J. Anat. (1995) 186, pp. 143–151, with 20 figures Printed in Great Britain

Muscle differentiation and morphogenesis in the regenerating tail of lizards

LORENZO ALIBARDI

Department of Histology and Embryology, University of Sydney, Australia

(*Accepted 18 August 1994*)

ABSTRACT

The differentiation of muscles in the lizards *Anolis* and *Lampropholis* with tails that had regenerated for 21–50 d was investigated by light and electron microscope autoradiography using tritiated thymidine. At the apex of the regenerating tail, groups of 4–8 myoblasts of the promuscle aggregates fused to produce bundles of myotubes whose multiple labelled and unlabelled nuclei appeared to be distributed at random. The formation of the first myotubes and their growth is responsible for the formation of the myotome primordia and their separation from the intermuscular connective myosepta. More nuclei were added with the lengthening of the myotubes—up to 14–18 nuclei in the oldest proximal myotubes. At 4–5 h after injection labelled nuclei were found outside the myotubes while at 2–6 d after injection many labelled nuclei were observed in the myotubes, particularly near the two ends of the myotubular sarcoplasm contacting the myoseptum. This change from the initial distribution suggests that the growth of the myotubes takes place mostly at their terminals. There is an apparent correlation between the number of nuclei and the final length of the myotubes and myotome. The insertion of fibres with a similar number of nuclei and lengths into the pinnated connective myoseptum of the original musculature, the autotomy plane, probably determines the wave-like shape of the muscles within the regenerated myotomes.

Key words: Tail autotomy; myogenesis.

INTRODUCTION

Tail regeneration in lizards requires growth and development of much muscle tissue (Hughes & New, 1959; Shah & Chakko, 1968). Muscles initially appear as myoblast condensations just behind and lateral to the apical tail blastema (Brunetti, 1948; Simpson, 1965; Cox, 1969). Although original tail muscles form specific muscle groups, regeneration produces 10–16 symmetric muscle bundles of similar diameter and organised into myotomes (Quattrini, 1954; Shah & Chakko, 1968).

The method of tail loss affects the regeneration process. After experimental tail amputation, the damaged muscles are repaired by resident myosatellite cells (Kahn & Simpson, 1974; Simpson & Bayne, 1979). In contrast, natural or induced tail autoamputation (autotomy; Bellairs & Bryant, 1985) is followed by muscle regeneration mostly derived from the migration of myogenic cells from the connective intermuscular septum into the blastema (Brunetti, 1948; Quattrini, 1954; Simpson, 1965; Mufti & Iqbal, 1975; Simpson & Bayne, 1979). In autotomy, the tail splits along preformed breaking planes, thus avoiding injury to the musculature. There is no muscle contribution to the regenerative blastema by dedifferentiation (Kahn & Simpson, 1974).

Myogenesis has been extensively studied in vitro using lizard myoblasts (Simpson & Cox, 1972; Simpson & Bayne, 1979; Marusich & Simpson, 1983) but we lack cytological studies in vivo that describe the dynamic of the morphogenetic process that produces the orderly arranged myotomes in the regenerating tail.

The present autoradiographic study, the first in vivo on lizards using transmission electron microscopy, deals with the regeneration of muscles and the formation of the segmental myotomes during the first 50 d of tail regeneration, when 70–80% of the new tail length is restored.

Correspondence to Dr L. Alibardi, Neuromorphology Laboratory, Department of Biobehavioral Science, U-154, 3107 Horse Barn Hill Road, University of Connecticut, Storrs, CT 06269-4154, USA.

MATERIALS AND METHODS

This study was conducted on specimens of two common lizard species, the American Iguanid, *Anolis carolinensis* and the Australian Scincid, *Lampropholis delicata*. The animals were kept in cages at 22–33 °C, with a photoperiod of at least 12 h and were fed with a diet of insect larvae (maggots and mealworms).

The process of tail autotomy was induced, as normally occurs in the wild, by grabbing the tail of the animals. They easily release it, particularly at 27–33 °C (Bellairs & Bryant, 1985). The tail was left to regenerate for about 3 wk when the regenerating tail had reached 3–6 mm in length. At this stage 45 animals were injected with a single pulse of tritiated thymidine (6-^{3}H-thymidine, Amersham, specific activity 29 Ci/mM or 60–90 Ci/mM; dosage 10–15 μCi/g body weight). This dosage of tritiated thymidine had been successfully used in previous studies (Cox, 1969; Alibardi et al. 1992). The regenerated tails were collected at successive postinjection periods after killing the animals by decapitation.

Eight specimens of *Anolis* and 7 of *Lampropholis* were sampled 4–5 h after the injection, and tissues were obtained from the original stump of the tail as well as from the regenerating tail. Another 4 *Anolis* were sampled at 2 d postinjection, 4 *Anolis* at 4 d postinjection, 4 *Anolis* and 6 *Lampropholis* at 6 d postinjection, and 12 *Lampropholis* at 12 d postinjection.

The tissues were immediately fixed in cold Ringer solution at pH 7.4–7.6, containing 2.5% glutaraldehyde and 0.5% acrolein. Fixation was for 5–8 h at 0–4 °C. The tissues were then rinsed for 30–60 min in Ringer, postfixed in 2% OsO_4 for 2 h, dehydrated and embedded in Epon or Spurr resin.

After sectioning with the ultramicrotome, semithin (1–2 μm) and thin (50–80 nm) sections of selected areas of the samples were collected and dried over glass slides. Some slides had been previously coated with collodion in amyl acetate for the autoradiographic procedure using the dipping techniques (Weakley, 1981).

The slides were coated with Ilford Nuclear Emulsions (K5 or L4) in a dark room equipped with an Ilford filter n 904, and left to expose for 1–5 months.

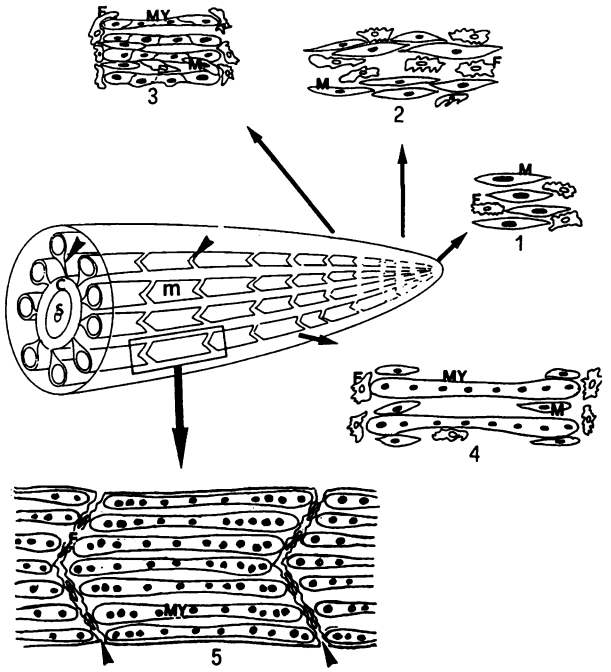

Fig. 1. Schematic drawing showing the progressive muscle regeneration to form myotomes (m) in the regenerating tail. C, cartilage. Arrowheads indicate the intermuscular connective and the myosepta. S, spinal cord. 1, promuscle aggregates; 2, piled myoblasts before fusion; 3, myotube formation; 4, myotube growth; 5, regenerated myotome. M, myoblasts; MY, myotubes; F, fibroblasts.

The slides were developed with D 19 Kodak and fixed with Agfa fixer. Semithin sections were weakly stained with 0.5% toluidine blue and observed by light microscopy for the visualisation of grains after autoradiography. For quantification, the number of labelled nuclei (from 300 unlabelled nuclei) was indicated as the 'percentage of labelling' (% L) and n the number of animals counted. A nucleus was considered labelled if more than 10 silver grains were present over it, against a background of 0–4 grains/25 μm^2.

The slides coated with a collodion membrane with the attached thin sections were used for the study by electron microscopy. The collodion membrane was stripped from the slide and left floating over double distilled water. The thin sections were then collected with grids and the collodion membrane was dissolved with amyl acetate and ethanol. The sections were lightly stained with uranyl acetate and lead citrate.

Fig. 2. *Lampropholis*. Two promuscle aggregates in the distal regenerating tail. Arrow indicates the developing intermuscular connective (myoseptum). Bar, 10 μm.

Fig. 3. *Lampropholis*. Dividing myoblasts (small arrow) in a promuscle aggregate in which no myotubes are visible. Bar, 15 μm.

Fig. 4. *Lampropholis*. Labelled myoblasts within a promuscle aggregate 5 h postinjection. Bar, 10 μm.

Fig. 5. *Anolis*. Electron micrograph of a labelled myoblast (arrowhead) in a promuscle aggregate 4 h postinjection. The cytoplasm of the myoblasts shows areas of initial myofibrillar aggregation (small arrows). Bar, 1 μm; inset, 0.25 μm.

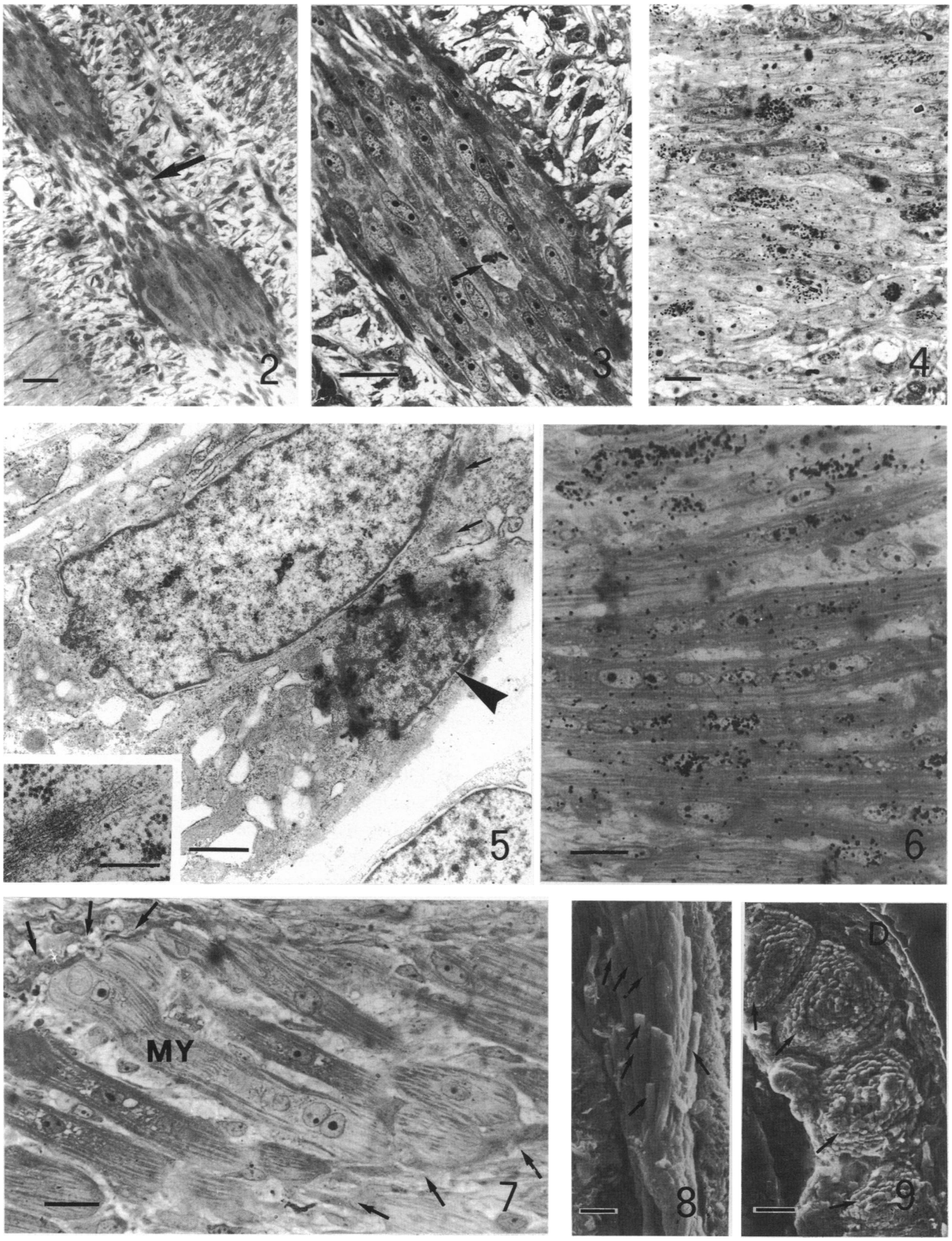

Fig. 6. *Lampropholis*. View of developing myotubes at 6 d after injection. Trace grains cover various myonuclei. Bar, 15 μm.

Fig. 7. *Lampropholis*. Developing myotubes (MY) between 2 consecutive myosepta (small arrows). Myofibrils and club-like ends are easily visible. Bar, 15 μm.

Fig. 8. *Lampropholis*. Scanning electron micrograph of myotubes (small arrows) in an early myotome. Bar, 10 μm.

Fig. 9. *Lampropholis*. Scanning electron micrograph of 4 myotube bundles (small arrows). D, dermis. Bar, 25 μm.

Sections were observed with either JEOL CX 100 or Hitachi 600 transmission electron microscopes (TEM).

Twenty samples of regenerating tails from animals of both species were fixed in 2.5% glutaraldehyde in Ringer (pH 7.4–7.6), postfixed in 1% OsO_4 in Ringer, dehydrated by ethanol, critical point dried and coated with palladium or gold for scanning electron microscope (SEM) observation (JEOL 35 and Isi-DS 130).

RESULTS

No labelled nuclei were seen either within or outside the muscle fibres of the normal tail muscles after injection (% L = 0; n = 5).

In the apical regions of the regenerating tail, within 0.5 mm from the tail apex, bipolar myoblasts bulged to form discrete fascicles of myogenic cells forming the 'promuscle aggregates' (Simpson, 1965; Simpson & Bayne, 1979) (Figs 1, 2). At 4–5 h after the injection of [^{3}H]thymidine many myoblasts within the promuscle aggregates were labelled (% L = 10.0–19.2% in *Anolis*, n = 5 and 9.8–15.1% in *Lampropholis*, n = 6) and many dividing cells were seen (Figs 3, 4). Labelled and mitotic myoblasts (5–6 × 30–50 μm) were distributed at random within the promuscle aggregates. In 3 wk regenerating tails (length 3–6 mm), 4–5 h after injection the % L in the regenerating muscles decreased, moving from the distal promuscle aggregates to the proximal regions where muscle myotomes were growing (1.8–5.3%, n = 6, in early myotubes of *Lampropholis* at 1.5–2.5 mm from the tail apex). The myoblasts of the promuscle aggregates had electron-pale cytoplasm and a well developed sarcoplasmic reticulum, whilst small myofibrils were sparsely aggregated (Fig. 5).

Myotubes resulted from the initial bulging of myoblasts (mixed with the fibroblasts of the blastema) and by their fusion into units that contained a fixed number of nuclei (initially 4–8; Figs 6, 7). Both thick (14–17 nm) and thin (6–11 nm) myofilaments were present inside the forming myotubes. These myofilaments were grouped into irregular bundles mixed with clear vesicles, tubules of smooth endoplasmic reticulum and nascent Z bands.

After 21–33 d of regeneration, the promuscle aggregates were seen at 150–300 μm from the tail apex. At this stage the first recognisable myotubes were observed at 1.5–2.5 mm from the tail apex, each myotube containing 4–8 nuclei (Fig. 7). These early myotubes stood out from the chaotic mass of fusiform myoblasts and fibroblasts of the more distal promuscle aggregates. The myotubes were organised in bundles, as observed in longitudinal and transverse sections (Figs 1, 7–9). Within the muscle bundle, the external myotubes often appeared less developed than the internal.

After about 25 d of regeneration, the oldest myotubes in the proximal regions contacting the original tail formed ordered and parallel cylinders with 8–12 nuclei (Figs 10, 11). These bundles followed the zigzag plane of the myoseptum of the original musculature. The club-like end of the proximal myotubes had more or less regularly spaced rows of narrow grooves, which were the invaginations of myoconnective junctions (Figs 11, 12).

Because the fusiform distal and proximal poles of the myotubes contacted the fibroblasts of the nascent intermuscular septa, the length of the myotubes determined the length of the developing myotome. Fibroblasts of the myosepta were smaller, contained parallel cisternae of endoplasmic reticulum and were darker than myoblasts.

Once myotubes had 8 nuclei, these were close to each other in a central row but with few myofibrils (Figs 6, 7). The length of these myotubes was 90–130 μm and their width 6–10 μm. At the 2 extremities of these myotubes the intermuscular connective tissue became better demarcated than at the preceding stages.

In the proximal regions of a 25–30 d regenerating tail, where the myotubes were already developed before injection, labelled cells were scarce 4–5 h postinjection (0.5–1.8%, n = 6). In contrast to the promuscle aggregates and young myotubes, in the proximal myotubes the pale labelled or dividing cells (myoblasts) were mostly near the 2 ends of the myotubes, facing the connective myosepta (Figs 13–15). Also most of dark labelled cells (fibroblasts) were adjacent to both ends of the myotubes.

Electron microscopy revealed that all dividing or labelled myoblasts 4–5 h postinjection were external to the myotubes, even if young myotubes were partly fused (Figs 16, 17). After 4–5 h or even days

Fig. 10. *Lampropholis*. Passage region between the muscle of the original tail (OM) and the first regenerate muscles (RM) that insert into the oblique myoseptum with club-like ends (arrowheads). Bar, 15 μm.

Fig. 11. *Anolis*. Scanning electron micrograph of a group of proximal myotubes with their club-like ends where myoconnective junctions appear as rows of grooves (small arrows). Some debris (arrowheads) remains from the intermuscular connective removed from the preparation. Bar, 5 μm.

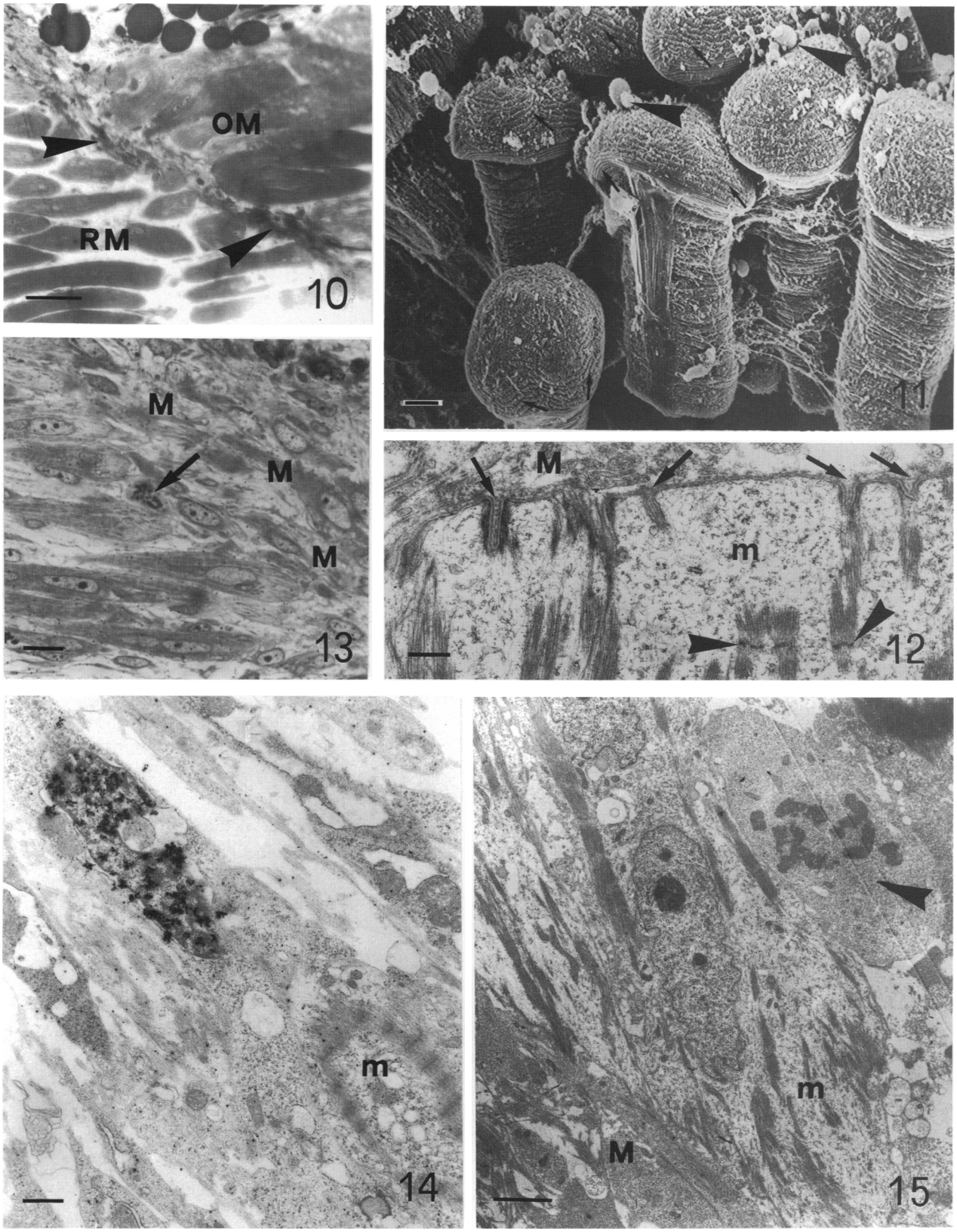

Fig. 12. *Lampropholis*. Transmission electron micrograph of a club-like muscular end of myotube (m). Arrows indicates the grooves of myoconnective junctions which face the connective myoseptum (M). Arrowheads indicate Z bands. Bar, 0.5 μm.

Fig. 13. *Lampropholis*. Dividing cell (arrow) near the myoseptum (M) at the club-like end of a myotube. Bar, 10 μm.

Fig. 14. *Anolis*. Electron micrograph of labelled muscle cell at the extremity of a myotube (m) 4 h postinjection. Bar, 1 μm.

Fig. 15. *Lampropholis*. Dividing myogenic cell (arrowhead) near the end of a myotube (m). M, connective myoseptum. Bar, 2.5 μm.

postinjection the old undamaged stump musculature completely lacked labelled myonuclei and labelled satellite cells.

After 2 d the first labelled nuclei (about one-third of all the labelled nuclei in *Anolis*) appeared in the regenerating myotubes (Fig. 18). In young myotubes, labelled nuclei were seen throughout the sarcoplasm 2, 4 and 6 d after injection (Fig. 6). In general the % L in the nuclei in myotubes increased 4, 6 and 12 d after injection as seen with the intracellularly labelled myonuclei and externally labelled fibroblasts, especially in the intermuscular septa. For instance 6 d after injection of 6 *Lampropholis*, the % L nuclei of myotubes (200–300 μm long) ranged from 3.6 to 7.2%, n = 6, and the % L in the connective tissue myoseptum ranged from 3.6 to 6.8%, n = 6. Therefore the % L increased both in the myotubes and in the connective myosepta.

Labelled or dividing darker cells (fibroblasts) were also present among the fibres. In the myosepta fibroblasts were orthogonally oriented with respect to the myotubes and were surrounded by numerous collagen fibrils (Figs 7, 13, 20).

Labelled myonuclei were evident 6–12 d after injection in the cytoplasm of myotubes, especially near their ends (Figs 19, 20).

While the labelled nuclei were randomly distributed in young myotubes (4–8 nuclei, length 100–140 μm; Fig. 6), in the more differentiated proximal myotubes with 10–18 myonuclei, at 6–12 d postinjection they were mostly in the cytoplasm near the ends facing the intermuscular septa (Fig. 20).

DISCUSSION

The present study helps to clarify the morphogenetic mechanism that leads to the organisation of the musculature within the regenerated tail of lizards.

Among previous studies (Brunetti, 1948; Simpson, 1965; Shah & Chakko, 1968; Cox, 1969; Mufti & Iqbal, 1963) that by Hughes & New (1959) on the gecko *Sphaerodactylus* put forward a hypothesis about muscle segmentation during tail regeneration; observing 'pyknotic nuclei' in the sites of myosepta, they suggested that 'cell degeneration is one factor by which the muscle fibres become separated from each other'. In the present study no pyknotic nuclei were seen whilst in the same areas darker fibroblasts built up the connective myotome. The study also stresses that the myotomal-like organisation of the regenerated muscles of lizards takes place because of the difference in fate of the myogenic and the connective cells within the promuscle aggregates (Brunetti, 1948).

Because cells of different potentiality are mixed together in the apical region of the regenerating tail (the 'blastema'; Bellairs & Bryant, 1985; Alibardi & Sala, 1988) it is also likely that the myoblasts and fibroblasts are initially mixed in the promuscle aggregates. Due to the different cell–cell affinities within the promuscle aggregates, the myoblasts recognise each other and start to fuse. The myogenic cells segregate from the fibroblastic cells when groups of 4–8 myoblasts fuse into myotubes whose lengths represent the initial lengths of the ordered myotomes (see Fig. 1). Therefore the young myotubes (90–130 μm in length) that will later incorporate new myoblasts for growing, arise from an initial fusion of a fixed number of myoblasts. It is likely that the few nonfusing cells (fibroblasts) simply remain among the myotubes, mostly near their ends forming the connective tissue myosepta.

This morphogenetic mechanism appears different from that for segmental muscles of other vertebrates. During the development of myotomes in the trunk of *Xenopus*, long mononucleated myoblasts first appear and then extend over the entire length of the somitic myotomes (Muntz, 1975). Later the mononucleated myofibres become multinucleated by the addition of new cells at their ends (Muntz, 1975) or even by amitosis (Boudjelida & Muntz, 1987). Similarly, in the zebra fish *Brachidanio* the initial formation of the myotomes starts with the formation of a single mononucleated myoblast and later, the fusion of 2–3 other myoblasts forms a multinuclear myotube with the extension of the whole myotome (Waterman, 1967). Therefore in both *Xenopus* and *Brachidanio* the myotome starts with a single myoblast that later becomes multinucleated. In contrast, both in the chick (Gonzalez-Santander et al. 1993) and in lizards, as presently observed, the myotome is multinucleated from the beginning.

Fig. 16. *Anolis*. Transverse section of myotubes (m) 4 h postinjection. The electron micrograph shows 2 labelled myoblasts containing myofilaments (MY). Bar, 0.5 μm. The inset (bar, 10 μm) shows that all labelled cells are outside the myotubes (m).

Fig. 17. *Anolis*. Higher magnification of 2 myoblasts at 4 h postinjection, showing tracts of the plasma membrane (small arrows) still dividing the 2 cells. Bar, 5 μm.

Fig. 18. *Anolis*. Section of labelled extrasarcoplasmic (small arrows) and intrasarcoplasmic (large arrows) nuclei 2 d after injection. Bar, 10 μm.

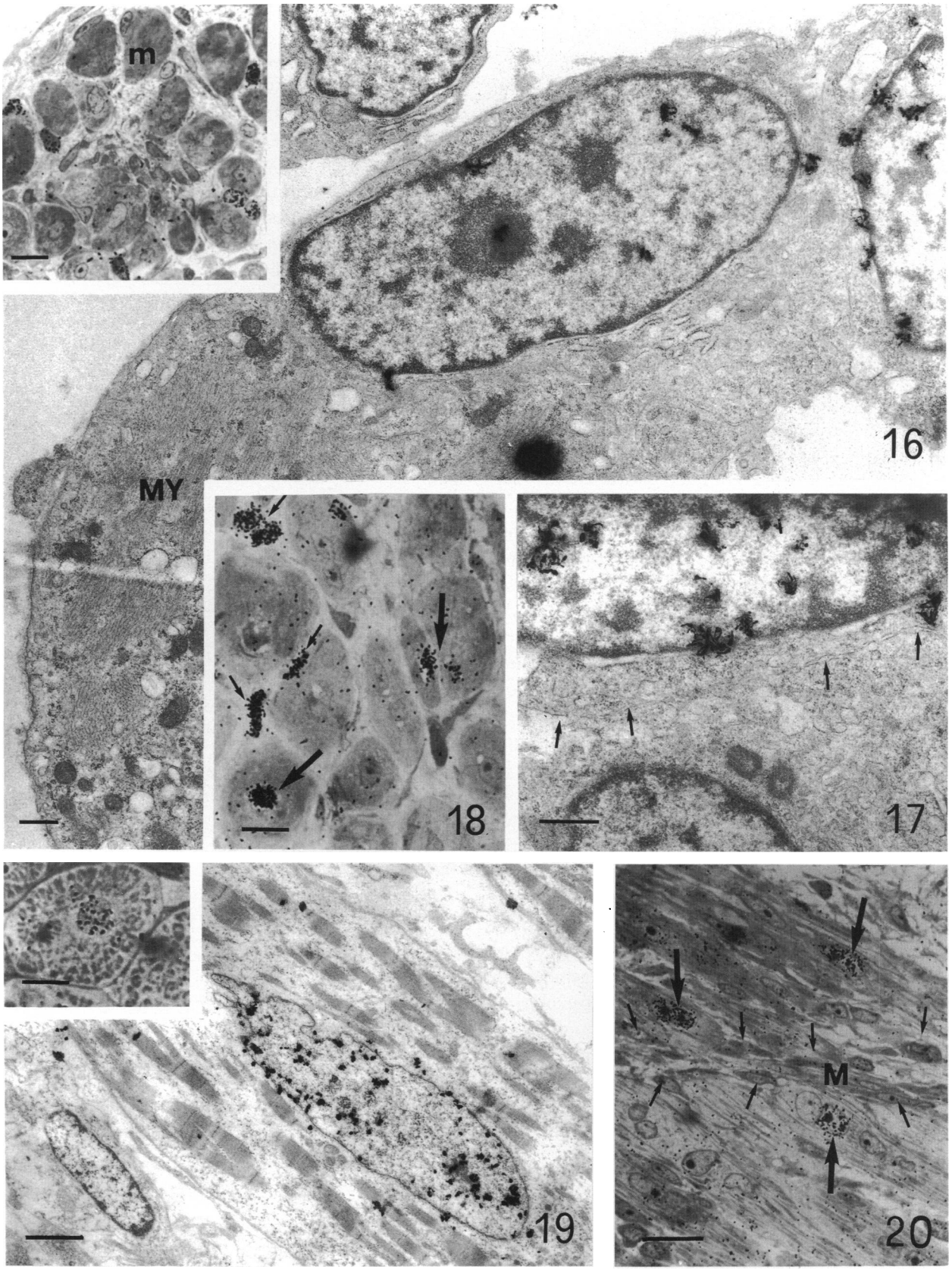

Fig. 19. *Lampropholis*. Electron micrograph of labelled nucleus 6 d after injection. Bar, 2 μm. The inset (bar, 5 μm) shows a cross section of a labelled central nucleus among the myofibrils in a myotube 6 d after injection.

Fig. 20. *Lampropholis*. Labelled myonuclei (large arrows) 12 d after injection in the extremities of proximal myotubes contacting the myoseptum (M, outlined by small arrows). Bar, 15 μm.

Despite these differences, the formation of myotubes between the connective terminals (myosepta) is always essential for the appearance of the ordered proximodistal myotome sequence. In order to maintain this segmental organisation during tail regeneration or in the embryo, most of the new myoblasts are added near the ends of myotubes resulting in long multinucleated fibres (see Fig. 1).

This mode of addition of new myogenic cells and myofibres during muscle growth has been reported for other segmental (Goldspink, 1972, 1980; Muntz, 1975) or nonsegmental muscles (Kitiyakara & Angevine, 1963). The number of nuclei in the muscle fibres increases with ageing (Goldspink, 1972; Enesco & Puddy, 1974). This has also been shown during tail regeneration in the skink *Lygosoma* and in the gecko *Sphaerodactylus* in which the number of nuclei within the myotube increases from 2–3 (Simpson, 1965) to 24 in the fully mature regenerate (Hughes & New, 1959).

In the present study no more than 18 nuclei were found in myotubes after 50 d of tail regeneration, when the new tail was not yet completely regrown (myotomes up to 350 μm long). Interestingly, however, in all the myotubes the number of nuclei increased similarly with age (4–8 in the youngest myotubes to 18 in the oldest, proximal ones). Various studies have convincingly established a direct correlation between the number of nuclei and the length and girth of myofibres, numbers of sarcomeres and myofibrils (Enesco & Puddy, 1964; Goldspink, 1972, 1980).

The addition of new nuclei to myotubes is correlated with the synthesis of new mRNA and contractile proteins that organise other sarcomeres inside the fibres with the final result of an increment in size and girth. Thus if the number of nuclei in each myotube of a lizard tail is generally constant, the length of the myotubes may also be fixed, and the attachment of myotubes along the zigzag arranged myoseptum of the old musculature could result in the zigzag myotome arrangement of the regenerated musculature (Fig. 1).

The present in vivo study has shown that the complete penetration into the myotubes of new cells takes about 2 d but that the fusion process in young myotubes is already visible 4–5 h after injection of [^{3}H]thymidine. This result is in agreement with the in vitro observation that myogenic cells fuse into myotubes 12–72 h after being transferred from the 'not fusion' into the 'fusion medium' (Cox, 1968; Simpson & Cox, 1972).

The timing of fusion in these heterotherm vertebrates is similar to that for the myotubes of the rat (Moss & Leblond, 1970, 1971).

The behaviour of the myoblasts in the promuscle aggregates initially resembles the clustering of myoblasts in vitro where they form colonies (Simpson & Cox, 1972; Chlebowski et al. 1973). Aggregates of rounded cells in the centre of the colonies are myosin-positive and it is in this area that the first myotubes with 2–3 nuclei appear (Chlebowski et al. 1973; Bayne & Simpson, 1977). In vitro, the number of nuclei in the myotubes usually ranged from 20 to 30, but exceeded 100 nuclei in the irregular network of myotubes after days of maintenance in the artificial medium (Cox, 1968). In vitro myogenesis (where no fibroblasts are present) never produces myotubes ordered into myotomes, suggesting that in vivo fibroblasts participate in the morphogenetic process of segmentation.

ACKNOWLEDGEMENTS

This research was supported by a scholarship from the Italian CNR, the University of Illinois at Chicago, USA and the Australian DEET in exchange with the Italian MAE and MURST. I am also grateful to the Department of Histology and Embryology and the Electron Microscopy Unit of the University of Sydney, Australia for support, to Dr R. D'Orazio and Mr C. Friso for the drawings and Dr A. Pearson (UWI, Jamaica) and Mr K. Bittman and Mr S. H. McKamey (UC, Storrs, USA) for reading the manuscript.

REFERENCES

ALIBARDI L, SALA M (1988) Fine structure of the blastema in the regenerating tail of the lizard *Podarcis sicula*. *Bollettino di Zoologia* **55**, 307–313.

ALIBARDI L, GIBBONS J, SIMPSON SB JR (1992) Fine structure of cells in the young regenerating spinal cord of the lizard *Anolis carolinensis* after H^3-thymidine injection. *Biological Structure and Morphogenesis* **4**, 45–52.

BELLAIRS A D'A, BRYANT SV (1985) Autotomy and regeneration in reptiles. In *Biology of Reptilia* (ed. C. Gans), pp. 303–410. New York: Wiley & Sons.

BOUDJELA H, MUNTZ L (1987) Multinucleation during myogenesis of the myotome of *Xenopus laevis*: a qualitative study. *Development* **101**, 583–590.

BRUNETTI PM (1948) Istogenesi e morfogenesi dei muscoli nella rigenerazione della coda di lucertola in seguito a mutilazione spontanea. *Monitore Zoologico Italiano* **57** (suppl.), 244–248.

CHLEBOWSKI JS, PRZYBYLSKI RJ, COX PG (1973) Ultrastructural studies of lizard (*Anolis carolinensis*) myogenesis in vitro. *Developmental Biology* **33**, 80–99.

COX PG (1969) Some aspects of tail regeneration in the lizard *Anolis carolinensis*. I. A description based on histology and autoradiography. *Journal of Experimental Zoology* **171**, 127–150.

ENESCO M, PUDDI D (1964) Increase in the number of nuclei and

weight in skeletal muscles of rats of various ages. *American Journal of Anatomy* **114**, 235–244.

GOLDSPINK G (1972) Postembryonic growth and differentiation of striated muscles. In *The Structure and Function of the Muscles* (ed. G. H. Bourne), 2nd edn, vol. I, part I, pp. 179–236. New York, London: Academic Press.

GOLDSPINK G (1980) Growth of muscles. In *Development of Specialization of Skeletal Muscles* (ed. D. F. Goldspink), pp. 19–35. Cambridge: Cambridge University Press.

GONZALES-SANTANDER R, TOLEDO LOBOS MV, MARTINEZ ALONSO FJ, MARTINEZ CUADRADO G (1993) Fusion mechanism of the myoblasts in the myotome of the chick embryo. *Histology and Histopathology* **8**, 471–490.

HUGHES A, NEW D (1959) Tail regeneration in the geckonid lizard *Sphaerodactylus*. *Journal of Embryology and Experimental Morphology* **7**, 281–302.

KAHN EB, SIMPSON SB JR (1974) Satellite cells in mature, uninjured skeletal muscle of the lizard tail. *Developmental Biology* **37**, 219–223.

KITIYAKARA A, ANGELINE DM (1963) A study of the pattern of postembryonic growth of *M. gracilis* in mice. *Developmental Biology* **8**, 322–340.

MARUSICH MF, SIMPSON SB JR (1983) Changes in cell surface antigens during in vitro lizard myogenesis. *Developmental Biology* **97**, 313–328.

MOSS FP, LEBLOND CP (1970). Nature of dividing nuclei in skeletal muscles of growing rats. *Journal of Cell Biology* **44**, 459–462.

MOSS FP, LEBLOND CP (1971) Satellite cells as the source of nuclei in muscles of growing rats. *Anatomical Record* **170**, 421–436.

MUFTI SA, IQBAL J (1975) Tail regeneration after amputation in *Hemidactylus flaviviridis*. *Pakistan Journal of Zoology* **7**, 15–28.

MUNTZ L (1975). Myogenesis in the trunk and leg during development of the tadpole of *Xenopus laevis* (Daudin 1802). *Journal of Embryology and Experimental Morphology* **33**, 757–774.

QUATTRINI D (1954) Piano di autotomia e rigenerazione della coda dei sauri. *Archivio Italiano di Anatomia ed Embriologia* **59**, 225–282.

SHAH RV, CHAKKO TV (1968) Histological observations on the normal and regenerating tail of the house lizard, *Hemidactylus flaviviridis*. *Journal of Animal Morphology and Physiology* **15**, 26–39.

SIMPSON SB JR (1965) Regeneration of the lizard tail. In *Regeneration in Animals and Related Problems* (ed. V. Kiortis & H. A. L. Trampbush), pp. 431–443. Amsterdam: North-Holland.

SIMPSON SB JR, BAYNE EK (1979) In vivo and in vitro studies of regenerating muscle in the lizard *Anolis*. In *Muscle Regeneration* (ed. A. Mauro), pp. 189–199. New York: Raven Press.

WATERMAN RE (1969) Development of the lateral musculature in the teleost, *Brachydanio rerio*: a fine structural study. *American Journal of Anatomy* **125**, 457–494.

WEAKLEY BS (1981) *A Beginner's Handbook in Biological Transmission Electron Microscopy*, 2nd edn. Edinburgh: Churchill Livingstone.