Tail regeneration in the lizards *Anguis fragilis* and *Lacerta dugesii*

By SUSAN V. BRYANT and A. d'A. BELLAIRS, F.L.S.

Department of Anatomy, St Mary's Hospital Medical School, London, W.2

Rates of tail regeneration in the Madeira wall lizard (*Lacerta dugesii*) and the slow-worm (*Anguis fragilis*) were studied.

L. dugesii regenerates very rapidly, the new tail sometimes attaining a maximum rate of growth of 2·6 mm a day during the fifth week after autotomy. By the twelfth week 90% of the original tail length has been replaced. Average regeneration rates of samples of lizards were reduced after repeated autotomies, but our investigation of this problem was probably complicated by another factor, the amount of tail lost, and is inconclusive.

The tip of the regenerate grows more rapidly than the rest; no elongation occurs at its cranial aspect.

Anguis, even when kept at 27°C, regenerates its tail very slowly, the best performance observed being a new tail of 5 mm after 14 weeks. The longest natural regenerate seen (16 mm) may have taken several years to produce in the wild.

The histological features of regeneration in *Anguis* are basically similar to those in other lizards. The new osteoderms are formed entirely in the subepidermal tissues but have a regular relationship with the scales. Some nerve fibres are regenerated with the ependymal tube.

The scales on the lizard's regenerating tail develop in a different manner from those in the lizard embryo and show suggestive resemblances to mammalian hairs.

INTRODUCTION

Although the slow-worm (*Anguis fragilis*) can shed its tail and has well-developed planes of autotomy, it is generally regarded as a poor regenerator. Malcolm Smith (1954) wrote that: 'The power of regenerating the tail which the Lacertids possess has been largely lost by the Slow-worm. In this animal healing of the stump is slower and the original length of the tail is never attained'. A study of the rate and histological features of regeneration in *Anguis*, and a comparison with lacertids, especially the Madeira wall lizard (*Lacerta dugesii*) which grows a new tail very quickly and completely, seemed likely to be of interest. Observations on the latter, more typical lizards will be described first.

MATERIAL AND METHODS

Twenty-eight adult slow-worms and 36 Madeira wall lizards were used. Most of the former were kept artificially heated at about 27°C, and were fed on earthworms and slugs; others were kept in an outdoor vivarium during the summer. The Madeira wall lizards were kept at 27 to 30°C and fed on mealworms and small locusts; individuals were identified by amputating digits. Despite its shyness, this species does well in captivity so that fairly uniform standards of health can be maintained. Autotomy was induced by suddenly twisting the tail. Most of the sectioned regenerates were stained with azan or with haematoxylin and eosin.
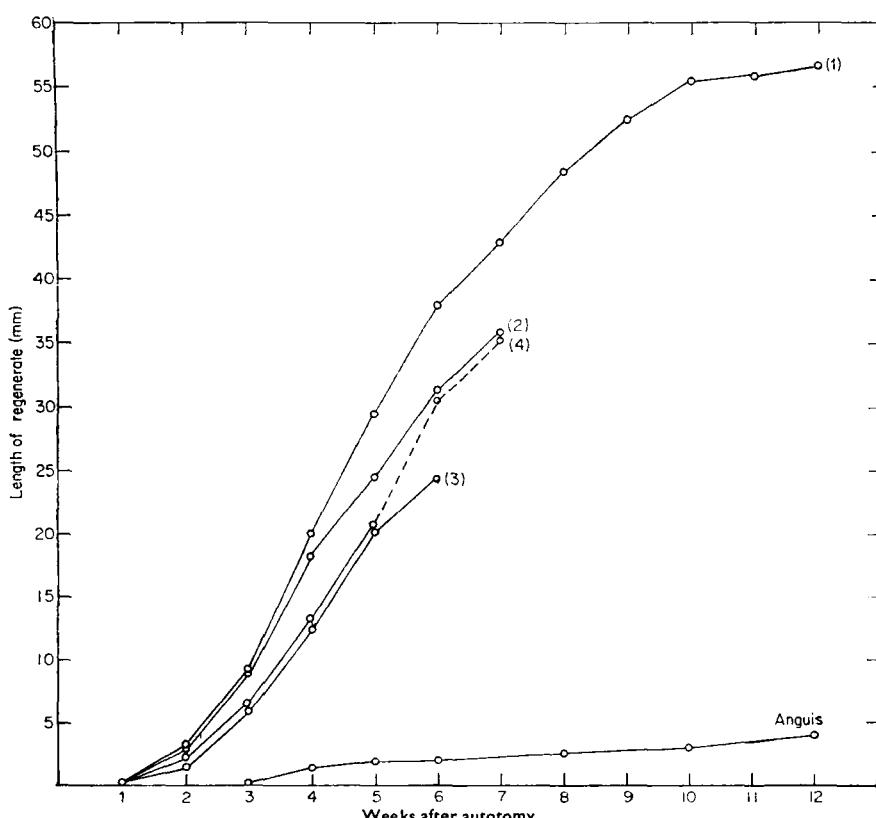
RATES OF REGENERATION

Lacerta dugesii

The following experiments were carried out:

(I) Autotomy was induced in ten lizards with complete tails showing no evidence of previous injury; portions of 45 to 70% of the tail were lost. The regenerates were measured at weekly intervals for three months. In the course of the seventh and later weeks four lizards broke off tiny pieces from the tips of their regenerates through accidental injury. The growth rates were then much reduced and the readings for these specimens after the time of injury have been omitted from our results. The results are illustrated graphically in Text-fig. 1 which shows the average rate of growth for the group as a whole.

There was always a latent period of some six to ten days before the regenerate began to increase visibly in length. The period of most rapid growth was between the third and

Text fig. 1. Graph showing growth in length of tail regenerates of *Lacerta dugesii*, and the single, most rapidly regenerating individual of *Anguis fragilis*, after autotomy at week 0.

(1) Average growth rate of 10 lizards (*L. dugesii*) after first autotomy of complete original tails. Rates during the 7 to 12 week period were only observed in six specimens.

(2) Average growth rate of 14 lizards after second autotomy cranial to a mature regenerate.

(3) Average growth rate of eight lizards after third autotomy, induced six weeks after the second autotomy, the growth of which is shown in (2).

(4) Average growth rate of 19 lizards after the third or fourth autotomies, induced at intervals of one to four weeks after the previous autotomy. Six of these lizards were observed only for five weeks; the broken line shows the average further growth rate for the remaining 13.

Anguis. Growth rate of fastest slow-worm, kept at 27°C.

sixth weeks after autotomy, when the average rate was about 9 mm a week. Maximum weekly rates of up to 18 mm were seen during the fifth week, and the length of the regenerate at this time was a reliable indication of the overall regeneration rate of any individual. This varied considerably, the fastest specimen having a regenerate of 40 mm after five weeks, while the slowest had one of 18·5 mm. The growth rate in all the lizards fell off gradually after the sixth week, and during the twelfth week only about 1 mm on average was added. By this time the regenerate measured about 90% of the portion lost in all cases.

(II) Autotomy was induced in 14 lizards with mature regenerates; as in later experiments the fracture took place through the original tail some 5 to 15 mm proximal to the regenerate. Six of these individuals had already been used in experiment (I) and had undergone their first autotomy 12 weeks previously, while the remainder had lost their tails and grown new ones before we obtained them. This experiment therefore involved a second autotomy after a substantial but in some cases, an unknown, period. Average figures during the ensuing four to seven weeks' growth were somewhat lower than the average after first autotomy (Text-fig. 1). Three lizards, however, had growth rates higher than the highest after first autotomy, while two others from the sample previously used in (I) surpassed their earlier performances. These five specimens were all observed for six weeks.
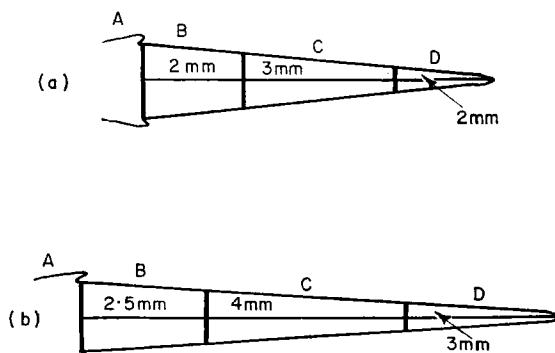
(III) Eight lizards used in (II) were again subjected to autotomy six weeks after the last. After this injury, representing a third autotomy in all, average growth rates were somewhat further reduced (Text-fig. 1). Two lizards regenerated more rapidly than the average after first autotomy, however, and one of these exceeded the fastest rate in (I).

(IV) Nineteen lizards were used to study the effect of repeated autotomy after a shorter interval. The experiment involved a third autotomy for 12 of these, and a fourth for seven, including injuries which had occurred before capture in some cases. The intervals between the penultimate and last autotomies were one to four weeks. Average growth rates for this whole group up to five weeks were about the same as those obtained after the last experiment (III). However, five of these lizards regenerated faster than the average after first autotomy in experiment (I) and three of these were faster than the fastest in (I). The high rates of these individuals were mainly responsible for the rise in average growth rate after the fifth week, when six of the original 19 specimens were eliminated (see Text-fig. 1 and item four of its legend). Of the five lizards with the fastest growth rates, three were among the sample which had suffered a total of four autotomies, the last three being only separated by intervals of one and three weeks.

(V) The tails of four additional *Lacerta dugesii* were amputated under ether anaesthesia at an intervertebral site, between adjacent autotomy planes. As in *L. vivipara*, the arrangement of the caudal scale rows serves as a guide to the level of amputation. Normal regenerates were formed, and grew at rates comparable with those in experiments (III) and (IV). In two cases the latent period was longer than usual, being about 18 days. Regenerates which grew to about 15 mm after seven weeks were also obtained after intervertebral amputation in two specimens of *L. vivipara*.

(VI) An attempt was made to study relative growth rates of different parts of some of the regenerates. These were between 4 and 40 mm in length and two and five weeks in age, and in most cases were undergoing rapid overall growth. They were marked with paint at intervals along their length. The first mark was placed at the junction of the regenerate with the original tail, the second 2 mm caudal from this, and the third 2 mm cranial to the tip (Text-fig. 2). After two days the distances between the marks were measured and the marks erased; new ones were then placed in the designated positions. In all, 21 separate observations, distributed among five lizards, were made.

It was found that the mark at the junction of the normal tail with the regenerate always remained in its position, indicating that new tissue was not being formed on the cranial end of the regenerate.

Text-fig. 2. Diagram to show the rates of growth of different parts of tail regenerate of *Lacerta dugesii*. (a) Shows the positions of the marks on the day of marking, and (b) their positions two days later.

Rate of increase in length of portion A = 0 mm/day
Rate of increase in length of portion B = 0·125 mm/day
Rate of increase in length of portion C = 0·16 mm/day
Rate of increase in length of portion D = 0·25 mm/day
Rate of increase in length of regenerate as a whole = 0·17 mm/day.

The distances between the first and second, and the second and third marks altered appreciably during the two-day periods so that it was possible to measure the absolute increase in length of each portion of the regenerate. From this the rate of elongation of each portion, per mm per day, could be calculated. It was found that in all but one case the small terminal region elongated at a faster rate per mm of tissue than the proximal portion, in most cases (16 out of 21) about twice as fast. In 18 cases also the rate of the tip was greater than that of the regenerate as a whole. Rates of growth of the middle and basal portions were nearly equal, but the middle portion grew a little faster.

Anguis fragilis

Autotomy in *Anguis* was more difficult to induce by twisting the tail laterally than by by bending in the dorsiventral plane. Some of the animals had old regenerates, but in view of the slowness and variability of replacement, no correlation with previous autotomy was attempted. The latent period was difficult to assess, but is probably some three to six weeks; because of the slow-worm's burrowing habits the scab becomes encrusted with soil particles and persists for much longer than it does in *Lacerta*, making both gross observation and histological preparation very difficult.

Weekly readings for the first 12 weeks' growth of the tail of the individual which regenerated most rapidly are shown on the graph in Text-fig. 1. After 12 weeks at 27°C the new tail was 4 mm long; it increased to 5 mm after 14 weeks, but had undergone no further growth after a total period of five months from autotomy. The average regenerate lengths for ten slow-worms kept at 27°C were 0·75 mm after four weeks and 1 mm after six weeks. Five of these animals, including the individual whose record is shown on the graph, had an average regenerate length of 2·2 mm after 14 weeks. Slow-worms kept out of doors regenerated even more slowly, the regenerates of four specimens being 0·5 mm long on average after six weeks; in three of these the new tail was only 1 mm long after 16 weeks. Two further specimens had regenerates of about 3·4 mm after being kept out of doors for seven and three weeks respectively, and then for a further 13 weeks at 27°C.

Mature slow-worm regenerates may be difficult to distinguish with the naked eye. In some cases, however, they have dark patches on the ventral surface, or the whole ventral surface is darker than the adjacent part of the original tail. The new scales are usually a

little smaller than the normal and differently arranged, so that it is possible to see under a lens where the pattern of the original tail ends and the regenerate begins.

Through the kindness of Miss A. G. C. Grandison we have been able to study the collection of slow-worms in the British Museum (Natural History). Of 26 adult specimens obtained in various parts of Great Britain, 16 had regenerates varying between 0·5 and 16 mm in length; only two of these had regenerates of over 10 mm. Two of the continental specimens examined had regenerates of 14 and 16 mm. It is interesting that all these four slow-worms had apparently lost at least half of the original tail, assuming that the latter measured 160 to 200 mm, an average range of tail length for well-grown specimens. The amount replaced, as represented by these unusually long regenerates, could not have amounted to more than some 14 to 20% of the portion lost.

HISTOLOGICAL FEATURES

The histological features of tail regeneration in lacertid and other lizards have been described by many workers, including Woodland (1920), Kamrin & Singer (1955), Hughes & New (1959), Moffat & Bellairs (1964) and Simpson (1965). It is clear from our own observations on 14 specimens, and from the short description by Ali (1950) of a mature regenerate, that the formation of the new tail of *Anguis* conforms to the general lacertilian pattern. Certain points of interest, however, may be described.

A longitudinal section through the tail of *Anguis* one week after autotomy is shown in Pl. 1, fig. 1. The wound surface is partly covered by a scab and the small fragment of vertebra cranial to the autotomy plane can be seen still articulating with its neighbour at the procoelous intervertebral joint. The broken end of the spinal cord is obscured, but examination of adjacent sections shows that the spinal canal is dilating to form the ependymal sac described by Hughes & New (1959) and other authors. At the margins of the wound the new epidermis has already begun to grow across the raw surface beneath the scab.

In Pl. 1, fig. 3, the tail of another specimen kept for six weeks after autotomy at 27°C is shown. Remains of the scab are still present but the broken vertebra and the adjacent surfaces of the intervertebral joint are no longer visible; the absence of a zone of articular cartilage on the caudal end of the last remaining vertebra suggests that the whole joint region has undergone absorption or sloughing. The end of the spinal cord has now grown back to form a thin-walled tube lined with ependyma, and this is surrounded by a zone of dedifferentiated, blastematous tissue. The epidermis covers the end of the tail and has become keratinized; a layer of keratin has become partly detached with the scab. This epidermis is considerably thicker than normal and represents the apical cap which is so characteristic of the early regenerates of amphibians and lizards. This section is comparable with that of a somewhat retarded three weeks regenerate of *Lacerta vivipara* obtained after intervertebral amputation and figured by Moffat & Bellairs (1964, Pl. 1, D). In the latter, however, the apical cap was much smoother and more rounded, and there was a greater development of blastema. More advanced conditions were seen in another regenerate of about the same age, in which irregular new scales were developing (see p. 303) and a cartilage cylinder had already formed.

Mature regenerates of 3·5 and 11 mm are shown in Pl. 1, figs 7 and 6; the latter was grown in the wild and is the longest regenerate which we have examined microscopically. The new scales have acquired a more regular arrangement and new osteoderms, smaller and less regular than those of the original tail, have appeared beneath them. Study of younger regenerates shows that the osteoderms ossify directly in the dermal tissues and are not preformed in cartilage. Although they arise quite independently of the epidermis, their distribution conforms with that of the scales, and it is possible that there is some causal relationship between the development of the two sets of structures.

The tip of these mature regenerates has now developed into a smooth, conical and well

keratinized terminal scale without underlying osteoderms except at its base; its epidermis is not appreciably thicker than that elsewhere. The tissue beneath this terminal scale and around the ependymal sac consists mostly of differentiated connective tissue and little if any blastema is present. The cartilage cylinder is massive and that of the 11 mm specimen shows slight peripheral calcification. It is traversed by the ependymal tube which issues from its caudal end and swells out to form the ependymal sac (Pl. 1, fig. 6). Selective stains show that some nerve fibres from the spinal cord have regenerated and pass back with the connective tissue around the ependymal lining of the tube (Pl. 1, fig. 2).

Outside the cartilage cylinder is a zone of connective tissue containing many blood vessels, pigment cells and nerve fibres derived from the last one or more pairs of spinal nerves of the original tail. This region also contains the newly formed bundles of muscle fibres which do not appear to have a strict segmental arrangement. The bands of fatty tissue which surround the vertebrae of the original tail have not differentiated in the regenerate; they are normally less well developed in *Anguis* than in *Lacerta*.

DISCUSSION
Rate and quality of regeneration

In the kinds of experiments which we have performed, necessarily on limited numbers of animals, the biologically significant data are probably the average rate of regeneration for the group, and the rate of the fastest specimen; the latter may at least give some indication as to the best performance of which the species is capable. Both the maximum (2·6 mm) and the average (1·3 mm) daily rates in *Lacerta dugesii*, calculated from the weekly increments during the peak period, compare favourably with figures recorded for other lizards: 1·36 and 2 mm for other lacertids, and 0·47, 0·7 and 1·12 mm for various geckos, which are usually regarded as good regenerators (see Hughes & New, 1959). *L. vivipara* can probably regenerate at least 0·7 mm a day during the phase of active growth (see Moffat & Bellairs, 1964). These figures are, however, based on group averages or isolated specimens and may not represent the maximum possible rates for the species.

Hughes & New (1959) found that the rate of regeneration in geckos (*Sphaerodactylus*) was retarded after second and third autotomies. The reduction in the average growth rate which we observed in all samples subjected to successive autotomy, even after an interval as long as 12 weeks in some cases, suggests a similar tendency. Nevertheless, our results are inconclusive. There were numerous exceptions to this trend, especially among the groups which had undergone three or four autotomies with intervals of only one to four weeks between the last two. In fact some of these individuals, treated in a fashion which might be expected to have the greatest effect on regeneration, had maximum growth rates slightly higher than any of those observed after first autotomy.

These inconsistencies can perhaps be related to the recent work of Tassava & Goss (1966) who showed that in *Anolis* the rate of regeneration varied directly with the amount of tail removed. Our own findings also indicate this, though they are based on a smaller sample of lizards. Of the ten *L. dugesii* used in experiment (I), the six which had lost 55 to 70% of their tails regenerated more rapidly in every case than those which had only lost 45 to 50%. Unfortunately it was impossible to draw a similar correlation between growth rate and amount of tail lost in many of the lizards which had undergone more than one autotomy; some of the lizards which had lost the greatest amounts of tail were among the slower regenerators.

It seems likely that regeneration rate depends on the interplay of a number of factors: repetition of injury, amount of tail removed, individual health and variation, and perhaps small fluctuations in environmental conditions such as temperature. The relative importance of these can only be evaluated by the study of larger numbers of individuals under circumstances which are critically controlled.

Jamison (1964) found that intervertebral amputation greatly retarded or inhibited regeneration in several non-lacertid genera of American lizards. Our own experiments, together with those of Woodland (1920) and Moffat & Bellairs (1964), show that the operation does not greatly affect regeneration in *Lacerta* and *Hemidactylus*, though in the former at least, it may somewhat delay it.
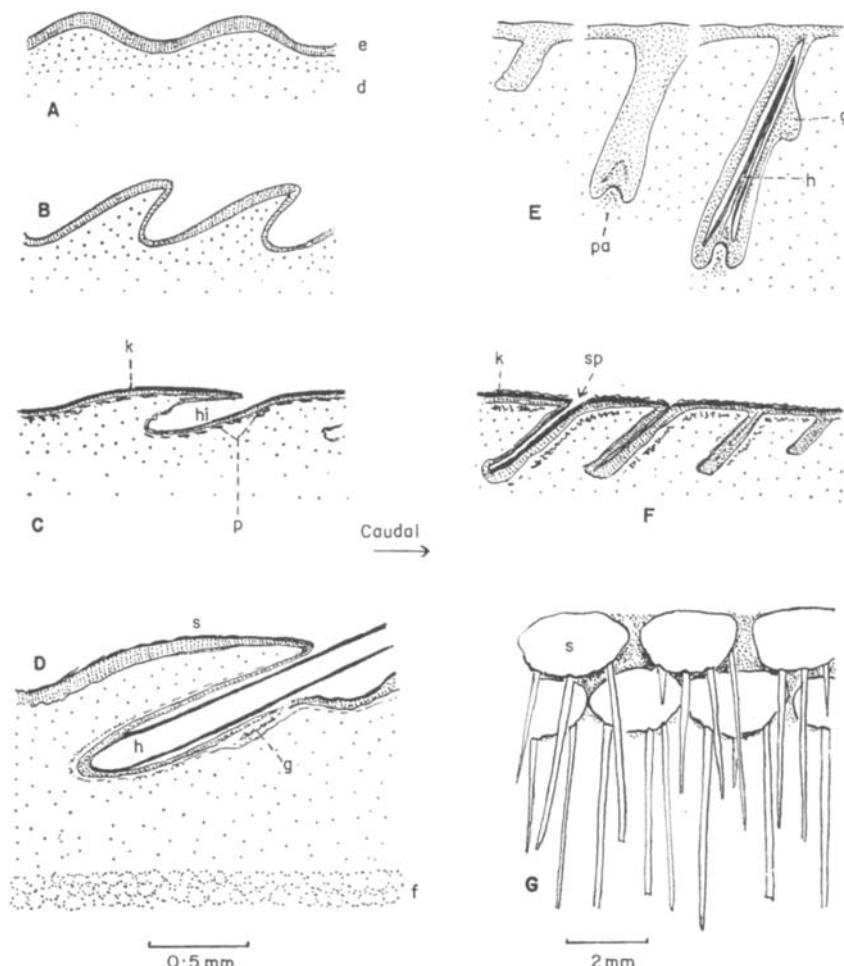
In the matter of regeneration, the slow-worm clearly deserves its name for slowness, but it originally seemed possible that this was simply due to its habits. It seldom basks and spends most of its time beneath stones or burrowing, so that it probably lives within a lower temperature range than typical lizards found in the same locality. Our figures suggest a tendency for *Anguis* kept at 27°C to regenerate more rapidly than those in an outdoor vivarium but there can be little doubt that the low growth rate, which is much lower than that recorded for any other lizard with regenerative capacity, is a basic character of the species. Our fastest specimen took 14 weeks to grow a new tail of 5 mm, and achieved a maximum rate of about 0·07 mm a day during the ninth week after autotomy, when the regenerate elongated by 0·5 mm. This was well above the average rate even for the other artificially heated specimens. If these figures are any indication of growth rates in the wild, they suggest that a natural regenerate of over 10 mm may well take several years to develop, allowing for quiescent six-month periods of hibernation. It is perhaps significant that *Anguis* has a rather slower rate of general growth than the other English lizards, taking at least seven or eight years to reach full size (Smith, 1954); its longevity, with a recorded maximum of 54 years, is certainly exceptional.

From the anatomical standpoint the regenerate of the slow-worm is neither more nor less perfect, save in the matter of size, than that of other lizards. It contains all the characteristic components of the saurian tail regenerate, with the possible exception of the fat-bands. The development of a typical ependymal tube and the regeneration of some nerve fibres around it are of interest, since there is evidence that both nerves and ependyma play an important causal role in initiating regeneration (Kamrin & Singer, 1955; Simpson, 1965). One has the impression, however, that in *Anguis* differentiation of the tissues predominates over proliferation so that although the volume of blastema remains small it produces new tissues such as muscle and cartilage relatively quickly. The histological appearances of the two mature regenerates described do not suggest that either is undergoing rapid growth of typical regenerative character. It seems possible that any further growth would have been mainly of interstitial type, involving a gradual increase in the size of the regenerate as a whole.

Scales and hairs

Although regenerating scales have been described by several workers, it has not, to our knowledge, been pointed out that they develop in a strikingly different fashion from those in lizard embryos. The scales of the regenerating tail arise as ingrowths into the dermis from an originally flat epidermal surface which is already keratinized (Text-fig. 3, Pl. 1, fig. 4). Subsequently each ingrowth splits to give rise to the greater part of the inner surface of one scale and of the outer surface of the next; the tissue at the bottom of the split will become the hinge region. Keratin appears as a sheet down the middle of the ingrowth, and when the split takes place a thicker layer of this substance is left on the outer than on the inner scale surface. Owing to the craniocaudal gradient of scale formation all stages in this process can be seen in a single section of a large *Lacerta* regenerate (Text-fig. 3F). The process is essentially similar in *Anguis*, but owing to the smaller size of the regenerate the gradient of differentiation is less obvious.

In the embryo, on the other hand, as Maderson (1965) has shown, the scales arise as a series of elevations which soon become asymmetrical and slanted backwards, forming what he calls 'perfect embryonic scales' (Text-fig. 3B). No epidermal ingrowth into dermis
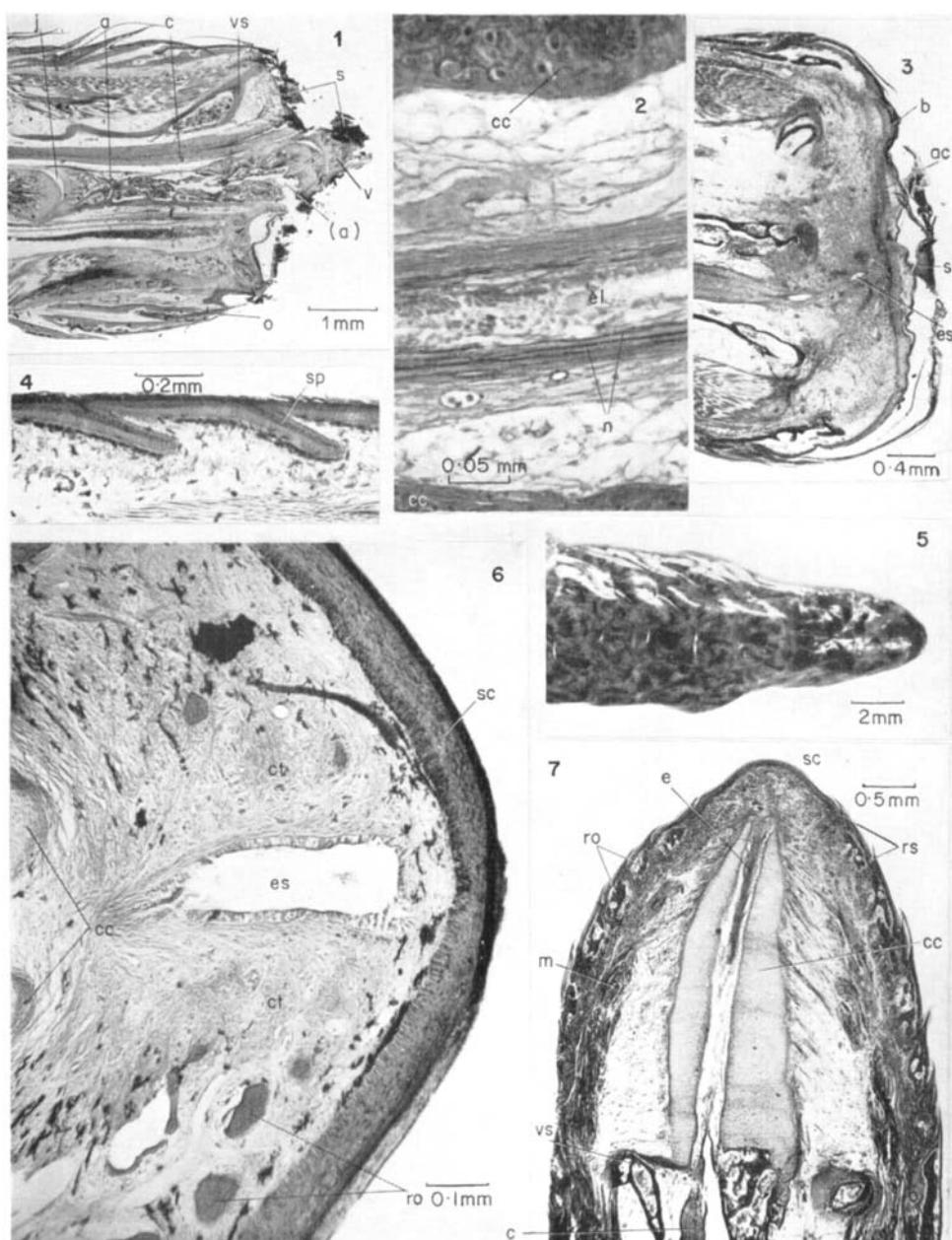
Text-fig. 3. Scales and hairs.

A, early; B, intermediate, and C, adult stages in the development of caudal scales of normal lizard embryo; shown in longitudinal section. D. Section through tail skin of desman (*Myogale*) showing hair emerging from beneath posterior edge of scale. The hair bulb and deeper parts of the hair are not shown. E. Diagram showing three stages in the development of a mammalian hair, in section. F. Longitudinal section through scales of a regenerating lizard tail, showing cranio-caudal sequence of differentiation. G. Surface view of portion of tail skin of desman, showing relation of hairs to scales.   Only two rows of scales are shown. d, Dermis; e, epidermis; g, sebaceous gland; h, hair shaft; hi, hinge region of scale; k, keratin; p, pigment cells; pa, dermal papilla of hair; s, scale; sp, split in keratin of regenerating scales; f, fat.

NOTE: The arrow 'caudal' only refers to A-F, the mm scales to D and G.

is involved in their formation, and no substantial keratinization takes place until the scales have virtually reached their definitive form.

In some respects the histological appearance of regenerating scales is much more like that of developing mammalian hairs than that of embryonic lizard scales. This finding is of interest in connexion with the view that hairs originated in the evolutionary sense from localised regions of the epidermis in the hinges of reptilian scales (Spearman, 1964). The topographical relationship between hairs and scales in pangolins and other mammals which possess both structures lends some support to the theory. In the desmans (*Myogale*),

for example, the tail is covered by scales of remarkably reptilian appearance with hinge regions between them. The hairs, usually three to five in number, emerge from the hinge beneath the overhanging posterior edge of each scale. The suggestive resemblances between regenerating scales, developing hairs and the desman's tail skin are illustrated in Text-fig. 3.

REFERENCES

ALI, S. M., 1950. Studies on the anatomy of the tail in Sauria and Rhynchocephalia. IV. *Anguis fragilis. Proc. Indian Acad. Sci.*, **32**: 87–95.

HUGHES, A. & NEW, D., 1959. Tail regeneration in the geckonid lizard, *Sphaerodactylus. J. Embryol. exp. Morph.*, **7**: 281–302.

JAMISON, J. P., 1964. Regeneration subsequent to intervertebral amputation in lizards. *Herpetologica*, **20**: 145–149.

KAMRIN, R. P. & SINGER, M., 1955. The influence of the spinal cord in regeneration of the tail of the lizard, *Anolis carolinensis. J. exp. Zool.*, **128**: 611–627.

MADERSON, P. F. A., 1965. The embryonic development of the squamate integument. *Acta zool., Stockh.*, **46**: 275–295.

MOFFAT, L. A. & BELLAIRS, A. d'A., 1964. The regenerative capacity of the tail in embryonic and post-natal lizards (*Lacerta vivipara* Jacquin). *J. Embryol. exp. Morph.*, **12**: 769–786.

SIMPSON, S. B., 1965. Regeneration of the lizard tail. In *Regeneration in Animals and Related Problems*. (Ed. V. Kiortsis & H. A. L. Trampusch.) Amsterdam, North-Holland Publ. Co.

SMITH, M., 1954. *The British Amphibians and Reptiles* (2nd ed.). London, Collins.

SPEARMAN, R. I. C., 1964. The evolution of mammalian keratinised structures. *Symp. zool. Soc. Lond.*, No. 12, 67–81.

TASSAVA, R. A. & GOSS, R. J., 1966. Regeneration rate and amputation level in fish fins and lizard tails. *Growth*, **30**: 9–21.

WOODLAND, W. N. F., 1920. Some observations on caudal autotomy and regeneration in the gecko (*Hemidactylus flaviviridis*, Rüppel), with notes on the tails of *Sphenodon* and *Pygopus. Q. Jl microsc. Sci.*, **65**: 63–100.

EXPLANATION OF PLATE

PLATE 1

All figures except 4 and 5 are of longitudinal sections through the tail of *Anguis fragilis.*

Fig. 1. One week after autotomy showing scab and broken vertebra. The break (a) is an artefact.

Fig. 2. Ependymal tube of 11 mm regenerate showing accompanying nerve fibres stained black. Fitzgerald silver technique.

Fig. 3. Six weeks regenerate, 0·8 mm long, showing apical cap, blastema and dilated ependymal tube and terminal sac.

Fig. 4. *Lacerta vivipara.* Skin of tail regenerate showing scale formation.

Fig. 5. *Anguis.* External appearance of 5 mm regenerate seen from below.

Fig. 6. Tip of 11 mm regenerate showing ependymal sac and terminal scale derived from apical Cap.

Fig. 7. Sixteen week regenerate, 3·5 mm long, showing cartilage cylinder and new osteoderms. a, Autotomy split (fracture plane) in vertebral body; ac, apical cap; b, blastema; c, spinal cord; cc, cartilage cylinder; ct, connective tissue; e, ependymal tube; el, ependymal tube lumen; es, ependymal sac; j, intervertebral joint; m, regenerated muscle; n, nerve fibres; o, osteoderm; ro, regenerated osteoderms; rs, regenerated scales; s, scab; sc, scale derived from apical cap; sp, region of future split between adjacent scales; v, vertebra; vs, vertebral spine.