey & Younkin, 1978). The mechanism of these effects is unknown.

Neural control of AChR and AChE. The neural control of AChE has certain features in common with that of AChR. In each case, a local interaction with the muscle fibre surface which seems not to depend on muscle activity occurs at a very early stage in synapse formation and determines the spatial distribution of the molecules (Lømo & Slater, 1980). This is followed by an effect mediated by muscle activity which influences the presence or absence of the molecules. In the case of AChR, the molecules are present on the surface of the inactive muscle fibre during the early stages of nerve–muscle interaction. The muscle activity generated by the developing n.m.j. blocks the synthesis of AChR molecules (Hall & Reiness, 1977) and this leads to their eventual loss from the extrajunctional region of the muscle fibre membrane, but not from the junctional region where their distribution has been stabilized by the nerve. For AChE, the effect of activity is opposite to that for AChR in that activity is required for the appearance of the enzyme molecules at the end-plate. As a result, the enzyme is absent at the immature n.m.j., where it might interfere with the action of the small amount of transmitter released, and only appears once transmission is adequate to generate appreciable muscle activity.

Additional evidence for a reciprocal effect of activity on the presence of AChR and AChE has been found in a number of other situations. For example, after complete muscle paralysis by denervation or chronic nerve conduction block, there is a marked increase in ACh sensitivity (Axelsson & Thesleff, 1959; Lømo & Rosenthal, 1972) and a marked decrease in junctional AChE activity (Guth, Albers & Brown, 1964; Butler, Drachman & Goldberg, 1978; Cangiano, Lømo, Lutzemberger & Sveen, 1980). Muscle disuse resulting from spinal cord section has similar effects but they are less pronounced, possibly because muscle inactivity in this case is less complete (Solandt & Magladery, 1942; Johns & Thesleff, 1961; Guth, Brown & Watson, 1967). Tenotomy on the other hand, which causes muscle atrophy but does not affect extrajunctional ACh sensitivity (Lømo & Rosenthal, 1972) is without effect on junctional AChE (Guth *et al.* 1967).

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EXPLANATION OF PLATES

PLATE 1

A, Methylene Blue stained fibular nerve fibres overlying ChE plaques 14 days after cutting soleus nerve. Note ectopic position of nerve fibres and underlying ChE plaques to the left of oblique band of original denervated endplates which are devoid of nerve fibres except for a narrow strand which may or may not contact original end-plates. *B* and *C*, axons apparently terminating at immature (left) and mature (right) ChE plaques 14 and 109 days respectively after cutting the soleus nerve. *D*, muscle fibre teased from the region of ectopic fibular nerve junction 21 days after cutting soleus nerve. ChE plaque (upper arrows) overlies dark spot (lower arrow) made by injection of dye at a site where a high sensitivity to ACh as well as focal m.e.p.p.s could be demonstrated. Horizontal bars represent 500 μm (*A*) and 50 μm (*B–D*).

PLATE 2

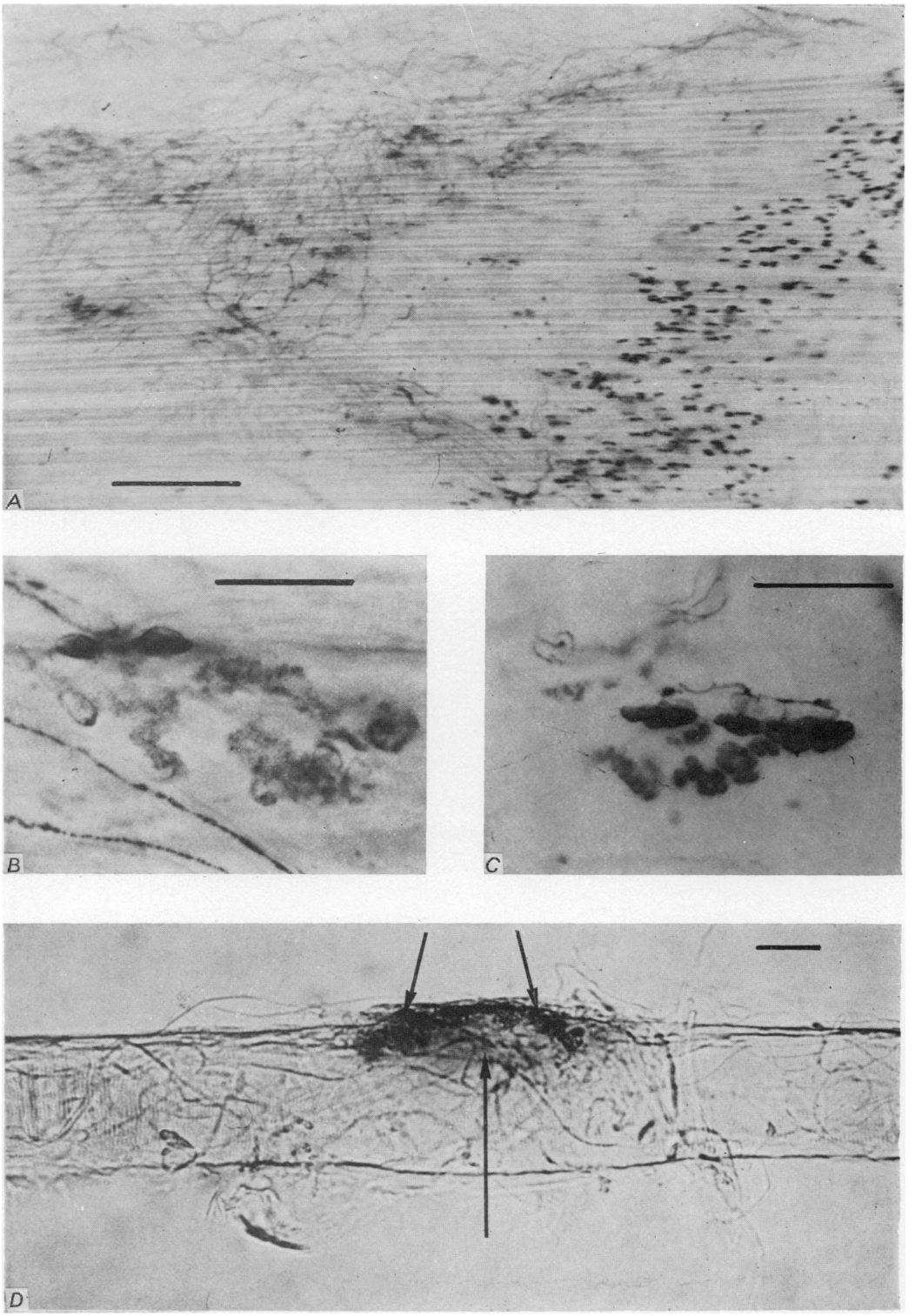
ChE plaques in the region of fibular nerve growth 13 (*A*), 22 (*B*) and 315 (*C*) days after cutting the soleus nerve. Pairs of smaller frames to the right show plaques from the same region (above) and from the original soleus end-plate region in the same muscle (below) at higher magnification.

PLATE 3

ChE plaques in the region of the degenerated fibular nerve after cutting first the soleus nerve and 2–7 days later the fibular nerve in stimulated muscles (*A*, *C* and *E*). Lack of ChE plaques in non-stimulated but otherwise similarly treated contralateral muscles (*B*, *D* and *F*). Plaques in the upper right corners of *A* and *D* are denervated, original, soleus end-plates. Single, large, dark mark in *A* is artifact after dye injection. Fibular nerve cut 2 (*A* and *B*), 3 (*C* and *D*) and 7 (*E* and *F*) days after cutting soleus nerve. Muscles in *A*, *C* and *E* stimulated for the last 8 (*A*), 7 (*C*) and 12 (*E*) days. Acute experiment 11 (*A–D*) and 19 (*E–F*) days after cutting soleus nerve.

PLATE 4

ChE plaques in the region of the fibular nerve growth in stimulated muscles with fibular nerve cut (*A–G*) and in a non-stimulated muscle with fibular nerve intact (*H*). Fibular nerve was cut 2 (*A*), 3 (*B* and *C*), 4 (*D*) and 7 (*E–G*) days after cutting soleus nerve. Muscles were stimulated for the last 8 (*A*), 7 (*B*), 4 (*C*), 31 (*D*), 12 (*E* and *F*) and 6 (*G*) days. Acute experiment was 11 (*A* and *B*), 8 (*C*), 35 (*D*), 19 (*E* and *F*), 22 (*G*) and 57 (*H*) days after cutting soleus nerve.



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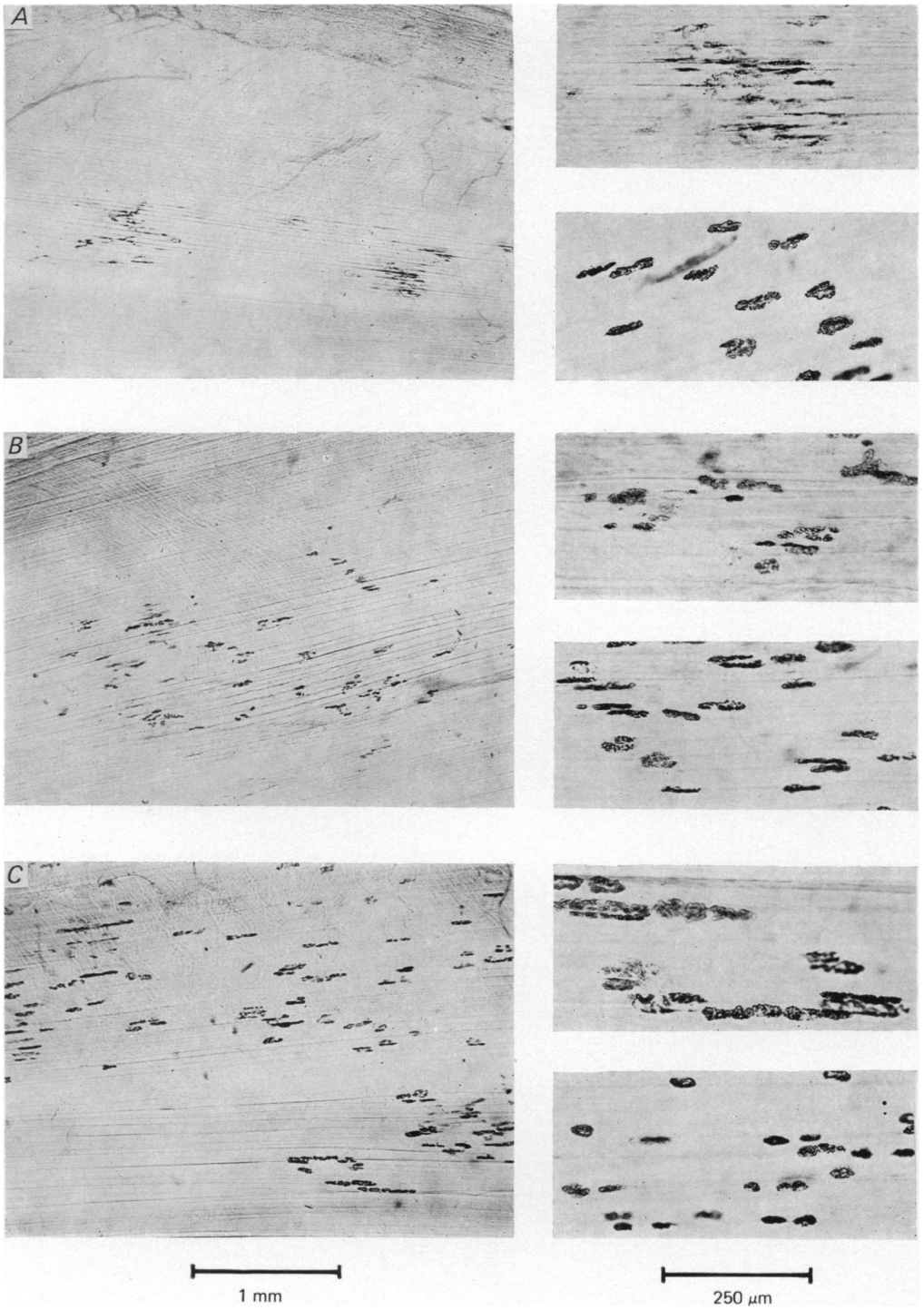

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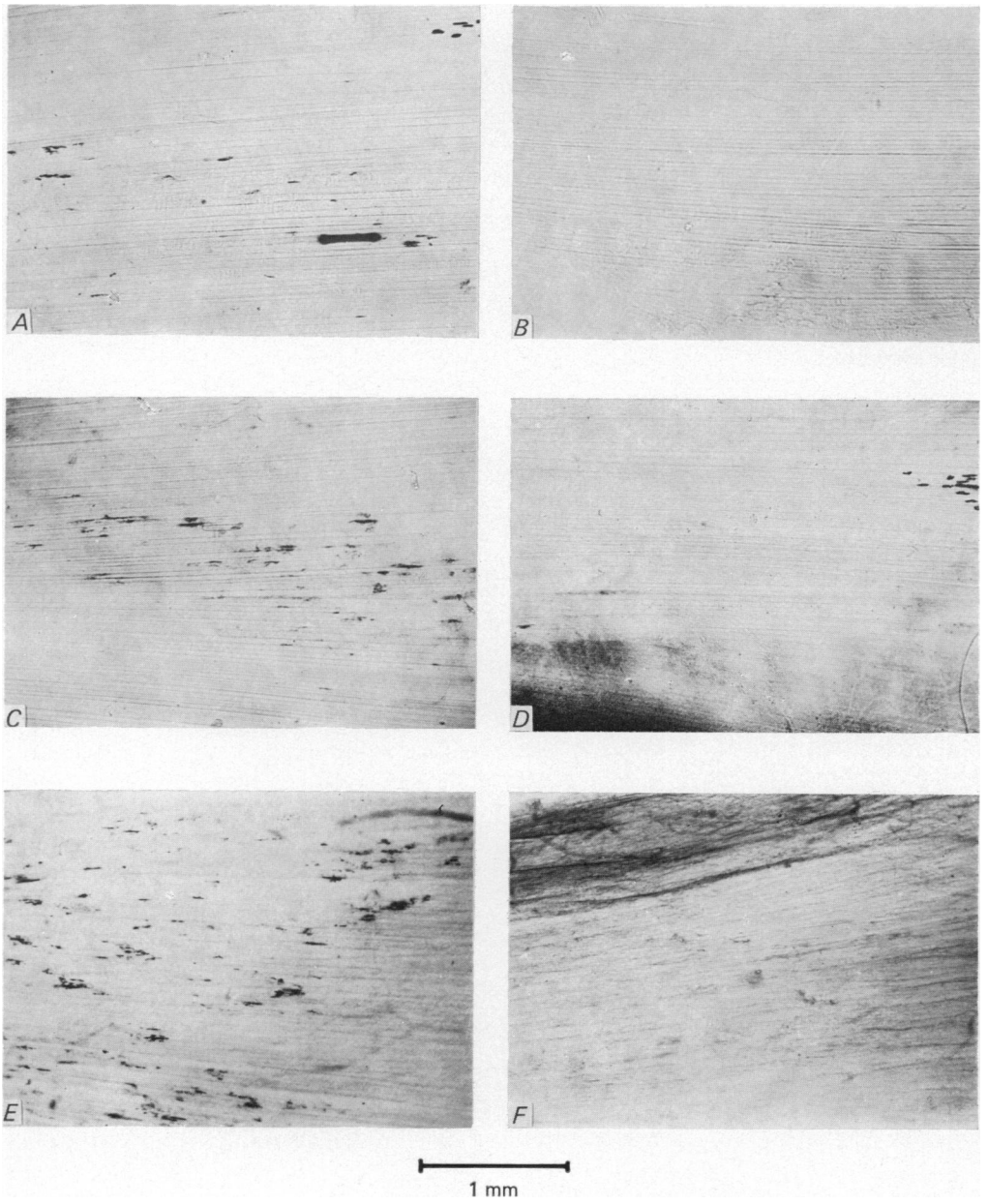
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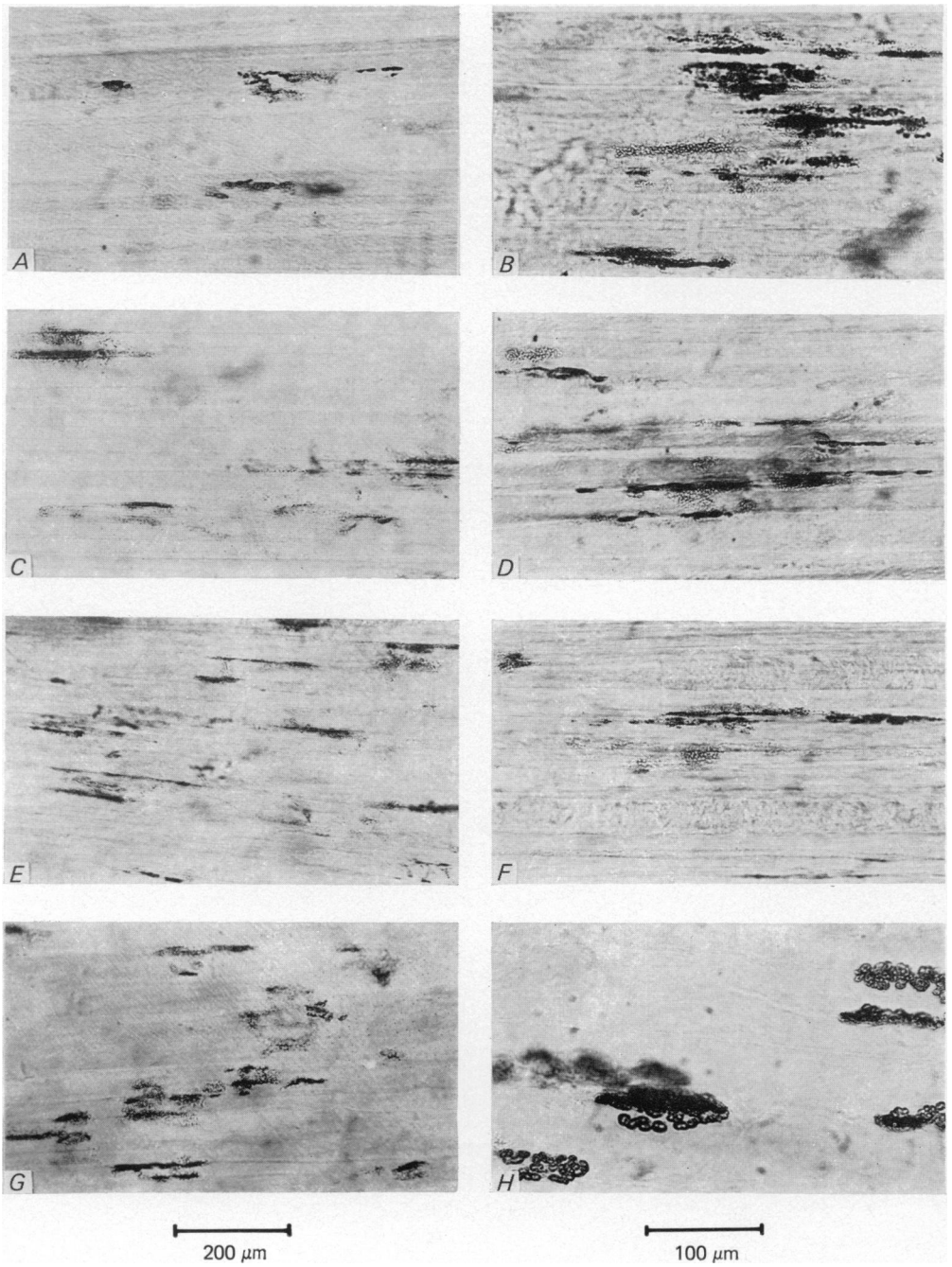

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