Tail Regeneration in the Geckonid Lizard, *Sphaerodactylus*

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WITH ONE PLATE

INTRODUCTION

IN the *Histoire de l'Academie Royale des Sciences* for 1686 (vol. ii, p. 7, published 1733) under the heading of 'Diverses Observations Anatomiques' is an account of the researches of three savants, Thevenot, Du Verney, and Perrault, on regeneration of the tail in the European Green Lizard. Perrault found that in the new tail, instead of vertebrae, 'il n'y avoit qu'un cartilage de la grosseur d'une grosse épingle'. It was not until nearly a century and a half later that this basic observation was extended when Dugès (1829) described this element of the regenerate as 'un cartilage d'une seule pièce, blanc, flexible, fistuleux, et rempli d'un prolongement du cordon ou faisceau nerveux rachidien'. Not before the main framework of microscopical anatomy had been built in succeeding generations was the exact nature of this nervous component determined. Gegenbaur (1862) realized that although it was a continuation of the spinal cord, the full medullary structure was lacking. In the next year Heinrich Müller (1863) described how it was made up of an 'Epithel umgebener Kanal' covered by a layer of longitudinal nerve fibres, between which and the inner epithelium were cellular elements, 'nicht mit Sicherheit als Nervenzellen anzusprechen'. On the other hand, Fraisse (1885) illustrated neuroblasts within the neural tube in tail regenerates of *Lacerta ocellata* and of the slow worm. Since the work of Hooker (1912), however, it has generally been agreed that this structure is a continuation of the ependymal epithelium, infiltrated with some glial elements, and covered with fibres from the white matter.

Fraisse's treatise was a general monograph on regeneration, mainly in Amphibia and Reptiles. In the lizard tail he described and illustrated the arrangement of the muscle bundles and the development of the scales. He saw that the

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regenerate is innervated from spinal nerves near the junction of new and old tissue. His longitudinal section through a regenerating tail of *L. agilis* shows branches of the last pair of spinal nerves entering the new growth and running between the cartilaginous tube and the annular zone of muscle bundles. This picture of the nervous system of the regenerate was not extended for nearly thirty years. In 1912 Hooker described the descent of branches of two pairs of spinal nerves into the new tail, while Terni (1920) showed that in *Gongylus ocellatus* three pairs of spinal nerves send branches into the regenerate. In a further paper, Terni (1922) described how fibres of the para-aortic sympathetic trunks grow into the new tissue, but are not accompanied by autonomic neurones.

Comparatively little has been written on the microscopical features of the early stages of regeneration and the histogenesis of the various tissues. Fraisse (1885) considered that each was derived from cells which originated from the corresponding tissue in the stump. For instance, he believed that special cells, the 'Muskelkörperchen', migrated out from the original musculature to form the new muscle fibres. White (1915, 1925), however, describes how beneath the epidermis which grows over the broken surface of the tail there accumulates a 'mass of undifferentiated cells mixed with pigment cells' which constitutes a blastema, within which the new tissues differentiate. The views of Quattrini (1954) on this question recall those of Fraisse. He considers that cells emerge from the intermuscular septa of the stump and form the muscles of the regenerate, and that the periosteum of the broken vertebra contributes formative cells for the new skeletal tube.

The present paper is concerned with tail regeneration in the small West Indian Geckonid *Sphaerodactylus*, chiefly with regard to the early phases of the process. Some points were compared with similar events in *Aristelliger*, the 'Croaking Lizard'. Some preliminary experiments on the effect of the treatment of the regenerate with nitrogen mustard are also described. The purpose of these experiments was to compare the effect of this substance on the regenerating tail with its action on the limb-buds of *Xenopus* tadpoles (Hughes & Tschumi, 1958), in the first instance with regard to the nervous system of the regenerate.

MATERIAL AND METHODS

Individuals of the smaller species of *Sphaerodactylus* (*goniorhynchus* or *argus*) were collected at various places in Jamaica, mostly within a few miles of the University College of the West Indies, at Mona, St. Andrew. The majority were found beneath the trash under coco-nut palms. The length of the animals used varied between 30 and 40 mm.

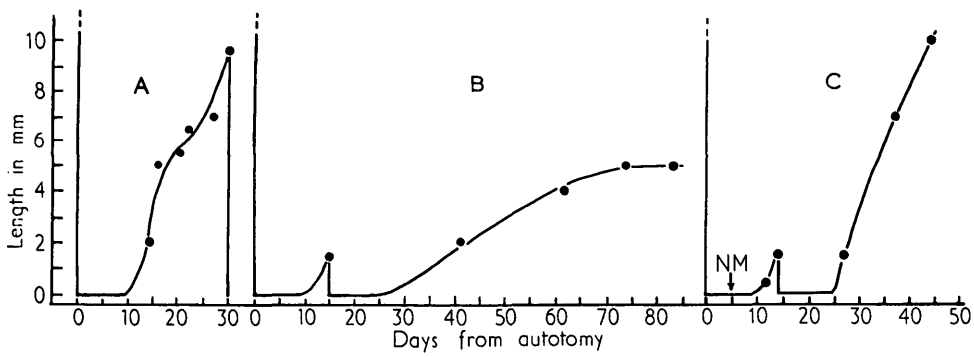
The animals were kept in the laboratory in small museum jars covered by wire gauze and kept on a diet of larvae and adults of local species of *Drosophila*. *Sphaerodactylus* readily sheds portions of tail about 10 mm. in length in response

to gentle traction on the extremity; approximately this length of tail was removed in all the experiments here described. Seven to ten mm. of tail remained between cloaca and the plane of autotomy. Measurements were made of the growth of the regenerate, samples of which were again 'plucked' at various stages. The animals were kept at room temperature varying between 25° and 30° C. The material was fixed in Bouin's fluid at full strength for 24 hours, followed by dehydration and infiltration with wax at 60° C. Sections were cut in an air-conditioned room at 21° C. The sections were stained either with Delafield's haematoxylin and eosin, or were silvered by either Holmes's or Bodian's methods. In treating the regenerating tails with nitrogen mustard it was found possible to immobilize the animal by wrapping cotton wool around the trunk and limbs. Solutions of methyl-bis (βchlorethyl) amine hydrochloride at various concentrations were applied for periods of up to 5 minutes. The treated regenerates were plucked after various intervals and cut into serial sections, which were impregnated with silver.

RESULTS

The rate of regeneration in Sphaerodactylus *and other lizards*

There are three phases in the growth of the regenerating tail. First there is an interval during which the blastema is being formed and in which little or no growth is visible externally. Then follows a period of rapid elongation, the rate



TEXT-FIG. 1. Length of individual tail regenerates in *Sphaerodactylus*. Vertical lines indicate autotomies, at the first of which (0 in abscissa) 10–12 mm. of original tail was shed. A, growth of first regenerate. B, growth of second and third regenerates. C, growth of second and third regenerates as in B except for treatment with 1 : 500 nitrogen mustard for 10 minutes (NM) during second period.

of which finally decreases as the regenerate approaches its full length. The first two of these phases in the normal growth of a first regenerate in *Sphaerodactylus* are shown in Text-fig. 1A. Repeated plucking of the regenerate results in a much slower rate of growth (Text-fig. 1B).

In the experiments with nitrogen mustard, the regenerating surface was

treated with the substance at various times after plucking of the original tail. When the nitrogen mustard was applied during the first phase of regeneration, growth was either halted or much slowed down. If, however, after such a period of dormancy the last segment of the tail was itself plucked, then normal regeneration ensued. In the two experiments of this kind the rate of growth was the highest ever observed (Text-fig. 1c).

The maximum rate of growth of a normal first regenerate in *Sphaerodactylus* was about 0·47 mm. per day. In *Anolis carolinensis*, Kamrin & Singer (1955) measured rates of growth during the second period of 0·4 mm. per day. Gosse (1851) observed in *Thecodactylus* (*Aristelliger*) a maximum of about 0·7 mm. per day; in *Hemidactylus flaviviridis* a rate of 1·12 mm. per day can be deduced from Woodland's (1920) drawings. In *Lacerta* there are records of still more rapid elongation, namely 1·36 mm. per day (Hooker, 1912) and 2·0 mm. per day (Holfert, 1869, quoted by Fraisse, 1885, p. 34). The seventeenth-century observations of Thevenot and Perrault (see Introduction) also suggest figures of this order for European lizards.

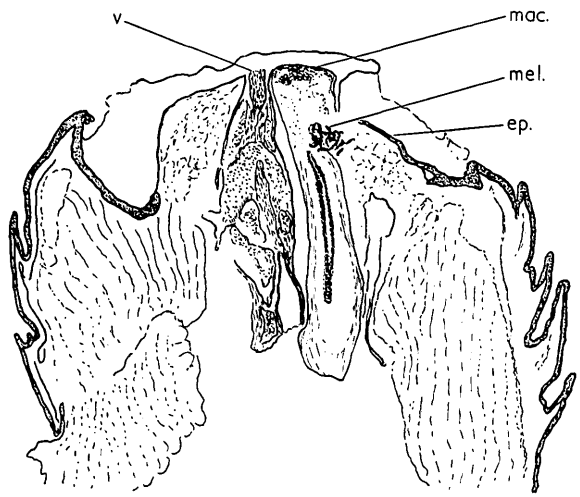
Histological observations

The microscopical structure of the regenerating tail during the earliest phases of the process will be described from a series of specimens taken at various times and cut into longitudinal sections.

Two days after the tail has been shed the soft tissues of the healing stump are covered by a crust of necrotic cellular material through which the broken surface of the autotomized vertebra projects. Beneath this covering, cellular reactions to the injury are already apparent. At the margins of the broken surface blood-vessels are dilated, and from them lymphocytic elements are spreading over the inner surface of the protective crust. Within the neural canal of the broken vertebra, the spinal cord has withdrawn some 60 or 70 μ from the surface. The open end of the central canal has begun to dilate, and beyond it cells from various sources are accumulating. Some are microglial elements of the cord; others are lymphocytes from dilated vessels of the meninges. From these membranes come also melanocytes, which are beginning to collect in a compact group on the dorsal surface of the cord. There are occasional mitotic figures among these cells within the neural canal.

A day later (Text-fig. 2), the cord has retreated still farther within the neural canal, the open end of which is occupied by a mass of cellular material, now much denser than on the previous day. There is a tight knot of melanocytes on the dorsal surface of the cord at its hinder limit. Beneath the external crust, a dense layer of macrophages is loaded with cellular debris. Round the edge of the wound, the epidermis has begun to proliferate, and in a longitudinal section a tongue of ectodermal cells is seen on each side turning sharply inwards and growing over the outer surface of the intact tail musculature and perivertebral fat and beneath the necrotic tissue to the outside. The inner edge of this circle of

ectoderm advancing centripetally meets in the centre on the 4th day, when a thin layer of epithelium covers the neural canal and the plug of new tissue within it. On the 5th day, this new epidermis becomes everywhere much thicker, and within it the distinction between an inner germinative layer and an outer *stratum corneum* becomes clear. The outer edge of the new epidermis joins the old in an even thicker rim which gradually extends more deeply into the zone of regeneration. The new epidermis remains much thicker than the old until the time when it undergoes differentiation into scales.

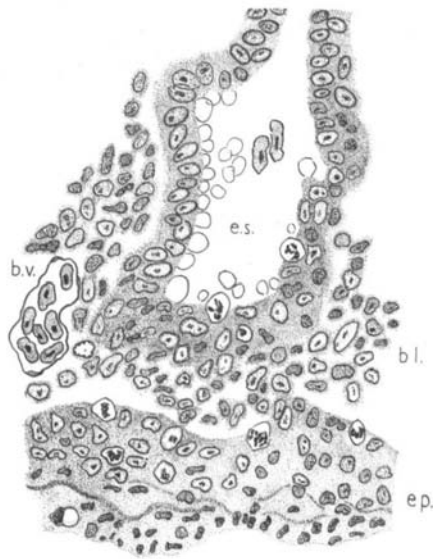


TEXT-FIG. 2. Vertical longitudinal section through stump of tail of *Sphaerodactylus*, 3 days from autotomy. *ep.*, regenerating epidermis; *mac.*, layer of macrophages over open end of neural canal; *mel.*, knot of melanocytes at broken face of spinal cord; *v.*, autotomized vertebra. × 32.

However, the most striking new development at this stage is the outgrowth of the ependymal epithelium into a flask-shaped sac, comparable to that which has been observed in the regeneration of the cord in the tail of a Urodele (Duesberg, 1925). In *Sphaerodactylus* the sac extends axially outwards towards the germinative layer of the epidermis on the 4th day. At the base its wall consists of a single layer of columnar epithelium. Towards the tip it is surrounded by a dense crowd of new cells between the inner members of which and the ependymal epithelium no clear distinction can be drawn. Within the wall of the ependymal sac mitotic figures are far commoner than at any other site. The whole appearance suggests that this sac is an active centre of proliferation, and that at this stage it is the main site of the production of new cells for the whole zone of regeneration (Text-fig. 3). Already at 5 days the cells constitute a dense blastema in the form of an inverted bowl, the edge of which approaches the outer zone of the tail musculature. The individual cells of the blastema are remarkable for their variety in appearance in that their nuclei differ both in size and in the density of their

contents. Over the inner surface of the blastema, melanocytes are migrating outwards from the tight group of earlier stages on the dorsal surface of the nerve-cord.

From the inner surface of the cells of the ependymal sac at 5 days, vesicles bulge into the lumen of the sac (Text-fig. 3). These are apparently a sign that cerebro-spinal fluid is being secreted by the epithelium. Occasionally blood-corpuscles are seen within the lumen.



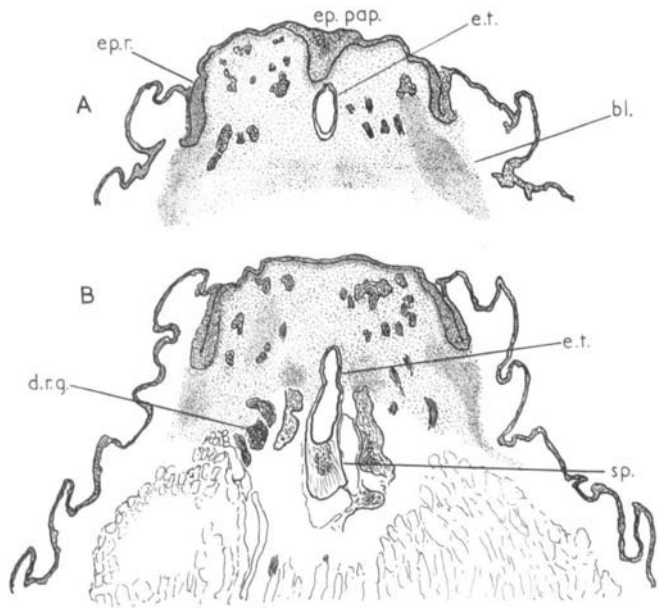
TEXT-FIG. 3. Part of median longitudinal section through regenerating tail area of *Sphaerodactylus* 5 days after autotomy showing ependymal sac (*e.s.*) close to regenerated epidermis (*ep.*), and the origin of cells of the blastema (*bl.*); *b.v.*, blood-vessel. × 333.

On the 6th day the influence of the ependymal sac, now an elongated tube, is apparent in another respect. At the centre of the new epidermis is an inward prolongation in the form of a papilla, pointing towards the tip of the sac (Text-fig. 4). The two structures are not in actual contact, but are separated by a few cells of the surrounding blastema. It may be that the ependymal sac at this stage stimulates a zone of the epidermis lying nearest to it to a maximal rate of proliferation. Melanocytes have by now entered the new epidermis and are particularly numerous within the central papilla.

The regenerative blastema is now nearly 0·5 mm. thick at its axis. Inwards it presents a deepening concavity. The outer surface is limited by the deep rim of epidermis at the junction of new and old ectoderm. Below this rim is a specially dense ring of blastema, the *Anlage* of the musculature of the regenerate. Within the remainder of the blastema, two sets of structures are apparent: a plexus of irregularly dilated blood-vessels, especially concentrated near the epidermis, and the growing longitudinal branches of the spinal nerves, which will be described in detail below.

Within the next 5 days signs of cellular differentiation within the blastema become apparent as the regenerate begins to grow in length. The dense ring of blastema from which the musculature is formed becomes a hollow cone, and its constituent cells differentiate into elongated myoblasts. They become sharply distinct from the cells of the surrounding blastema. Mitoses are more common among the myoblasts than in the blastema generally; the axes of the mitotic figures of the myoblasts coincide with the general direction of growth (Plate, fig. B). They increase mainly by accretion; at the tip are the youngest myoblasts, hardly distinguishable from the cells of the surrounding blastema. Differentia-

tion is most advanced at the base of the cone, and here the future segmentation of the muscle bands is already foreshadowed. These bands, when seen in transverse section, vary in number between ten and thirteen. In *Sphaerodactylus*, twelve is the usual number. At 11 days (Text-fig. 5) a wide space still lies between the myoblastic cone and the original musculature; it is occupied by a loose mesenchyme which is heavily infiltrated with lymphocytes, as is the whole zone

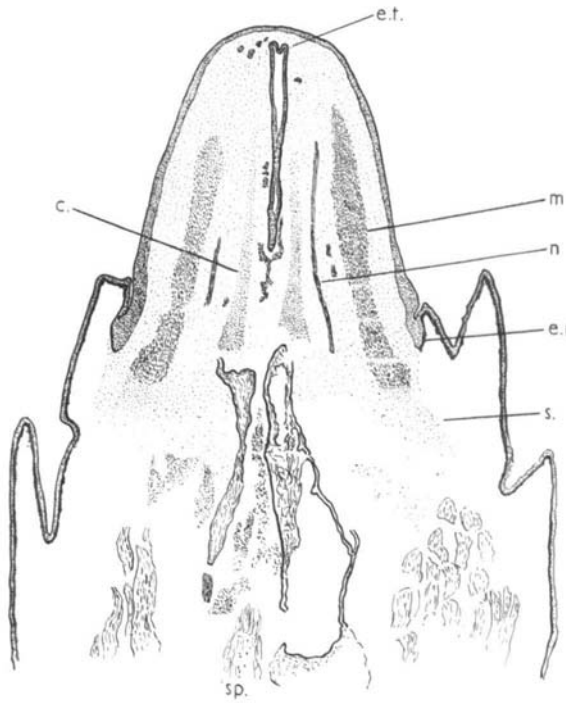


TEXT-FIG. 4. Part of horizontal section through regenerating tail area of *Sphaerodactylus* 6 days after autotomy. (A) Near tip of ependymal tube, and (B) at its base. *bl.*, blastema, containing nerves and blood-vessels and bounded by denser myogenic ring; *d.r.g.*, dorsal root ganglion; *e.t.*, ependymal tube; *ep. pap.*, epidermal papilla; *ep. r.*, epidermal rim; *sp.*, spinal cord. ×42.

of junction between new tissue and old. The myogenic region of the regenerate is derived from blastemal cells, and is independent of the original musculature of the tail. The latter must however exert an influence on the division of the cone of myoblasts into muscle bands, for each new band becomes a continuation of an old one.

The ependymal sac, extended at 11 days into a tube nearly a millimetre in length, is surrounded by a further condensation of mesenchyme, consisting also of elongated cells; here, however, their axes are all in radial directions. These cells are the chondroblasts of the cartilaginous tube. By this stage there has differentiated a coarse plexus of blood-vessels surrounding the ependymal sac. The epidermis of the regenerate has the form of a thick hollow cone, the base of which is the peripheral rim of earlier stages. In this region, the outer surfaces of new and old epithelium join at the base of a deep annular fissure. Within the

stratum germinativum mitotic figures are common everywhere, but they are especially prominent within the massive basal rim. Here melanoblasts are most frequent, both in the future dermal layer of the regenerate and also within the epidermis, into which pigment cells can be seen to migrate.

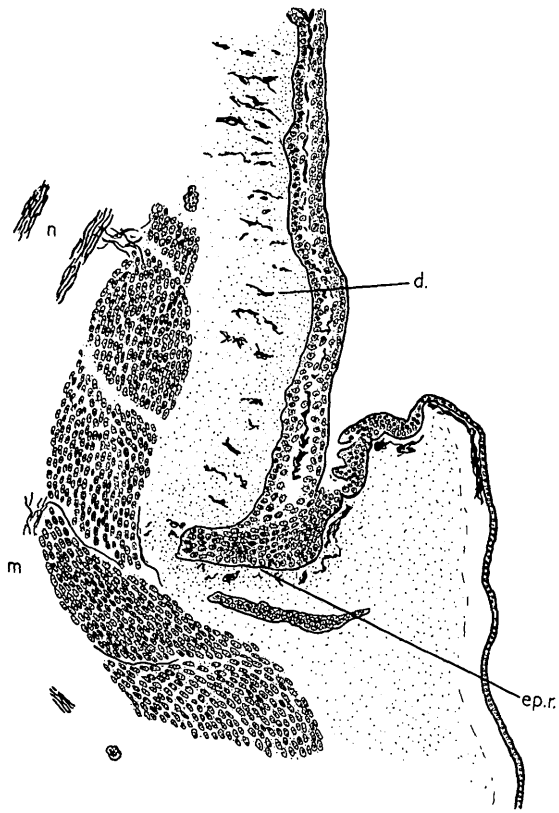


TEXT-FIG. 5. Vertical longitudinal section through 1-mm. tail regenerate of *Sphaerodactylus*, 11 days from autotomy. *c.*, developing cartilaginous tube; *e.t.*, ependymal tube; *e.r.*, epidermal rim; *m.*, myogenic band; *n.*, longitudinal nerve; *s.*, space between new and old muscle infiltrated with lymphocytes; *sp.*, spinal cord. ×43.

At 15 days (length of regenerate 1·5 mm.) the chondroblasts of the skeletal tube are beginning to lay down their intercellular matrix. The tube remains open at the tip for some days, where the vascular network at the tip of the regenerate joins with the peri-ependymal plexus. Within the myoblastic bands segmentation now extends nearly to their apices. Melanoblasts in both dermis and epithelium reach to the tip of the regenerate (Text-fig. 6).

In succeeding days the inner surface of the epithelium becomes uneven, and these irregularities develop into a series of internal ridges which in longitudinal section recall the now well-known internal cristae of mitochondria. The dermis is thus moulded into a series of bays, each of which will be the core of a future dermal scale (Text-fig. 7). Within each bay is a large vascular space, and at the

base of the epidermal cristae is a dense mesenchyme. The epidermal segmentation into scales does not correspond with that of the muscle segments, for the former are the more frequent.



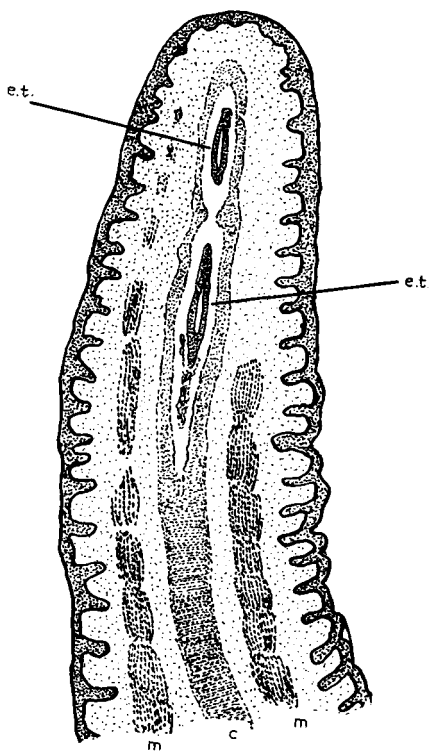
TEXT-FIG. 6. Part of longitudinal section through base of 1·5-mm. tail regenerate of *Sphaerodactylus* 15 days from autotomy. (Same specimen as in Plate, fig. E.) *d.*, dermis with melanocytes; *ep. r.*, epidermal rim; *m.*, segmented muscle band with nerve fibres in intersegmental plane; *n.*, longitudinal nerves. ×121.

In regenerates between 5 and 6 mm. in length, the whole development of the skin is carried to a further stage. All but the youngest epidermal cristae become tilted so that they point away from the tip of the regenerate. In this way the imbricated arrangement of the scales of the regenerate is foreshadowed. Within the *stratum corneum* of the developing scales, dense lamellae of keratin are formed, but the whole outer surface of the regenerate is still covered by an even surface of cornified cells which fill the spaces between the young scales. At 7 mm. all that remains of this original outer layer is a hyaline cuticle. By this stage the outermost layer of the dermis is densely infiltrated by melanocytes, processes

from which extend into the dermal core of the scale. As the scalation develops, the epidermis becomes much thinner than at earlier stages and then, as in the adult epidermis, no longer contains any pigment cells.

The differentiation of muscle fibres

In the 1-mm. regenerate (11–12 days) the muscle columns are distinct from the surrounding mesenchyme (Text-fig. 5). They are made up of elongated myoblasts, and towards the base of each column the division into muscle segments is already apparent. At 1·5 mm. (15 days; Text-fig. 6) the myoblasts in the older segments are becoming aligned into longitudinal rows. During the next 2 days each of these rows becomes a continuous strip of cytoplasm with a row of nuclei down the middle. Within the denser cytoplasm on either side of the nuclei can be seen longitudinal myofibrillae. The myoblasts have thus fused into multinucleate muscle fibres. At first they are densely packed together.



TEXT-FIG. 7. Part of longitudinal section through 2·5-mm. tail regenerate of *Sphaerodactylus* 17 days from autotomy. The epidermal bays foreshadow the future scalation. *c.*, cartilaginous tube; *e.t.*, ependymal tube; *m.*, muscle band. × 50.

The regenerate is now elongating at its maximum rate; the greatest extension among the muscle segments is seen in that next but one to the original muscle of the tail. In a 5-mm. regenerate it is about 0·5 mm. in length, a threefold increase since the time when segmentation was first recognizable. The segment next to the original musculature of the tail is a specially short one, and serves to join the new band to the old.

As the muscle fibres elongate they become separated by clear spaces in which occasional fibroblast-like cells are seen. Pycnotic nuclei are so common at these sites as to suggest that cell degeneration is one factor by which the muscle fibres become separated from each other (Plate, fig. C). As the muscle fibre elongates, its nuclei increase from about twelve to twice this number. Mitotic figures gradually become less common, but do not cease before the muscle fibres become discrete.

In *Aristelliger* mitosis within the muscle segments continues for a longer

period after the first differentiation of fibres than in *Sphaerodactylus*, a distinction presumably correlated with the more rapid growth of the regenerate in the former. Most of the later mitotic figures remain orientated in the axial direction, but are generally to be found in cells lying either within the spaces between the muscle fibres or in contact with the surface of a fibre. Such cells show the rounding-off usual in the later stages of mitosis. We were not able to find an early prophase figure among nuclei within fibres to help us to decide whether the nuclei of the young muscle fibres can undergo division *in situ*. It seems most probable that in *Aristelliger* the muscle fibres grow in length by the intussusception of additional cellular elements derived from residual myoblasts within the muscle segments. Although mitotic figures are abundant among cells between the muscle fibres, there is no general increase in their density at these sites, while the nuclei within the muscle fibres steadily increase in number. It is further possible that new muscle fibres may also be formed to add to the number of those already differentiated in a similar way to that which Couteaux (1941) has described in mammalian myogenesis.

In *Sphaerodactylus* pycnotic nuclei are still seen within the muscle segments of regenerates in which multinuclear fibres have differentiated. Counts of the fibres at comparable levels in transverse sections of regenerates of different ages indicate that their total number decreases in the final stages of development. At this time the diameter of the fibres increases from about 60 μ to about three times this value. The spaces between the fibres are thus largely obliterated.

The first sign of cross-striation within the fibres is seen as a dotting of the myofibrils in the oldest segment of a 3-mm. regenerate. In a silvered preparation of a 7-mm. specimen, the *A*-disks are clearly distinguishable in the more proximal segments among fibres which have reached 100 μ in length. In mature Lacertilian muscle the nuclei remain at the axis of the fibre.

In the regenerating tail it is probable that the multinucleate muscle fibre is formed in the first place by the coalescence of myoblasts. The extensive literature on the histogenesis of muscle is generally divisible between descriptions of the fusion of myoblasts into muscle fibres (Godlewski, 1902; Asai, 1914) and, on the other hand, their origin from single myogenic cells (Iwanaga, 1925). It is probable that during the course of evolution there has arisen diversity in this respect. Franz (1915) finds that the muscle fibres of *Triton* are developed from uninuclear myoblasts, while in Arthropods the fibres have a multicellular origin. In the developing chick, both Meves (1909) and Weed (1937) agree that the single myoblast is the formative unit, but diverse opinions have been expressed on myogenesis in mammalian embryos (Baldwin, 1913; Assai, 1914). The regenerating lizard's tail provides extremely favourable material for the study of the formation of muscle fibres, and would repay investigation by more detailed methods.

The development of the nervous system of the regenerate

In *Sphaerodactylus*, as in *Gongylus* (Terni, 1920), branches of three pairs of spinal nerves run across each autotomy septum. When the tail is shed, fibres of the severed proximal ends of all these nerves resume growth and enter the zone of healing. In *Sphaerodactylus* these growing neurites are approaching the surface of the wound within 2 days of autotomy. When this becomes covered, on the 4th day, by a layer of new ectoderm, a plexus of fine nerve fibres can be traced among the cells of the inner surface of the new epithelium (Plate, fig. D). At their roots the growing fibres of the spinal nerves become swollen and at fixation are made irregular in outline. This feature is more marked in ventral roots than in dorsal roots; it is evident as early as the 4th day, and is still more marked on succeeding days (Plate, fig. A). As the ependymal sac develops, fibres of the white matter grow over the surface of this structure. These cord neurites begin their new growth on the 4th day; each is then dilated at the tip.

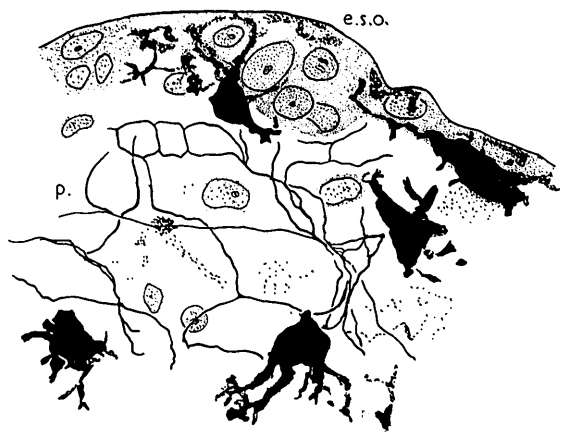
Within the dorsal root ganglia the perikarya show signs of the demands which the new growth of fibres imposes. Two days from autotomy all traces of Nissl substance vanish from the cytoplasm and the nucleus is then liable to an artefact of fixation in which the contents contract round the swollen nucleolus, leaving either an empty space around the inner margin of the cytoplasm or a number of large vacuoles (Plate, fig. A). Recovery of the perikarya is largely complete during the second week of regeneration. Distortion of the cell-body at fixation is absent in dorsal root ganglia not concerned with regeneration. A further feature to be seen within the dorsal root ganglia during regeneration is a heavy infiltration of lymphocytes from surrounding tissues during the 2nd and 3rd weeks after autotomy (Plate, fig. G).

As the apical blastema thickens there is a corresponding elongation of the nerve trunk. Each of the last three pairs of spinal nerves sends four backwardly directed branches into the regenerate. As Terni (1920) has shown in *Gongylus*, the last pair innervates that sector on either side of the regenerate which includes the median horizontal plane. Branches of the next pair traverse the adjacent sectors, while the mid-vertical plane is supplied by the most anterior of the three pairs of spinal nerves. The full number of 24 longitudinal nerves is seen at the base of the regenerate, but only about half of these reach the neighbourhood of the tip. These longitudinal nerves run in the loose mesenchyme between the cartilaginous tube and the inner surface of the myogenic zone. Smaller branches are given off which run between the developing muscle bands and towards the epidermis.

The nerves within the regenerate resemble those of an embryo in that they consist of fibres accompanied by immature Schwann cells, the distribution of which remains uniform in density during the continued elongation of the nerve (Plate, fig. I). Mitotic figures among the Schwann cells are very rarely seen, yet their total number is continually augmented, presumably from the blastema at

the tip of the regenerate, which must be made up of cells of various presumptive potencies.

Branches of the longitudinal nerve trunks pass outwards between the muscle bands towards the surface of the regenerate. At 3 mm., fibres from these sensory nerves have everywhere reached the epidermis into which fibres have hitherto penetrated only at the tip. As soon as the future scalation is foreshadowed within the outer layers of the regenerate, a sensory nerve enters the dermis of each young scale and gives off a plexus of fine fibres which run towards the surface. In an 11-mm. regenerate, at the tip of each scale can be seen a developing epithelial sense-organ consisting of a cluster of cells thicker than the surrounding



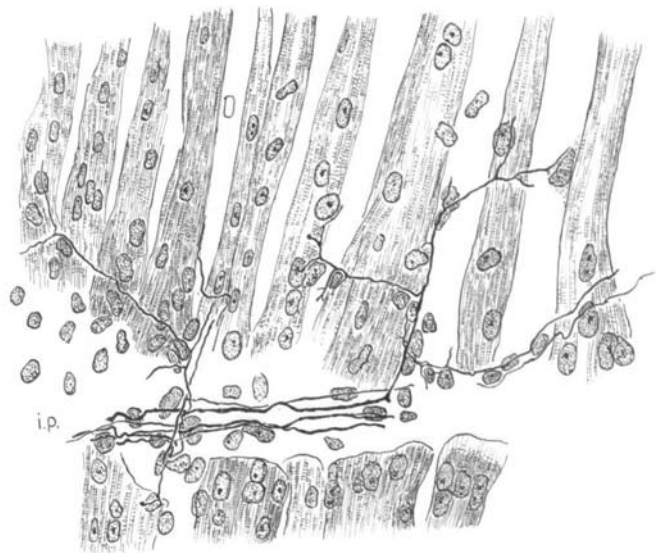
TEXT-FIG. 8. Part of section through scale of 11-mm. tail regenerate of *Sphaerodactylus* at 22 days showing epidermal sense organ (*e.s.o.*) at tip of scale. *p.*, dermal plexus of nerve fibres. ×864.

ectoderm, and beneath which the sensory plexus is especially prominent (Text-fig. 8). These tactile organs are completed by the acquisition of hair-like prolongations of the surface cuticle, forming organs of the type which Schmidt (1921) has described in *Calotes* and other lizards. These processes are readily seen in the shed cuticle; the earliest stage at which we have seen them in a regenerate was at 45 days.

As soon as the segmentation of the muscle bands is detectable, and while their constituent cells are still in a myoblastic stage, single nerve fibres leave the longitudinal trunks and enter the myocommatal-like intersegmental planes. Such neurites will become the motor fibres of the regenerate. They first enter the base of the muscle bands at 15 days, the regenerates being 1·5 mm. long (Text-fig. 6; Plate, fig. E).

Gradually, more fibres enter the intersegmental planes accompanied by Schwann cells. In 4-mm. regenerates the whole length of each muscle band is segmented and has received motor fibres in all intersegmental planes.

A further stage in the development of the innervation of the musculature is



TEXT-FIG. 9. Part of longitudinal section near tip of muscle band of 11-mm. tail regenerate of *Sphaerodactylus* at 22 days showing intersegmental plexus of motor fibres (*i.p.*) with Schwann cells spreading along muscle fibres. × 500.

TEXT-FIG. 10. Part of longitudinal section through oldest segment of muscle band of 11-mm. tail regenerate of *Sphaerodactylus* at 22 days. Motor fibres, accompanied by Schwann cells, have migrated along muscle fibres and are forming end-plates. × 1,280.

TEXT-FIG. 11. Mature motor end-plate within original musculature of tail of *Sphaerodactylus*. × 1,200.

reached by the migration of the motor fibres, accompanied by Schwann cells, to either side of the plane of entry and along the surface of the muscle fibres (Text-fig. 9). The motor fibres soon become contorted and twisted round the Schwann cells (Text-fig. 10). Together these two components gradually assume the form of the mature end-plates characteristic of Lacertilian muscle (Text-fig. 11) as described by Bremer (1882) and more recently by Wilkinson (1931). In an 11-mm. regenerate only in the most mature muscle segments has the motor innervation spread along the whole length of the muscle fibres.

In other vertebrates it is not generally agreed that the nuclei of motor end-plates are always derived from Schwann cells, though Tiegs's (1953, p. 125) review of this subject suggests that they are the most probable source of these elements. In the regeneration tail of the lizard, thanks to the entry of motor nerves and Schwann cells into the muscle segments from the ends of the muscle fibres, it seems clear that at least some end-plate nuclei come from Schwann cells, though the possibility cannot be excluded that fibroblasts of the inter-segmental planes may contribute thereto.

Development of function in the neuromuscular system of the regenerate

The activity shown on autotomy by an original tail or a mature regenerate, free of cortical control, is extremely violent. In *Aristelliger*, for instance, one sees circular waves of contraction propagated round the tail with the effect that the shed member rotates briskly on the ground for some minutes. In an immature regenerate movement is less evident, and may be no more than a faint uncoordinated twitching of each muscle band. In *Sphaerodactylus* this is seen in a shed regenerate 7 mm. long, but one of 4 mm. remains immobile.

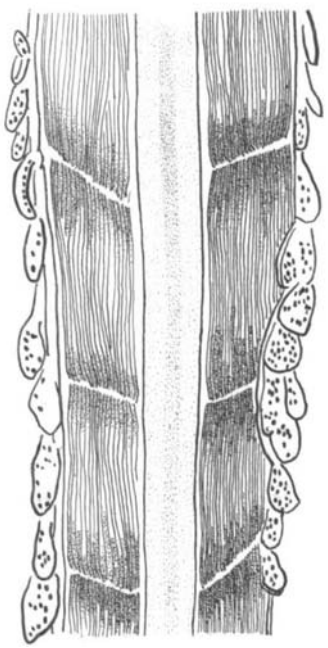
A further approach to this general question was made by the application of the diazonium method for the detection of esterases (Lewis, 1958) to developing regenerates of various ages. At autotomy the shed organ was plunged in chilled formalin and bisected along a median plane with a razor blade. Lewis's procedure was then followed, and the cut surface of the regenerate was finally examined for the appearance of the reddish-brown product which indicates the presence of an esterase and, in this connexion, presumably of cholinesterase (Text-fig. 12). In a 7-mm. regenerate (30 days), a strong reaction was evident between each of the older muscle segments, but in a 4-mm. regenerate, where no movement was seen on plucking, a faint coloration was only just detectable in the intersegmental planes. At this stage motor fibres have not spread beyond these sites, and their endings are still in an undifferentiated condition.

The effect of nitrogen mustard on tail regeneration

The experiments on the effect of nitrogen mustard on tail regeneration were divided into two groups. In the first the substance was applied 5 days after autotomy at a stage when the wound is covered by epithelium beneath which is a regenerative blastema in its earliest stages. Of eleven animals treated in this

way, in two there was no growth externally visible in the 3rd week. In the remaining nine the regenerate began to grow after the usual 10–11 days after autotomy, but elongated at rates below the normal for first regenerates. In the second series of experiments a group of regenerates 4–5 mm. in length were treated with nitrogen mustard and fixed at intervals from 2 to 13 days after treatment.

In the two animals of the first group which did not display growth, it was seen after sectioning that in both a large flask-shaped ependymal sac had been formed. In one the blastema consists only of a dense mass of undifferentiated cells in which are large sinus-like blood-vessels. In the other a more normal blastema is present in which a myogenic zone of denser cells is recognizable. The ependymal sac is densely clothed with fibres of the white matter, some of which branch outwards in an irregular fashion through the blastema. Schwann cells are noticeably deficient, but pigment cells are abnormally abundant.



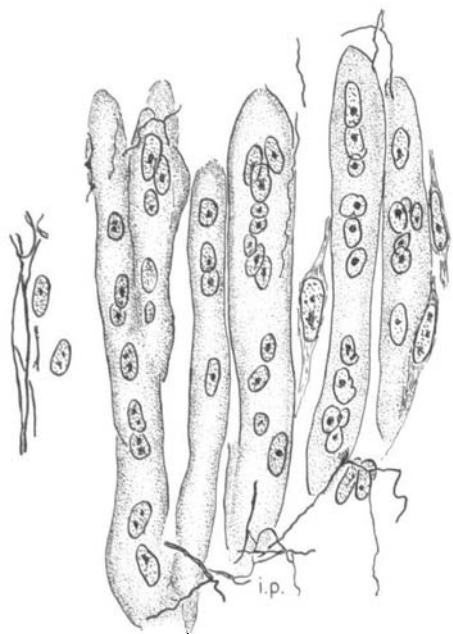
TEXT-FIG. 12. Part of cut surface of bisected 9·5-mm. tail regenerate of *Sphaerodactylus* showing the result of a diazonium reaction for esterases. The coloured reaction product is confined to the end regions of the muscle fibres. × 40.

Of the group of nine regenerates which grew at sub-normal rates under the influence of the nitrogen mustard, five examples were fixed and sectioned at lengths which varied from 0·5 to 4·0 mm. In all of them the component tissues of the normal regenerates were present, though with some degree of distortion in texture and arrangement. The degree of histological development corresponds with that of normal examples of the same age irrespective of the sub-normal length of the treated regenerates.

One feature again common to this group of regenerates was a superabundance of melanocytes. At the tip they are present in such excess as to largely obscure everything else. In a 1·5-mm. regenerate (25 days) appearances suggest that the pigment cells are migrating along the ependymal tube towards the tip and out beyond it at the open end of the surrounding cartilage. The base of the tube is also surrounded by melanocytes, many of which do not yet contain pigment granules at their full density. Elsewhere in the regenerate they are more than usually abundant in the loose parenchyma between the cartilaginous tube and the muscle zone. Into both of these differentiated structures occasional melanocytes have penetrated. In younger members of this series (0·5 mm., 15–17 days), the blood-vessels are dilated into large sinuses. There are also numerous large empty lacunae within the mesenchyme of the regenerate. In both of these

examples nuclear effects of the nitrogen mustard are apparent in the presence of both pycnotic nuclei and cells in abnormal mitosis with scattered chromosomes unattached to a mitotic spindle.

Within the dorsal root ganglia of the stump the effect of fixatives on the perikarya associated with the regeneration described above (p. 292) is seen to a greater extent than in normal regeneration. Some of the corresponding motor-cells within the cord are also affected. However, there was no evidence at any



TEXT-FIG. 13. Parts of a terminal muscle segment of a 7-mm. regenerate of *Sphaerodactylus* 7 days after treatment with 1 : 1,000 nitrogen mustard. The intersegmental plexus of motor nerves (*i.p.*) is almost entirely without Schwann cells. × 546.

TEXT-FIG. 14. Young melanocytes within the parenchymatous connective tissue of a 5-mm. tail regenerate of *Sphaerodactylus* 5 days after treatment with 1 : 1,000 nitrogen mustard. Pigment granules are still separately discernible. × 1,233.

stage of the degeneration of neurones under the influence of the nitrogen mustard such as Hughes & Tschumi (1958) have demonstrated in the immature larval spinal ganglia of *Xenopus laevis*.

In the second series of experiments the substance was applied to regenerates 4-5 mm. long in which the constituent tissues are already largely differentiated. Two days after application the first effects of the treatment have already spread throughout the regenerate. Pycnotic nuclei are seen most commonly in the muscle bands, the ependymal tube, and the epidermis. The nerve fibres round the ependymal tube no longer reach to its tip, and counts in transverse sections of the total number of fibres in the longitudinal trunks indicate a considerable thinning out in number at the tip of the regenerate though some fibres are still

to be seen within the epidermis. In all tissues the nitrogen mustard has affected either the cell surfaces or the intercellular matrix in such a way that contact between cells has been partly lost. The spaces between them are everywhere enlarged. On the 4th day after treatment the longitudinal nerve trunks share in this effect by assuming a characteristic frayed-out appearance. Between the fibres, Schwann cells are seen in degeneration (Plate, fig. F). In the succeeding days their rarity among the smaller bundles of nerve fibres is a striking feature (Plate, fig. H; Text-fig. 13). Elsewhere, from 4 days onwards, tissues begin to resume a more normal aspect although pycnotic nuclei remain common. The nerve fibres at the tip of the regenerate are soon restored. Nuclei of the outer layers of the *stratum corneum* become shrivelled and gradually sealed off from the healthy cells within by thick laminae of keratin. The dead material is sloughed off by the 6th day and the whole epidermis thereby becomes much thinner. Mitotic figures reappear among the cells of the *stratum germinativum* (Plate, fig. H), the nuclei of which are abnormally large though their mitotic figures do not suggest a polyploid condition.

On the 5th day the influence of the nitrogen mustard on the production of melanin becomes apparent. The new keratinous zone of the epidermis is uniformly black. Within the dermis, melanocytes are no less conspicuous than usual, but some of them are extending long-cell processes into the mesenchymatous tissue beneath, within which are also to be seen cells with nuclei similar to those of the surrounding fibroblasts but from which there radiate fine cytoplasmic filaments in which small pigment granules are sparsely distributed (Text-fig. 14). These are apparently cells which are differentiating into melanocytes. On the 7th day the cytoplasm of most cells which contain pigment is a uniform dense black (Plate, fig. H) though occasionally fibroblast-like elements containing a few granules are to be seen. The general proportion of melanocytes increases still further in succeeding days: in one specimen there is much extracellular pigment within the mesenchyme 13 days after treatment; young melanocytes are, however, still to be seen.

This apparent influence of nitrogen mustard in evoking the formation of melanin within mesodermal cells does not seem to be confined to the regenerate which was originally treated with the substance. In one animal a regenerate of 1·5 mm. grew in 25 days, 20 days after treatment. This was removed, together with two caudal segments of the original tail, and a further regenerate allowed to develop without further treatment. It grew to 3·5 mm. in 21 days. On sectioning it was found that melanocytes were abnormally abundant both around the ependymal tube and within the general parenchyma.

The effect of nitrogen mustard at the concentrations here used is apparently confined to young and rapidly growing regenerates. Two older examples were treated, one 10-mm. long, and the other so mature that the boundary between new and old tail was no longer traceable. On sectioning, no effect of the treatment was seen in either instance.

DISCUSSION

Hitherto, the regeneration of the lizard's tail has been discussed largely in terms of the anatomy of the regenerate and the latter's relationship with the normal tail. We can now turn to the further question of the development and histogenesis of the new tail in comparison with the corresponding processes in normal embryology.

From the regenerating blastema arise cells that form cartilage, muscle fibres, general mesenchyme, as well as Schwann cells and melanocytes. In ontogeny the neural crest is the main source of these latter two cell types, though there is evidence that in some vertebrates Schwann cells are not exclusively derived from the neural crest (Hörstadius, 1950, p. 31). As far as it is known the neurones of the dorsal root ganglia develop wholly from neural crest cells; yet in the regeneration of the tail of a Urodele fresh ganglia are formed by the outward migration of cells from the new spinal cord (Duesberg, 1925).

The regenerative blastema arises in the first place in close association with the ependymal sac. It is possible that cells which originate by mitosis within this neural epithelium may be in genetical continuity with those which at an early embryonic stage were formed together with the neural crest. A possible difficulty here is that, in a bifid tail, one branch may lack a cartilaginous tube and its contained ependymal prolongation. Brindley (1898) has described such an instance in *Mabuia carinata*, and Woodland (1920) has evoked this condition experimentally in *Hemidactylus flaviviridis*. However, nothing is yet known in such examples about the distribution or the origin of pigment cells or even how the growth of the accessory branch proceeds in the first instance.

In the regenerating tail of *Sphaerodactylus* the first melanocytes originate from the pigmented meningeal investment of the spinal cord, but the experiments with nitrogen mustard described in this paper show that some normally unpigmented cells within the general mesenchyme have the potentiality of forming melanin. This observation is paralleled by the experiments of Figge (1948), who found that in tadpoles of *Rana* which had been treated with indophenol dyes, melanin granules appeared within connective tissue-cells of the larva.

Woodland (1920), in discussing the differences between the normal and regenerated tails of a lizard, regards the latter as a 'cheap "jerry-built" structure only reproduced for the purpose of being shed'. The analogy has some value in relation to the respective time-scales on which each structure is laid down. Normal development in the Lacertilia is extremely slow, and extends over several months, whereas tail regeneration is complete within a few weeks of adult life. It is in this light that the different modes of histogenesis of normal and regenerated muscle are best viewed, irrespective of the relative worth of the final products, in which no difference is apparent on microscopical examination.

In the regenerate, the division into myotome-like segments persists during and after the formation of muscle fibres. Motor nerve fibres enter the developing

musculature at intersegmental planes, to which a positive esterase reaction at neuromuscular junctions is at first restricted. The normal development of muscle fibres in *Sphaerodactylus* is, however, similar to that of other Amniotes in that the original segmentation breaks down before the differentiation of muscle fibres, and when motor fibres enter the musculature the myocommata have long been unrecognizable.

Why one type of myogenesis should necessarily be more rapid than the other is yet impossible to explain, but the thesis that each is correlated with a different rate of ontogeny receives support from the corresponding developments in the tadpole of the Anuran *Xenopus*, where the crucial events are compressed into a few hours. Here, as Lewis & Hughes (1957) and Lewis (1958) have shown, the histogenesis of the trunk muscles is closely comparable to that in the Lacertilian tail regenerate; segmental divisions are retained, innervation is at first confined to the ends of the muscle fibres, and esterases first appear in the myocommatal planes. Further exploration of this subject demands an extended comparative survey of the functional aspects of myogenesis.

SUMMARY

1. Tail regeneration in the Geckonid Lizards *Sphaerodactylus goniorhynchus* and *argus* is described. The rate of growth of a first regenerate, after a latent period of 10–12 days, is about 0·47 mm. per day. Subsequent regenerates grow more slowly.

2. The tissues of the new tail are derived from a regenerative blastema which is formed beneath the epidermis covering the broken surface of the tail. A flask-shaped outgrowth of the ependymal epithelium is a centre for the proliferation of new cells.

3. The outer part of the regenerative blastema is a zone of myogenesis, which becomes divided into the same number of muscle bands as in the original tail. Each muscle band becomes segmented, and motor nerves enter the intersegmental planes. At these sites a positive reaction for esterases is first seen. Multinucleate muscle fibres, which stretch from one intersegmental plane to the next, arise by the fusion of myoblastic cells.

4. The regenerate is innervated by branches of the last three remaining pairs of spinal nerves. These branches keep pace with the growth of the regenerate, and form longitudinal nerves. Their terminal fibres enter the new ectoderm on the 4th day.

5. Nitrogen mustard applied in solution to the outer surface of the regenerating area may inhibit, or slow down, the new growth. Degeneration of each type of cell within the regenerate is caused by nitrogen mustard, but melanocytes become more abundant than normal.

6. A regenerate subsequent to one inhibited by nitrogen mustard grows as

fast, or even faster, than a normal first regenerate, and again contains an excess of melanocytes.

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EXPLANATION OF PLATE

FIG. A. Spinal nerve roots of tail of *Sphaerodactylus* which send fibres into a 6-day regenerate. Ventral root fibres run vertically in figure, dorsal root fibres emerge horizontally from ganglion on right. Ventral root fibres are more swollen than those of a dorsal root. Nuclei of perikarya of ganglion much vacuolated at fixation. Bodian. × 340.

FIG. B. Orientated mitotic figures among myoblasts in 2·5 mm. regenerate (17 days) of *Sphaerodactylus*. H. & E. × 340.

FIG. C. End stages of pycnosis round muscle fibres in 6-mm. regenerate of *Sphaerodactylus*. H. & E. × 340.

FIG. D. Fibres from longitudinal nerves among cells of inner layers of epidermis in 4-day regenerate of *Sphaerodactylus*. Holmes. × 340.

FIG. E. Motor fibres in intersegmental plane (horizontally across middle of figure) at base of muscle band in 1·5-mm. regenerate (15 days) of *Sphaerodactylus*. Holmes. (Same specimen as in Text-fig. 6.) × 340.

FIG. F. Longitudinal nerve in 3-mm. regenerate of *Sphaerodactylus* 4 days after treatment with 1:1,000 nitrogen mustard, showing degeneration of Schwann cells and fraying of nerve fibres. Bodian. × 340.

FIG. G. Part of dorsal root ganglion supplying 1-mm. regenerate (11 days) of *Sphaerodactylus* showing infiltration with lymphocytes. Nuclei of perikarya fixed without vacuolation. H. & E. × 340.

FIG. H. Area of dermis and epidermis (top left) from 7-mm. regenerate of *Sphaerodactylus*, 7 days after treatment with 1:1,000 nitrogen mustard, showing peripheral nerves with very few Schwann cells, frayed nerve trunk (bottom right), and new melanoblasts. Among the large epidermal nuclei is one in metaphase. To be compared with Fig. I. Bodian. × 340.

FIG. I. Area of dermis and epidermis (top left) of 7-mm. regenerate of *Sphaerodactylus* to serve as control to Fig. H. Note smaller size of epidermal nuclei and numerous Schwann cells on peripheral nerve branches. Bodian. × 340.

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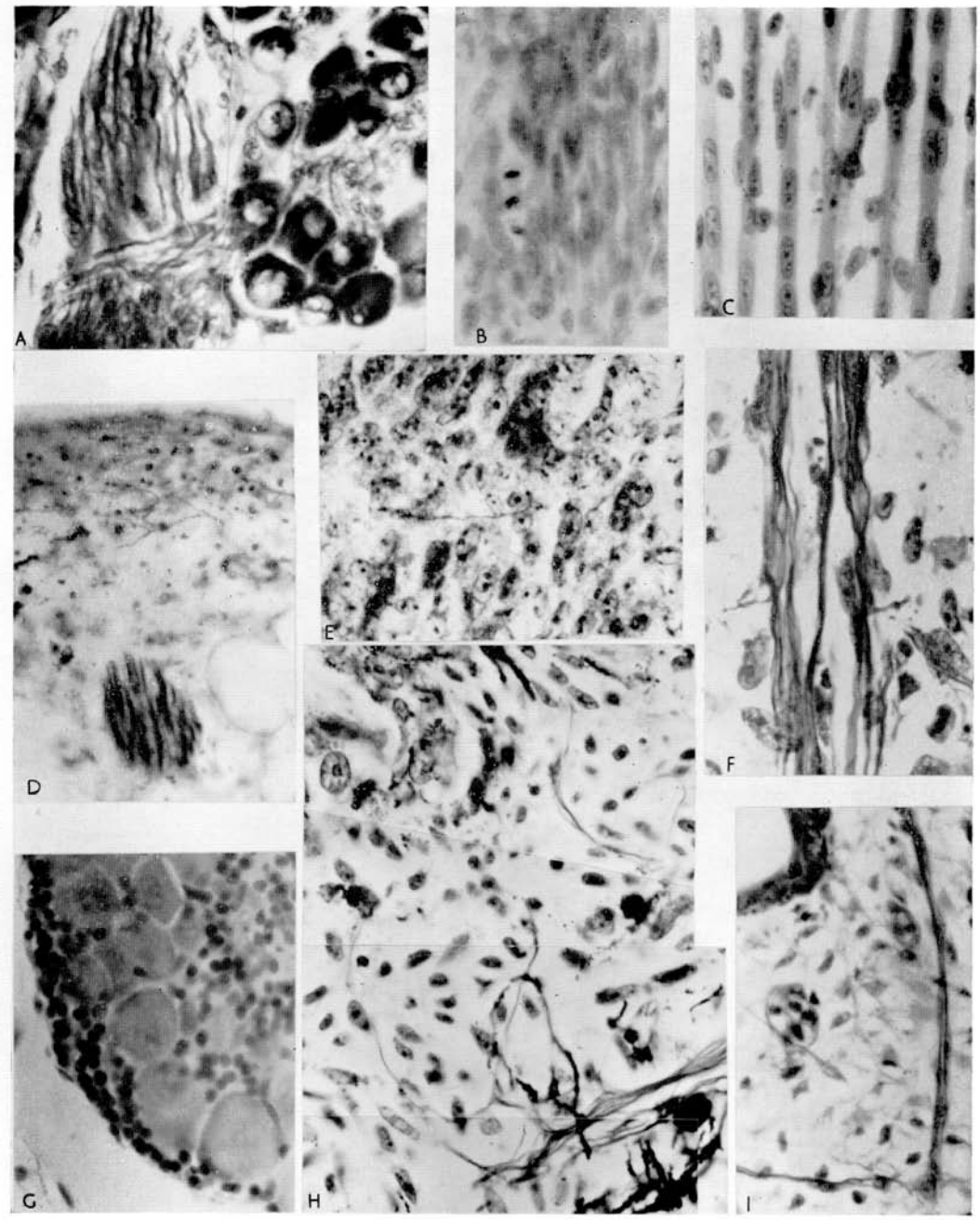

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