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The regenerative capacity of the tail in embryonic and post-natal lizards (*Lacerta vivipara* Jacquin)

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WITH THREE PLATES

INTRODUCTION

MANY studies have been made on autotomy and regeneration of the tail in lizards; for example those by Woodland (1920) and Hughes & New (1959) on geckos, by Slotopolsky (1922) on *Lacerta*, by Barber (1944) and Kamrin & Singer (1955) on *Anolis*, and by Simpson (1964) on *Lygosoma*. This work is concerned with the adult, and, so far as we are aware, no comparable studies on young and embryonic lizards have been made. Indeed, the application of experimental techniques to reptilian embryology is still in its infancy (see Holder & Bellairs, 1962, 1963).

In 1956 Panigel found that embryos of the common lizard (*Lacerta vivipara*) would develop, sometimes until hatching, when removed from the mother and kept under sterile moist conditions in a form of culture. Their yolk is sufficient for nourishment, and the rudimentary type of allanto-placenta which he describes seems to have no significant nutritive function. We have found that embryos of the slow-worm (*Anguis fragilis*) may also survive for long periods after similar treatment, but a more elaborate form of culture is necessary for their normal development (Raynaud, 1959).

Maderson & Bellairs (1962) removed part of the tail in a number of *Lacerta* embryos kept in Panigel culture, and found, rather surprisingly, that no regeneration took place. This was confirmed by Holder & Bellairs (1962) so that a more thorough investigation of the problem seemed desirable. It was found necessary to re-examine certain general aspects of autotomy and regeneration in lizards in order to assess the results of our experiments.

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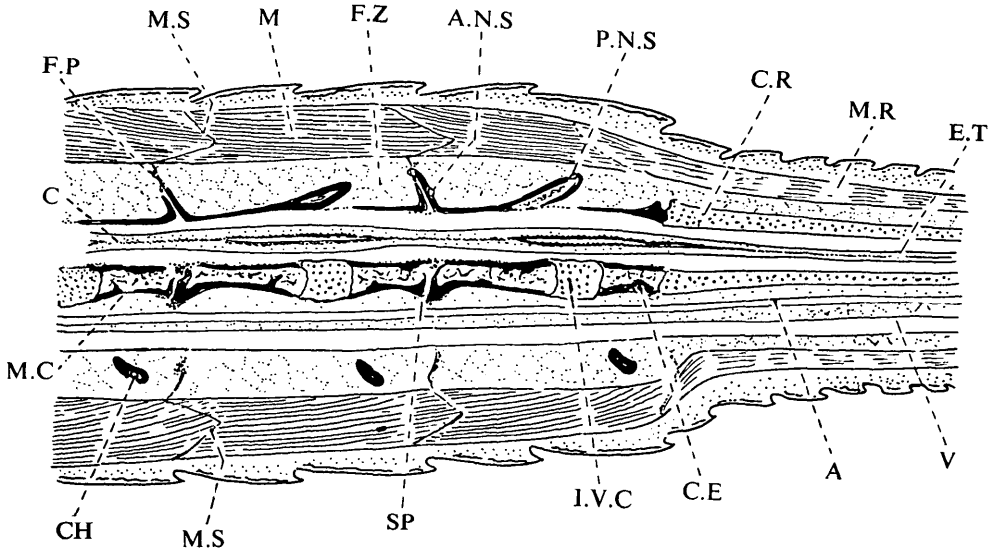
THE ANATOMY OF AUTOTOMY

In *Lacerta*, as in many lizards, the tail possesses a series of fracture planes through which breakage can occur with the minimum of damage. This may be due either to autotomy, which involves active contraction of the tail muscles, or to passive rupture produced by simple mechanical trauma. The anatomy of these planes in *Lacerta* has been described by Slotopolsky (1922) and Pratt (1946) and is shown in Text-fig. 1 here.



TEXT-FIG. 1. Diagrammatic longitudinal section near midline through tail and mature regenerate of adult *Lacerta vivipara*, after autotomy. × 14. *Abbreviations:* A, caudal artery. A.N.S, anterior neural spine. C, spinal cord. C.C, cartilage of vertebral centrum. C.E, centrum of broken vertebra. CH, chevron. C.R, cartilage of regenerate. E.T, ependymal tube. F.P, fracture plane in fat zone. F.Z, fat zone. I.V.C, intervertebral cartilage. M, caudal muscles. M.C, marrow cavity of centrum. M.R, muscle of regenerate. M.S, myoseptum. N.C, notochordal cartilage. P.N.S, posterior neural spine. SP, autotomy split in centrum. V, caudal vein.

Each of the caudal vertebrae, except for the first six or seven, has a split passing through it about halfway along its length or a little further forwards. The connective tissue around the margins of the split is continuous with a septum which passes outwards through the zone of fatty tissue surrounding the caudal vertebrae, and joins the transverse myoseptum between adjacent muscle segments. The vertebral split, the connective tissue septum in the fat zone and the myoseptum together make up a single plane of fracture which traverses most of the tissues of the tail. It does not pass across the spinal canal, however, and contains a gap in the ventral mid-line which transmits the caudal artery and vein.

The fracture planes in the fat and muscle are differentiated well before birth, but the formation of the vertebral splits is not completed until early post-natal life and is associated with the process of ossification. At birth the tail vertebrae

are still for the most part cartilaginous (Plate 2, Figs. F, G), but the centra have a thin shell of perichondral bone and the neural arches are partly ossified. Around the middle of each centrum the notochord has chondrified to form a structure known as the notochordal cartilage or septum which is characteristic of the developing tail vertebrae of lizards which possess fracture planes. Gadow (1896) believed that the tissues of tail regenerates were derived from persistent 'quasi-embryonic cells' of the notochordal cartilage, but his views are not substantiated by more recent work.

The fracture planes in the vertebral centra appear to arise as the result of the invasion of the bone and notochordal cartilage by vascular connective tissue. In late embryos and new-born lizards they are represented by small splits in the perichondral bone of the centra with connective tissue extending into them (Plate 2, Fig. F). Similar conclusions were reached by Howes & Swinnerton (1901) in their monograph on *Sphenodon*, which also has caudal fracture planes. The split in the neural arch appears to arise separately from that in the centrum, the two joining up behind the transverse process in early post-natal life.

The arrangement of the caudal scales in *Lacerta vivipara* is useful as a guide to experimental amputation. The scales are arranged in regular transverse rows or rings around the tail, except in its basal region. Every two of these transverse rows corresponds with a single body segment, and hence with a single vertebra. Owing to their mode of development, however, the vertebrae are intersegmental in position. The scale rows alternate slightly in antero-posterior length, a row of longer scales being followed by a row of shorter ones. The difference in length over the dorsum of the mid-caudal region of adult lizards is 0·1–0·2 mm. A section made behind a long scale row passes intravertebrally through the region of the split, much as in normal autotomy. A section made behind a short row passes through or near the massive intervertebral cartilage which separates each vertebra from its neighbours. The difference in scale lengths is shown in Text-fig. 1, and, on the dorsal surface of the tail, in Plate 2, Fig. F. It is not always apparent in longitudinal sections, however, since some of the scales may be cut obliquely.

REGENERATION IN POST-NATAL LIFE

Regeneration was observed in the tails of eleven adult, two young (probably 8–10 months old) and six baby lizards, after induced autotomy or after amputation with a scalpel or scissors under ether anaesthesia; this relaxes the tail muscles and prevents autotomy from taking place. Some of the animals were subjected to injury more than once; in the case of some of the babies previous amputations had been performed before they hatched in Panigel culture. The regenerates of five of the adults and all the babies were serially sectioned.

The objects of these experiments were as follows:

(i) to determine whether the power of regeneration is present in the new-born;

(ii) to confirm the findings of Woodland (1920) and Slotopolsky (1922) that regeneration would occur after amputation at sites other than those of the fracture planes;
(iii) to define clearly recognizable criteria of regeneration;
(iv) to determine the period after injury when these first become apparent.

The results are shown in Table 1. The figures are too irregular to allow the compilation of critical growth data such as were obtained by Hughes & New (1959) for *Sphaerodactylus*. They show, however, that regeneration occurs at all stages in post-natal life and after both autotomy and amputation either at an intravertebral or an intervertebral site. Evidence for the last point was obtained from only three specimens, but seems conclusive; the cut off portion of the tail posterior to the site of amputation was sectioned as well as the regenerating stump (Plate 1, Figs. D, E).

The histological features of regeneration resemble those described by White (1925) in this species, and by Hughes & New and others. At first the tail stump is covered by a clot of blood and cellular debris which is soon sloughed off. Epidermis appears over the wound surface and soon becomes modified to form a thick cap similar to that described in regenerating amphibian limbs (see Singer & Saltpeter, 1961). This cap, which has been called the apical cap, has a very characteristic appearance (Plate 1, Fig. D; Plate 2, Fig. J) and differs strikingly from the epidermis of normal scales. Beneath the thickened epidermis

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EXPLANATION OF PLATES

All photos of *Lacerta vivipara*. All numbers of embryos refer to Table 3, p. 777. All sections are longitudinal (sagittal), near midline.

Abbreviations: A.N.S., anterior neural spine. B, raw band. BL, blastema. C, spinal cord. CH, chevron. C.R, early cartilage of regenerate. EP, epidermis of regenerate (apical cap). E.T, ependymal tube. F.Z, fat zone. H.P, hemipenis. I.V.C, intervertebral cartilage. M.R, muscle of regenerate. N.C, notochordal cartilage. NO, notochord. PA, epidermal papilla. S, strand of tissue leaving spinal cord. SP, autotomy split in centrum. T, tag.

PLATE 1

FIG. A. Embryo 6, after hatching, showing effects of amputation of tail at Dufaure-Hubert stage 30. × 4. The right hind limb was amputated at the same time in one of several preliminary experiments on limb buds. There is no regeneration of either extremity after 19 days culture at 28°C. Plate 3, Fig. K, shows section of tail.

FIG. B. Embryo 15 after hatching, showing very short non-regenerating tail with raw band (B) and hemipenes (H.P). × 15 Stage at operation, 31: survival time 21 days at 28°C. The right hind leg has been cut off after death for better view.

FIG. C. Embryo hatching in Panigel culture. × 3·3. The piece of handkerchief was applied to prevent prolapse after operation.

FIG. D. Adult lizard 18 (Table 1, p. 773); section showing regeneration of tail amputated intervertebrally, after 24 days. × 12.

FIG. E. Proximal end of cut off tail of lizard 18, showing that level of amputation passed just behind an intervertebral cartilage. × 12. The diagram below shows the relationship of the two pieces of tail.

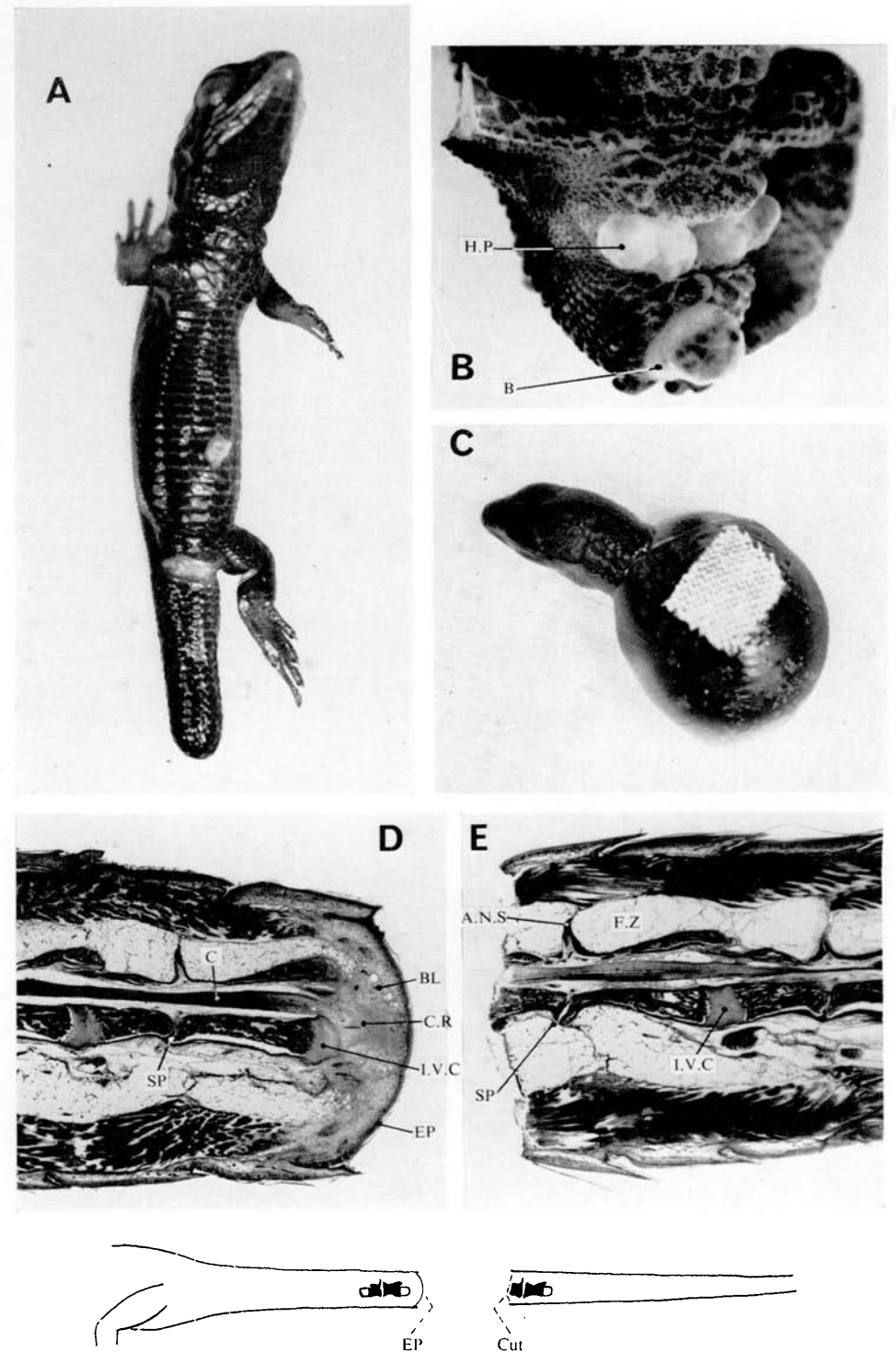
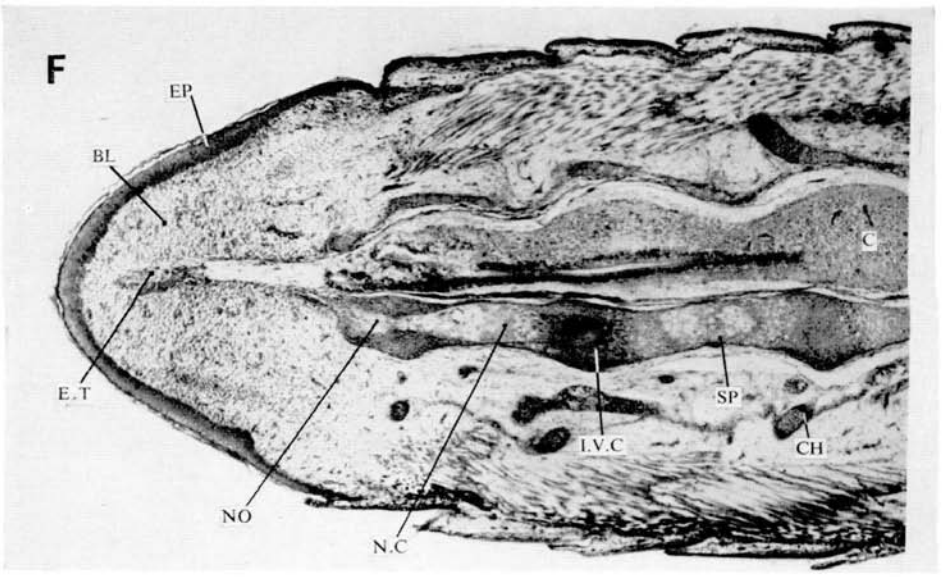
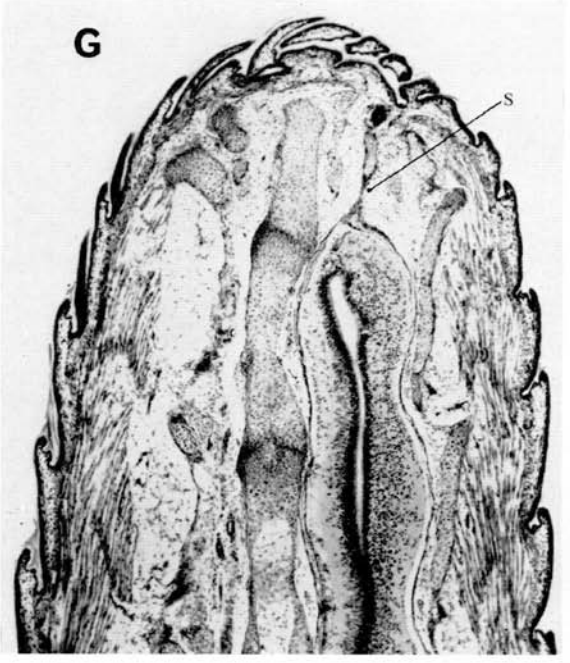
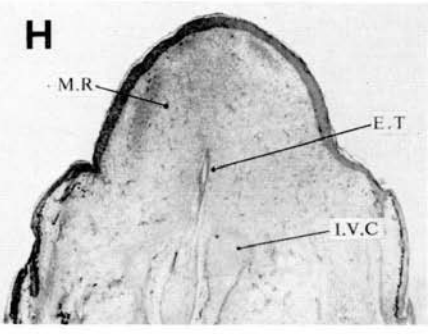


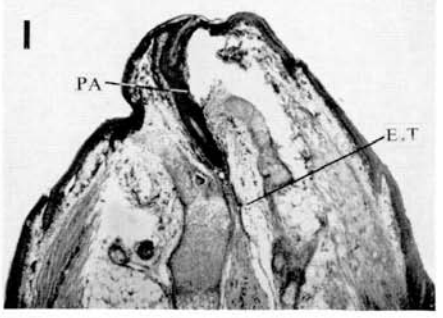
PLATE 1

LYNETTE A. MOFFAT (*née* HOLDER) *and* A. D'A. BELLAIRS (*Facing page 772*)









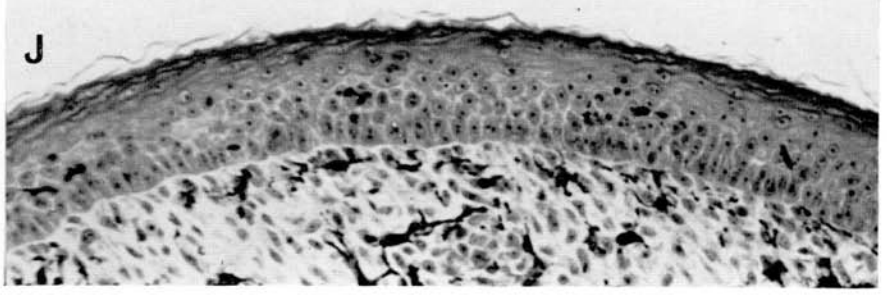


PLATE 2

LYNETTE A. MOFFAT (*née* HOLDER) *and* A. D'A. BELLAIRS

(*Facing page 773*)

TABLE 1

Length of regenerate (mm.) after autotomy and amputation in post-natal life

Lizard No.	*Age, etc., at operation*	*Days after injury*			
		13–16	19–21	22–27	31–36
After autotomy or passive rupture					
1	Adult		1·5	2·5	
2	Adult		3	5	
3	Adult		2		12
4	Baby (P) 57 d (iii)			0·5	
5	Baby (B) 9 d (ii)			2	
6	Baby (B) 0 d				2
After intra-vertebral amputation					
7	Young	Early			
8	Young	0·5			
9	Adult		1·5		7·5
10	Adult		2		12·5
11	Adult		2·5		10
12	Adult		3		12
13	Adult		2		6·5
14	Adult		2		10·5
15	Baby (B) 0 d			0·5	
16	Baby (B) 0 d	0·5			
16	Baby (B) 16 d (ii)	1			
17	Baby (P) 0 d (ii)		1		
After inter-vertebral amputation					
18	Adult			0·5	
19	Adult			1	
4	Baby (P) 36 d (ii)		0·5		

Abbreviations: B, normal birth in captivity; d, days old, after birth or hatching; 0 d, day of birth or hatching; P, hatched from Panigel culture; ii, iii, second or third operation.

PLATE 2

FIG. F. Embryo 55 after hatching; section of tail showing regeneration after amputation at stage 39–40, very late in embryonic life. × 50. Regeneration is seen after only 6 days in culture at 28° C.

FIG. G. Embryo 7 after hatching, showing typical appearance of non-regenerating scaly tail tip. × 54. Stage at operation, 30; survival time 19 days at 28° C.

FIG. H. Baby lizard, 4, 57 days old, showing regeneration 21 days after intervertebral amputation in post-natal life. × 30.

FIG. I. Baby lizard 4, 36 days old, showing failure of regeneration after amputation performed at stage 39–40 (as embryo 53, Table 3), 6 days before hatching. × 35. The section is damaged but a well developed epidermal papilla can be seen.

FIG. J. High power of thickened epidermis (apical cap) and part of blastema of post-natal 16-day regenerate. × 300. There are numerous melanocytes.

a cone of blastema cells develops and, owing to the growth of this, the spinal cord and the broken vertebra appear to retreat from the tip of the stump. Both the regenerating epidermis and the blastema contain many melanocytes which give the regenerate its dark colour. The broken end of the spinal cord dilates to form a sac which very soon grows back into the blastema as a long slender tube lined by ependyma and called the ependymal tube. The presence of these features in association may be regarded as a reliable criterion of early regeneration.

Muscle fibres differentiate in the blastema, and the new skeleton, which consists of an unsegmented tube of cartilage, develops around the ependymal tube and is attached at its proximal end to the broken vertebra or intervertebral cartilage (Text-fig. 1). Cartilage may begin to appear in adult and baby *Lacerta vivipara* when the regenerate is under 1 mm. in length (Plate 1, Fig. D; Plate 3, Fig. L). The formation of new scales from the thickened epidermis does not take place until a considerably later stage. The main nerve-supply of the new tail is derived from the spinal nerves above the site of injury, but Kamrin & Singer (1955) have shown that in *Anolis* some descending fibres regenerate with the ependymal tube.

The rate of regeneration, as Slotopolsky found in *Lacerta muralis*, varies a great deal. Probably it depends on both temperature and nutrition, but neither of these factors was controlled closely enough in our experiments to allow a comparative assessment. In both babies and adults, however, growth of the tail stump becomes externally visible some 14–21 days after injury, at least in animals which are feeding well. Histological evidence of regeneration, such as the appearance of the thick smooth epidermis and of the ependymal tube, can probably be detected somewhat earlier. As in the geckos studied by Hughes & New (1959) there seems to be a latent period of 10–12 days after injury during which the blastema is being formed and there is hardly any visible increase in length.

The figures in Table 1 suggest that the growth of the regenerate may be delayed

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PLATE 3

FIG. K. Embryo 6 showing absence of regeneration and strands of tissue (S) leaving tip of spinal cord. × 140. D, dermis. N.E, non-thickened epidermis.

FIG. L. Baby lizard 17 (Table 1), formerly embryo 14 and now 21 days old. There is a post-natal regenerate of 1 mm. after tail amputation 2 hr. after hatching. Regenerating muscle (M.R) is clearly seen and cartilage is just beginning to appear around the ependymal tube (E.T). × 35.

FIG. M. Embryo 14 after hatching, showing absence of regeneration and atypical epidermis at tail tip. × 39. Stage at operation 31: survival time 20 days at 28°C.

FIG. N. Embryo 3. Section of short raw tail showing absence of normal epidermis and tag (T) possibly derived from embryonic membranes. There is no regeneration. × 56.

FIG. O. Embryo 6 after hatching. Longitudinal section of thigh with femur, showing very loose subcutaneous tissue. × 33.

FIG. P. Malformed embryo with short tail, about stage 35.

FIG. Q. Egg in culture with embryo partly prolapsed at site of operation.

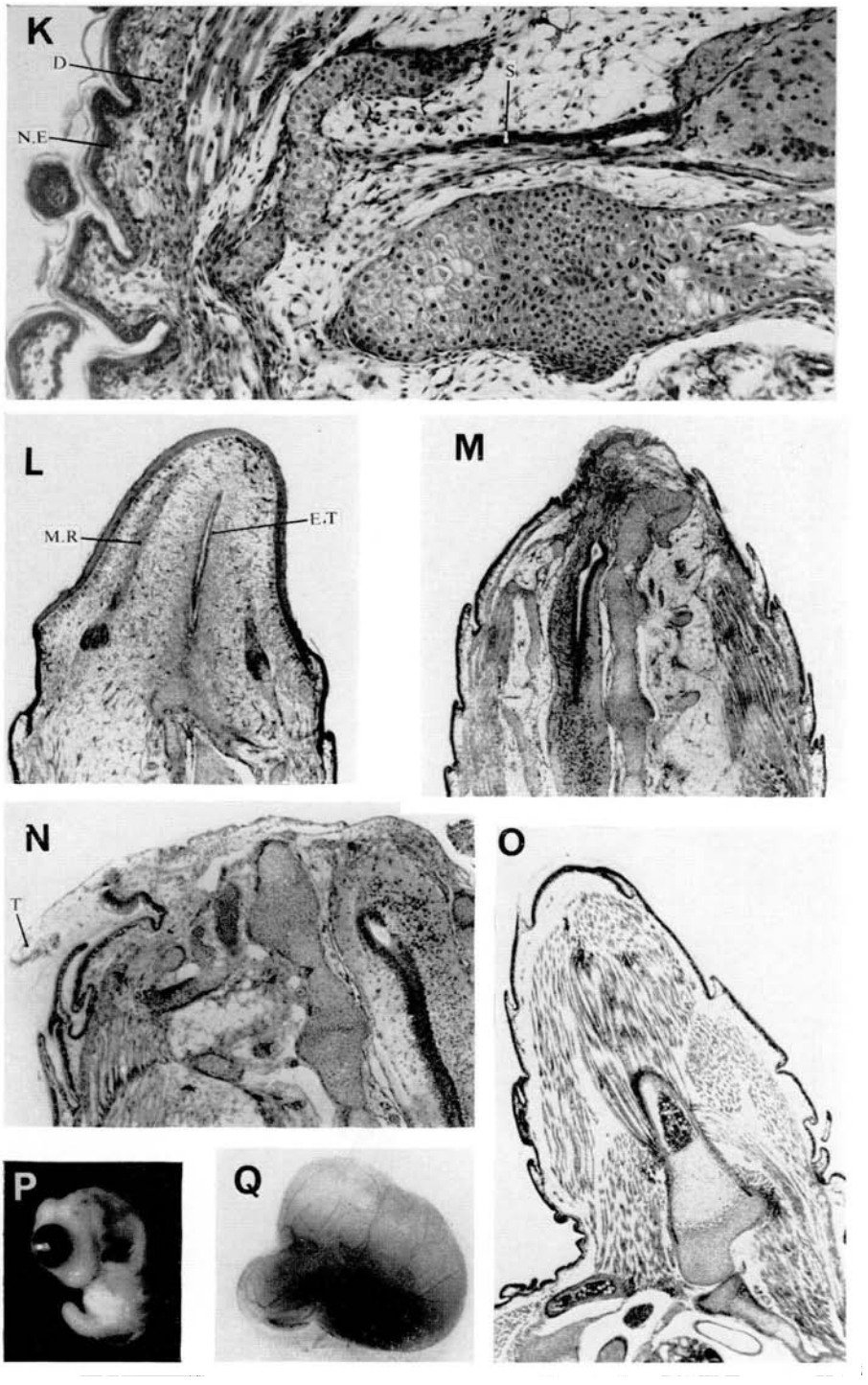

PLATE 3

LYNETTE A. MOFFAT (*née* HOLDER) *and* A. D'A. BELLAIRS (*Facing page 774*)

after intervertebral amputation, but they are certainly not conclusive on this point. The two adults subjected to this operation (Nos. 18 and 19) were taking little, if any, food, whereas most of the other lizards were feeding well.

EXPERIMENTS ON EMBRYOS

Material and Methods

Our technique for culturing the embryos is based on the work of Panigel (1956). We have previously described it briefly (Holder & Bellairs, 1962) but in view of the unfamiliarity of reptilian material in experimental embryology a fuller account may be given here.

In England *Lacerta vivipara* usually mates in April or May and the young are born after a gestation period of 10 to 12 weeks (Smith, 1964). Like most reptiles this lizard is hard to breed in captivity and it is necessary to collect pregnant females in order to obtain a supply of embryos; each female usually contains 4–8 fertile eggs. Collection of a suitable range of embryonic stages during the single annual breeding season may be difficult, and this undoubtedly constitutes a limitation of the method.

The lizards were killed with ether, the body was opened and the oviducts containing the embryos were removed and placed in a Petri dish partly filled with 0·6 per cent. NaCl. The oviduct was then peeled away from the eggs with watchmakers' forceps. One or more of the eggs were fixed so that the stage of development at the onset of culture could be ascertained and referred to the normal table by Dufaure & Hubert (1961). As a rule all the embryos in a litter are at about the same stage of development, but this is not invariably the case.

After liberation from the oviducts the eggs were transferred individually by means of a wide pipette to small Petri dishes, one egg to each dish. They were placed with the embryo uppermost and the yolk downwards to facilitate daily inspection. Each dish contained a circle of gauze moistened with about 3 c.c. of a mixture of chick albumen (1 part) and 0·6 per cent. NaCl (4 parts). Egg albumen is believed to have a fungicidal effect (Tokin, 1959) and our findings (Table 2) support this view. All glassware, instruments, gauze and saline were sterilized and the removal of the eggs was carried out under aseptic conditions. The embryos in their small dishes were finally placed in a large covered glass or plastic receptacle containing an open dish of distilled water to maintain humidity.

The embryos were kept at room temperature for about 24 hr. before operation. After such an interval the embryonic membranes, which include a thin shell membrane (Panigel, 1956), seem to become harder and the egg is easier to handle and less liable to collapse after the membranes have been cut. The operations were carried out under a dissecting microscope. An incision was made in the membranes, avoiding as far as possible the larger chorioallantoic blood-vessels. The tail was then withdrawn with watchmakers' forceps and broken or cut off with scissors above the forceps' grip. The tail stump was then replaced within

TABLE 2

Survival and hatchability of 1963 embryos after tail amputation

	Dufaure-Hubert stage at operation	
	30–32	39–40
Total number of embryos*	43	18
Dying within 3 days of operation	6	1
Becoming grossly abnormal	6	0
Put up without albumen	21	0
Without albumen infected	9	0
Put up with albumen	22	18
With albumen infected	3	0
Dying before reaching stage 37	13	0
Dying at stages 37–38	11	0
Dying at stages 39–40	12	0
Died hatching	3	4
Hatched alive	4	13

* 17 further embryos were used as standards for age assessment.

the egg (often with difficulty) and the rent in the membranes was closed with a small piece of sterile cotton handkerchief which soon became adherent to them (Plate 1, Fig. C). This dressing helps to prevent the embryo from prolapsing through the membranes. Other operations such as removal of limb-buds (see Plate 1, Fig. A) and parts of the head could also be performed, though less easily.

After operation the embryos may be kept at room temperature but develop more rapidly and without higher mortality if kept at a constant temperature of about 28° C. In these experiments they were allowed to develop until they either died or hatched. It was found that embryos which succeeded in hatching often died soon afterwards if they were not removed from their dishes. Drowning or asphyxiation in the moist sticky environment may have been the cause of death. Such mortality was apparently reduced if the embryos were transferred to fresh Petri dishes containing dry or only slightly moist gauze shortly before they were expected to hatch.

Panigel (1956, p. 595) states that in *Lacerta vivipara* embryonic development can occur outside the mother 'dès les premiers stades' and found that specimens placed in culture at blastodisc stages became little embryos with beating hearts after about a fortnight. The earliest embryos used with any success in these experiments, however, were at about Dufaure-Hubert stage 30, which is roughly equivalent to a chick embryo of 4 days. At this stage the tail is well developed and curled but its mesoderm is still segmented into somites. The limb-buds are paddle-shaped but not yet differentiated into upper and lower regions.

The total number of embryos subjected to tail amputation at all stages between Dufaure-Hubert 30 and the time of hatching was about 120 in 1962 and 61 in 1963. The immediate post-operative mortality was fairly low and the embryos

TABLE 3

Particulars of embryos and sectioned tails after amputation during embryonic life

		Days survival in culture			
Embryo No.	*Stage at operation*	*At room temperature*	*At 28° C.*	*Stage reached*	*Regeneration*
1	30	—	20	39–40	–
2	30	—	20	39–40	–
3	30	—	20	39–40	–
4	30	—	24	39–40	–
5	30	—	21	39–40	–
6	30	—	19	H	–
7	30	—	19	DH	–
8	30	—	24	39–40	–
9	30	—	19	39–40	–
11	30	—	17	39–40	–
12	30	—	10	37	–
13	31	36	—	39–40	–
14	31	—	20	H	–
15	31	—	21	DH	–
16	31	—	20	H	–
17	32	—	19	H	–
19	32	17	—	37	–
20	32	29	—	39–40	–
21	32	33	—	39–40	–
22	32	35	—	39–40	–
23	32	27	—	39–40	–
24	33	20	—	H	–
25	33	10	—	39–40	–
26	33	31	—	39–40	–
27	33	21	—	39–40	–
28	33	25	—	39–40	–
29	33	24	—	39–40	–
30	33	—	18	38	–
31	34	13	—	38	–
32	34	19	—	39–40	–
33	34	17	—	39–40	–
34	34	18	—	39–40	–
35	34	18	—	39–40	–
36	34	18	—	H	–
37	34	18	—	H	–
38	35–36	16	—	39–40	–
39	35–36	21	—	39–40	–
40	35–36	18	—	39–40	–
41	37–38	16	—	H	–
42	37–38	23	—	39–40	–
43	37–38	13	—	H	–
44	37–38	9	—	39–40	+
45	37–38	9	—	39–40	+
46	39–40	5	—	DH	+
47	39–40	9	—	H+7 d	+
48	39–40	0 (2 hrs.)	—	H+14 d	+

TABLE 3 (continued)

Embryo No.	*Stage at operation*	*Days survival in culture*		*Stage reached*	*Regeneration*
		At room temperature	*At 28° C.*		
49	39–40	7	—	H+27 d	–
50	39–40	5	—	H+21 d	–
51	39–40	—	7	H	–
52	39–40	—	7	H+11 d	–
53	39–40	—	6	H+36 d	–
54	39–40	—	6	H	+
55	39–40	—	6	DH	+
56	39–40	—	6	39–40	D
57	39–40	—	6	H	+
58	39–40	—	6	H	+
59	39–40	—	8	H+2 d	D
60	39–40	—	6	H+5 d	+
61	39–40	—	7	H+4 d	+

Abbreviations: d, days after hatching; DH, died hatching; H, hatched; +, positive regeneration; –, no regeneration; D, doubtful regeneration.

Note: Embryos 14 and 53 underwent further operations during post-natal life, and are shown as lizards 17 and 4, respectively, in Table 1.

seemed to be more resistant to haemorrhage and other injuries than chick embryos. A high proportion (over 60 per cent.) of the 1962 series, however, were killed by infection or prolapsed partly or completely from the egg (Plate 3, Fig. Q). In these latter cases adhesion bands, probably derived from the embryonic membranes often formed round the body or tail where it was extruded from the egg. Such embryos sometimes survived for long periods like those which became infected with fungus, but they developed gross abnormalities, especially of the hind end of the body (Plate 3, Fig. P) and seldom reached near-hatching stages.

The much better results obtained in 1963 are ascribed to improvements in technique, especially the use of the handkerchief dressing and the addition of albumen to the saline medium, which may have some slight nutritional benefit, as well as preventing infection. Table 2 shows the survival periods and hatchability of this series of embryos, which, unfortunately, contained no specimens between stages 32 and 39–40 at the time of operation. The histological data on the intermediate stages given in the next section are derived from the 1962 experiments. It is clear, however, that, as one might expect, the difficulties and hazards of the operation diminish the later the stage at which it is carried out. Nevertheless, embryos operated on at the earlier stages (30–32) often reached 39–40 before they died, and a few succeeded in hatching. Panigel found that even embryos which had not been subjected to operation often died shortly before they were ready to hatch.

After fixation the remaining portion of tail was removed from fifty-nine embryos in all, taken from both the 1962 and 1963 series, serially sectioned and stained with haematoxylin or by a trichrome method. Individual particulars of these embryos are given in Table 3. They had all survived for a minimum of 5 days after operation (generally much longer) and showed no gross abnormality of the tail region apart from that which could be ascribed directly or indirectly to the experimental injury. Some of them, however, had developed localized malformations not involving the caudal region, such as prolapse of the viscera and deformities of the hind limbs. One case of bilateral cleft palate, which may not be attributable to the effects of culture or operation, is reported elsewhere in a paper on reptilian teratology (Bellairs, 1964 or 1965).

Effects of tail amputation in embryos

It was found that a number of the embryos which were operated on at the earlier stages subsequently developed very short stumpy tails (Plate 1, Fig. B). These were obtained so often as to suggest that the accidental selection of too high an amputation site was not the only factor responsible. It is possible that a second amputation of the tail by bands derived from the embryonic membranes may have been the cause (see addendum).

In many other specimens tails of 15 per cent. or more of the normal length were obtained. In these, as in the very short-tailed examples, the organ usually ended in a blunt scaly tip (Plate 1, Fig. A) suggesting that complete healing had taken place. Only in certain specimens operated on at very late stages was a regeneration cone observed. The histological findings were very consistent in the great majority of cases. The following conditions were found in almost all the embryos operated on between stages 30 and 38, and which had survived in culture for periods of 13 to 36 days afterwards. They were also found in some of the specimens in which the operation had been performed at the very late stages 39–40.

The tip of the tail was covered by scales which were irregular in arrangement but on the whole similar in structure to normal scales above the region of injury (see Plate 2, Fig. G; Plate 3, Fig. K). The resemblance was evident in such features as the probable extent of keratinization, the structure of the epidermal and dermal layers and the distribution of the melanocytes. The remaining portions of the last caudal vertebra extended almost to the tip of the tail. They were sometimes distorted but it was usually possible to determine the approximate level at which it had been cut through. In some specimens the spinal cord also descended to the tip of the tail (Plate 3, Fig. N), but in the majority of cases it ended cranial to the cut surface of the vertebra. Strands of material which appeared to have grown out from it passed backwards to end in the tissues of the tail tip, sometimes becoming attached to a part of the vertebra (Plate 2, Fig. G; Plate 3, Fig. K). These strands were partly derived from

the meninges but in the absence of selectively stained material it was impossible to say whether they contained nerve fibres. The connective tissue near the site of injury resembled that elsewhere in the tail and there was no indication of blastema formation. The whole appearance of the region suggested that rapid healing had taken place and that regeneration would not have occurred however long the animal had survived. It contrasted strikingly with the picture of a typical regenerate (Plate 2, Fig. H; Plate 3, Fig. L).

Certain atypical features were seen in a few specimens which in other respects resembled the non-regenerating embryos just described. Patches of thickened epidermis differing from both the normal epidermis and from that of the apical cap of regenerates were occasionally seen. In embryo 14 (Table 3) epidermis of this type covered the small prominence at the extreme tail tip and appeared to be heavily keratinized (Plate 3, Fig. M). There were, however, no signs of regeneration although the embryo had lived for 20 days in culture at 28° C. after operation. This specimen was also interesting in being the only one among those operated on at a relatively early stage (stage 31) which we were able to keep alive for a considerable period after hatching. The tail was re-amputated 2 hr. after it had hatched, the cut off piece providing the section shown in Plate 3, Fig. M. Twenty days after this second amputation the lizard had developed a typical post-natal regenerate 1 mm. long containing immature muscle and procartilage (Plate 3, Fig. L). It is listed as No. 17 in Table 1.

In two of the embryos the cut spinal cord ended in a structure resembling the ependymal tube of a regenerate, instead of merely having strands of tissue leaving it. The presence of this feature in non-regenerating embryos is of interest since Simpson (1964) has shown that the ependymal tube is probably responsible for the initiation of tail regeneration and the differentiation of the cartilage tube in adult *Lygosoma*. In both embryos there was also a well developed ingrowth of the epidermis of the tail tip which seemed identical with a structure described by Hughes & New in early adult regenerates as the epidermal papilla (Plate 2, Fig. I). They suggested that its formation was stimulated by the activity of the ependymal tube. The papilla was also found in some of our post-natal regenerating specimens, but its significance is not clear.

In some embryos raw patches or bands appeared on the tail after operation. Sections of these showed that there was no normal epidermis over the affected region (Plate 3, Fig. N). The surface was covered by a thin layer of cells which probably represented an adherent portion of chorioallantoic membrane. Small tags of tissue, perhaps of the same origin, were also sometimes found projecting from the tail tip.

Interesting results were obtained from the group of sixteen embryos operated on in the final stages (39–40) of pre-natal life. Most of these hatched after about a week in culture. Several of them (see Table 3) failed to regenerate even after considerable periods of post-natal survival. No. 53 was a particularly striking example. The section figured in Plate 2, Fig. I, shows the result of the original

amputation performed in embryonic life, after 42 days, 36 of them representing post-natal survival. No regeneration has occurred although an ependymal tube and epidermal papilla have developed. This section was obtained by a second amputation at an intervertebral level on the 36th day of post-natal life; after a further 21 days autotomy was induced. After both these injuries regeneration occurred within 22 days (see Plate 2, Fig. H). This individual is listed as No. 4 in Table 1.

The majority of embryos operated on shortly before hatching reacted in a different fashion and showed good evidence of early regeneration, even in some cases after periods as short as 6 days in culture (Plate 2, Fig. F). The formation of an apical cap and blastema, of an ependymal tube, and occasionally of an epidermal papilla could be seen; a few of the regenerates contained immature muscle and procartilage. In two further individuals it was impossible to say whether or not regeneration was taking place and they are listed as doubtful in Table 3.

Regenerative changes were also found in two embryos (44 and 45) which were operated on at stages originally estimated (and shown in Table 3) as 37–38. The fact that both these specimens reached stage 39–40 after only 9 days at room temperature suggested that our original age assessment, based in any case on circumstantial evidence, had erred on the early side. Unfortunately our sample of this age group was too small to enable us to determine precisely the stage at which regeneration first becomes possible.

DISCUSSION

Validity of experiments on embryos

The regenerative or regulative capacity of early embryos before the period of organogenesis is unknown since we were unable to use them in our experiments. Our observations seem to show, however, that the power of tail regeneration possessed by young and adult lizards is absent throughout much of embryonic life but may develop shortly after birth.

Our results might be invalid because of some defect in the conditions of experiment, such as inadequate survival periods after operation; but this seems unlikely. Baby and adult lizards show undoubted regenerative changes after 2 to 3 weeks, despite fluctuations in their environmental temperature and food intake. Embryos operated on at stages 30–31, nourished by their own yolk and kept at a constant temperature of 28° C., fail to regenerate after 20 days, which may be equivalent to about 30 days at room temperature (see Table 3). Moreover, some of the specimens which were operated on as very late embryos began to regenerate after as little as 6 days.

The relationship of the amputation site to the autotomy planes, whether differentiated or presumptive, seems to have little bearing on the problem. Regeneration in the adult can take place at any site or level, even, according to

Slotopolsky and Woodland, after amputations through the base of the tail, producing a condition like that of our short-tailed embryos. This supports the prevalent view (Singer & Saltpeter, 1961) that the blastema of the regenerate arises by de-differentiation of tissues near the site of injury, rather than from persistently embryonic cells derived from the notochordal cartilage or other parts of the fracture plane.

The question also arises as to whether embryos raised in Panigel culture are in some way deficient as compared with normal embryos. This certainly appears to be the case with embryos of *Anguis*, which fail to reach their proper size and generally become abnormal after long periods in Panigel culture (Holder & Bellairs, 1962). Presumably the embryos of this lizard have a more intimate physiological relationship with the mother than those of *Lacerta vivipara*.

It is possible that embryos of *L. vivipara* which hatch after a considerable time in culture are slightly smaller on average than the new-born offspring of a normal gestation, but we have no conclusive evidence of this. The weights of four specimens, cultured after operation at stages 30–32 for 19–21 days at 28°C. ranged from 0·15 to 0·18 g. at hatching. This was within the range of variation (0·13 to 0·26 g.) of eleven specimens put up in culture at stages 39–40 which hatched after only about a week. Unfortunately we had no normal new-born young available for weight comparison, but the snout–vent lengths of all the cultured specimens were within the normal range (17–22 mm.) given by Smith (1964) for young of this species. The behaviour of Panigel hatchlings does not differ noticeably from that of babies born in captivity; they can usually be induced to feed on *Drosophila*, green aphids and tiny spiders. Even if Panigel embryos prove to be marginally 'subnormal' in certain respects it does not follow that such a basic function as regeneration would be completely suppressed. In fact, two of our embryos (Nos. 14 and 53) which did not regenerate after amputation during embryonic life produced normal regenerates after post-natal amputation or autotomy.

Causes of change in regenerative capacity of embryos

Our experiments were designed primarily to determine whether there was a genuine failure of regeneration after the longest possible survival periods. Unfortunately they provide only a slender basis for discussion as to the causes of this failure. Further investigation is necessary, especially of the reactions of the nervous system and of the epidermis; it is well known that these both play an important part in regeneration (see Singer & Saltpeter, 1961). The work of Barber (1944) suggests that the clue to the problem may well lie in the relationship of the skin to the deeper tissues, and in the existence of some critical balance between regeneration and healing.

Barber compared the response to amputation of the non-regenerating fore-limbs and the regenerating tails of adult *Anolis* lizards. The initial effects were

similar in both; a scab formed, and beneath this a thin layer of epidermis spread across the wound. The tissues of the cut limb, however, shrank rapidly so that the bone projected slightly from the stump, and the wound surface was soon closed by the apposition of the surrounding edges of scaly skin. De-differentiation of the wound tissues occurred only to a limited extent. The edges of the tail wound, on the other hand, remained apart and it is clear from Barber's picture that a typical apical cap was formed over its relatively wider surface. Beneath this, extensive blastema formation took place.

Barber attributed these differences in the reactions of the limbs and tail to differences in their structure. The skin of the tail is stiff because of the hardness of the scales and the fact that the dermis is firmly attached to the underlying muscles. The skin of the limbs is more flexible, the muscles are less compact, and the two are separated by large lymph spaces so that greater shrinkage of the tissues can occur after injury. Such differences are clearly visible in sections of lizards at the time of hatching (Plate 3, Fig. O).

The structure of the skin and subcutaneous tissues of reptiles changes markedly during later embryonic life. In *Lacerta vivipara* the scales begin to appear as elevations on the surface of the body around Dufaure-Hubert stage 35, but substantial keratinization of the epidermis probably does not take place until a considerably later stage. Shortly before birth the sub-epidermal tissues also become more closely attached to the muscles. It is possible that this general stiffening of the skin prevents the wound surface from being prematurely closed, so that the conditions necessary for regeneration are realized. In the earlier embryos the skin is less highly differentiated and the wound area is smaller; rapid healing by apposition of the cut epidermis may take place so that regeneration is prevented.

Our findings seem to conflict with the generally observed tendency among animals for regenerative capacity to decrease with age and extent of histological differentiation. As Needham (1952) points out, however, this tendency is not universal. The power of regeneration, like that of autotomy, is a product of natural selection. It is doubtless useful to the free-living amphibian larva and to the baby or adult lizard exposed to the jaws of predators; it has no relevance to the life of the amniote embryo developing within its egg or the body of its mother.

SUMMARY

1. The structure and development of the fracture planes in the tail of *Lacerta vivipara* are briefly described.

2. The histological features of normal tail regeneration in baby and adult lizards are described. Regeneration occurs after autotomy or after amputation through either an intravertebral or an intervertebral plane, and becomes clearly visible after 2 to 3 weeks.

3. The technique, based on the work of Panigel, of rearing embryos of lizards

in a form of culture after removal from the mother, and of operating on them is described.

4. In recent experiments over 40 per cent. of the embryos subjected to the operation of tail amputation at stages 30–32 in the normal table by Dufaure and Hubert survived to reach near hatching stages (39–40). A few hatched after periods of 17 to 24 days in culture at 28° C. Embryos operated on at very late stages generally succeeded in hatching.

5. The effects of tail amputation during embryonic life were studied histologically in fifty-nine individuals. Almost all the embryos operated on at stages younger than 39 failed to regenerate after up to 36 days in culture, and showed appearances suggestive of rapid healing. Most of the embryos operated on at stages 39–40, very late in embryonic life, showed early regenerative changes. Others showed failure of regeneration, as in the younger embryos, and in a few specimens the results were doubtful.

6. A possible reason for the lack of regenerative power in embryo lizards, and for its appearance at or shortly before hatching (or in nature, birth) is suggested. There is evidence that regeneration may depend upon the rate of healing and the extent of the wound surface. Substantial keratinization of the scales and stiffening of the sub-epidermal tissues occur towards the end of embryonic life. By preventing premature healing and helping to maintain a large wound surface such changes may provide the necessary conditions for regeneration.

7. From the standpoint of natural selection the lack of regenerative power of the lizard embryo is not surprising, since regeneration would have no survival value until post-natal life.

RÉSUMÉ

La capacité régénératrice de la queue du lézard embryonnaire et post-natal (Lacerta vivipara, *Jacquin*)

1. La structure et le développement des plans de fracture de la queue de *Lacerta vivipara* sont décrits brièvement.

2. Les caractéristiques histologiques de la régénération normale de la queue chez les lézards jeunes et adultes sont décrites. La régénération se fait après l'autotomie, ou après amputation à travers un plan intra- ou intervertébral, et devient évidente après deux à trois semaines.

3. La technique, basée sur le travail de Panigel, pour élever en culture les embryons de lézard qu'on a enlevés de la mère, et pour les opérer, est décrite.

4. Dans les expériences récentes, plus de 40% des embryons qui avaient subi l'opération de l'amputation de la queue aux stades de 30 à 32 du schéma de développement normale de Dufaure et Hubert, ont survécu presque jusqu'aux stades d'éclosion (39 à 40). Quelques-uns sont éclos après 17 à 24 jours en culture à 28° C. Les embryons opérés à des stades très tardifs ont presque toujours réussi à éclore.

5. Les effets de l'amputation de la queue pendant la vie embryonnaire, ont été étudiés histologiquement sur 59 individus. Presque tous les embryons opérés à des stades antérieurs à 39, n'ont pas régénéré pendant les 36 premiers jours de culture; il y avait plutôt des indices de cicatrisation rapide. La plupart des embryons opérés aux stades 39 à 40, très tard dans la vie embryonnaire, a montré les premiers changements régénératifs. D'autres n'ont pas régénéré, comme dans la cas des embryons plus jeunes, et chez quelques-uns, les résultats étaient douteux.

6. Une raison possible, du manque de capacité régénératrice chez les embryons de lézard, ou de son apparition au moment de l'éclosion ou juste avant celui-ci (dans la nature, la naissance), est suggérée. Il semble que la régénération puisse dépendre de la vitesse de cicatrisation et de l'étendue de la surface de la blessure. Vers la fin de la vie embryonnaire il se fait une kératinisation considérable des écailles, et les tissus sousépidermaux se raidissent. En empêchant la cicatrisation prématurée, et en assurant le maintien d'une surface étendue de blessure, de tels changements pourraient fournir les conditions nécessaires à la régénération.

7. Du point de vue de la sélection naturelle, le manque de capacité régénératrice de l'embryon de lézard n'est pas étonnant, car une telle régénération n'aurait aucune valeur pour la survie jusqu'à la vie post-natale.

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ADDENDUM

Experiments made since this paper went to press strongly suggest that in some cases constriction bands derived from the embryonic membranes develop around the tail stump and cut through it proximal to the site of experimental amputation. This would account for the appearance of embryos with very short tails. It is possible that this type of self-amputation, effected by ligature, might increase the speed of healing by apposition of the epidermis and hence prevent regeneration.