TAIL REGENERATION AFTER AUTOTOMY IN THE GECKONID LIZARD *BUNOPUS TUBERCULATUS*

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تجدد الذيل بعد الانفصال الذاتي
في البرص بونوبس تيوبركيولاتس

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قام الباحثان بدراسة تجدد الذيل في البرص بونوبس تيوبركيولاتس مورفولوجياً وهستولوجياً بعد حث الحيوانات على احـداث الانفصال الذاتي للذيل . وقد أزيل ٧٠٪ من طول الذيل لكل حيوان . وتمت دراسة مراحل التجدد المختلفة للذيل وهي : التئام الجرح ، فقد التميـز ، تكوين البلاستيما ، إعادة التميـز والنمـو . وقد أوضحت النتائج وأظهرت تكويـن البلاستيما القمية والتي ظهر بها تزايد عـددي للخلايـا . ومـن هـذه البلاستيما تميزت الأنسجة المختلفة للجزء المجدد من الذيل . وقد درس أيضاً معدل التجدد أو معدل استطالة الذيل أسبوعياً ولمدة ثمانية أسابيع بعد الانفصال الذاتي للذيل . وقد كان أعلى معدل هو ٣٦ , ٠ مم لليـوم ، ويعتبر معـدل الاسـتطالة في بونوبس بوجـة عـام بطئ أو أقل بكثير عنـد مقارنتـه بمعدلات التجدد أو الاسـتطالة التي درسـت أثنـاء تجـدد الذيل في السحالي الأخرى .

Key Words: Autotomy, *Bunopus*, regeneration, blastema, rate.

ABSTRACT

Tail regeneration in the geckonid lizard, *Banopus tuberculatus* was morphologically and histologically studied after induced atutomy. Seventy percent of the tail of each animal, by length, was removed by autotomy. Regeneration phases; wound-healing, dedifferation, blastema formation, redifferentiation and growth were investigated. The results have established the formation of an apical blastema within which cellular proliferation and differentialtion of the regenerate tissues have occurred. Rate of regeneration, or the elongation rate, was weekly studied for eight consectuive weeks after tail autotomy. The maximum rate observed was 0.36 mm per day, and the elongation rate in *Bunopus* was generally much slower than in other examined lacertilians.

INTRODUCTION

Lacertilian reptiles are the only vertebrates that can regenerate an appendage from a discrete preformed break (autotomy) plane. This singular adaptation is a protective mechanism which enables the lizard to twist off its tail when the appendage is seized by an enemy. The severed tail jumps about by reflex action and presumably occupies the attention of the hunter while the lizard escapes. The regenerate that forms after normal autotomy of the tail does not contain ventebrae; instead the vertebral column is replaced by an unsegmented cartilage tube (Duges, 1929). Many studies have been made on autotomy and regeneration of the tail in lizards; for example those by Woodland (1920) and Hughes

and New (1959) on geckos, by Slotopolsky (1922) and Moffat and Bellairs (1964) on *Lacerta*, by Simpson (1964) on *Lygosoma*, by Kamrin and Singer (1955) and Cox (1969) on *Anolis*, and by Magon (1977) on *Mabuya*. White (1925) described 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 tissued differentiate. Quattrini (1954) considered 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. Hughes and New (1959), working on tail regeneration in *Sphaerodactylus* demonstrated the formation

of a regenerating blastema from which arise the cells that form cartilage, muscle fibres, general mesenchyme, as well as Schwann cells and melanocytes. In the regenerating tail of *Lygosoma*, Simpson (1965) reported an accumulation blastema that is made up of cells of various sizes. The results of Cox (1969) strongly suggested that there is no apical blastema in the lizard tail regenerate. He proposed that the term blastma, as applied in amphibian limb regeneration, can not be applied in lizard tail regeneration. The growth of a normal regenerate results from extensive interstitial growth in the differentiating tissues and from subapical growth in the less differentiated areas of these tissues. The present work reports the results of experiments designed to test the ability of the geckonid lizard, *Bunopus,* living in Qatar, to regenerate tail after induced autotomy and the rate of the growth of the regenerating tail.

MATERIAL AND METHODS

Individuals of the adult gecknoid lizard, *Bunopus tuberculatus* were collected from different regions in Doha, Qatar. The body length, from snout to vent, ranged from 3.5-5 cm and the tail length, from vent to the tip of the tail, ranged from 3.5-4.5 cm. Forty adults, without previsouly regenerated tails, were selected. This species is a convenient gecko for experimental work, because it is avaialable in quantity from various areas in Doha, as is easily maintained for long periods of time in the laboratory. The animals were chosen to be nearly of the same size, they were kept in the laboratory in glass museum jars, each containing three or four animals. Heater was placed near the jars to raise their temperature and it was controlled to be $27 \pm 2°$ C, with 8-12 hours of light per day. The appetite of the animals was markedly depressed, and therefore, all animals were hand-fed a standard diet, one meal of meat daily. Water was provided in small dishes.

The tail autotomy was induced by pinching off tails, leaving the basal third of the tail as a stump which ranged from 10-15 mm in length, between cloaca and the plane of autotomy. This means that about seventy percent of the tail of each animal, by length, was removed by autotomy. No growth was observed during the first week after autotomy. Thereafter, measurements of the growth of the regenerate were made weekly through the following eight weeks after atutotomy. for histological observations, regenerating tails with at least one or two segments of the original tail stump were fixed in Bouin's fluid, for about 24 hours at room temperature, daily, 2-3 days or 7 days intervals. These tissues were declacified in 2% acid alcohol (nitric acid + 70% alcohol), dehydrated and infiltrated with paraplast. Serial longitudinal sections of 8-9 μm thick were cut in an air-conditioned room at 21-22° C, and stained with borax carmine-modified Azan. Samples of the regenerates were fixed in FAA (formalin, acetic acid, alcohol, 10:5:85 in volume) and stained with methylene blue (0.5% in 95% ethyl alcohol) to examine the formation of the regenerating cartilage, during the late phases of regeneration.

RESULTS

Histological observations:

The entire period of tail regeneration has been divided histologically into five different stages: wound-healting stage, dedifferentiation stage, blastema stage, redifferentiation stage and growth stage. However, the process of regeneration is, in fact, a continuous one.

Wound-healing and dedifferentiation stages:

Following autotomy there was a loss of blood but it is normally not excessive. By day one and two, blood alongwith other body fluids and necrotic cellular material formed a clot which dries into a hard wound scab (Fig. 1). This scab incorporated both the tip of the broken vertebra and the tips of the muscle bundles extending posteriorly beyond the level of skin and bone autotomy. At the margins of the broken surface underneath the scab and beyond the open end of the central canal, accumulated cells from various sources: lymphocytes; macrophages and microglial elements of the spinal cord. By day two, the number of phagocytic elements appeared to have increased in the area under the scab, and macrophages were loaded with cellular debris. This increase appeared to continue through days four or five. The perivertebral fat contained numerous lymphoid-like connective tissue cells either singly or in aggregates. During the first three days following autotomy, some of these lymphoid-like cells were found in the wound area. Osteoclasts were observed by day two and small areas of bone dissolution indicated that they were active. A day later, these osteoclasta were numerous all along the shaft of the broken vertebra, both inside and outside. By day three, melanocytes were found on the dorsal surface of the spinal cord. The spinal cord was already detached from the scab and appeared to be retracting. The ependyma was retracted from the end of the spinal cord. In fact, various injured tissues get detached from the cut surface. The spinal cord, vertebral column, muscle and fat layers, at the cut end of the original tail, showed a tendency for dedifferentiation. Slowly and with much basal cell division, the would epithelium, derived from the epidermis of the stump, migrated as a sheet under the scab (see Fig. 1). On the fourth day, an outgrowth of the epondyma appeared as a sac or vesicle (Fig. 2). On the 5th day, the new epidermis (wound epithelium) became everywhere much thicker to form a thick cap, and within it the distinction between an inner germinativum layer and an outer stratum corneum became clear. This cap, which has been called the apical cap, has a very characteristic appearance and differs strikingly from the epidermis of normal sclaes. The new epidermis remained much thicker than that of the stump until the time when scales differentiate (see Fig. 3). Within the wall of the ependymal sac, mitotic figues were observed. This sac appeared to be an active centre of proliferation (see Fig. 2).

Blastema stage:

At the end of the fifth day, and beneath the thickened epidermis, a cone of blastema cells developed. Both the regenerating epidermis and the blastema contained many melanocytes which gave the regenerate its dark colour. Many mitotic figures were recovered in this early blastema. The ependymal sac or vesicle very soon had grown back into the blastema as a long slender tube lined by ependyma and called the ependymal tube (Figs. 2, 6 & 9).

Redifferentiation and growth phases:

On the 7th day, within the blastema, there was a dense areas of blastema cells which represented the anlage of the musculature of the regenerate. The promuscle aggregates appeared under the wound epithelium (Fig. 3). By days 7 to 9, the young regenerate became 0.5 mm long and was covered with a black epithelium. The promuscle aggregates became differentiated into elongated myoblasts which extended towards the tip to the level of the ependymal tube and were much larger and distinct. Mitotic figures were more common among the myoblasts than in the blastema generally. At 10 to 11 days, a wide space was still observed between the regenerating myoblasts and the original (stump) musculature;

it was occupied by a loose mesenchyme. By days 11 to 14, the ependyma approached the epithelium and the regenerate began to enlarge. When the regenerate became 1 mm in length, the apical region, occupied by blastema cells, began to form an ordered aggregate around the outgrowing ependymal tube. The aggregate was occupied by spindle-shaped (fibroblast-like) cells, some stellate (mesenchyme-like) cells, and many melanocytes. The cells aligned themselves with their axes perpendicular to that of ependyma (Figs. 3 & 4). Over the next few days they differentiate into a clarly discernible procartilage tissue surrounding the ependymal tube, these chondroblasts formed the cartilaginous tube (Figs. 5, 6, 7 & 9). On the 16th day, this tube appeared still opened at the tip for some days (Figs. 5 & 6). The regenerating myoblasts appeared to be extending proximally into the autotomy cavities as a result of cell migration into these cavities (Fig. 7). Within stratum germinativum, mitotic figures were common everywhere. Melanoblasts were most frequent in the epidermis and in the future dermis. In the following days, when the regenerate reaches 2 mm in length, the inner surface of the epithelium became uneven, and these irregularities developed into a series of internal ridges. The dermis was moulded into a series of bays, each of which would be the core of a future dermal scale (Fig. 8). The dermal papillae were formed, increased in size, pushing the epidermis with scale as the projecting body. The dermis is mainly composed of fibroblasts which are differentiated into fibrocytes. Formation of various types of epidermal cells was observed. Externally the scale began to differentiate proximally after 30 to 40 days, when the regenerate was about 6 mm long. By the 45 to 55 days with regenerate of 7-8.5 mm in length, the epidermal ridges became tilted so that they pointed away from the tip of the regenerate. Within the stratum corneum of the developing scales, dense lamellae of keratin were formed, but the whole outer surface of the regenerate was still covered by an even surface of cornified cells which filled the spaces between the young scales. These scales were at first soft and small, but with the completion of differentiation they have expanded, along with the tail, to their mature size (Fig. 10). As the scalation developed, the epidermis became much thinner than at earlier stages and then the pigment cells were no longer observed in the epidermis. By stage 55-65 days, the regenerate was about 7-9.5 mm in length, the scale differentiation proceeded proximodistally, and the entire regenerate became gradually covered with scales.

Rate of regeneration:

During the growth of the regenerating tail, three periods were observed during which the rate of regeneration was changed. First, there was an interval during which the blastema is being formed and in which little or no growth was visible externally. Then there was a period of rapid elongation, the rate of which was very high. Finally in the third period the rate of regeneration decreased gradually as the regenerate approached its full length. Table 1 summarizes the changes in the rate of regeneration. The elongation or regeneration rate was somewhat varied among the operated animals. The regenerate length and regeneration rate, in Table 1, are the means obtained from twenty animals which were left to regenerate their tails till attaining nearly their original lengths. The data in Table 1 clearly indicate the continuous increase of regeneration rate during the period form the 2nd to the 5th week after tail autotomy. This period was occupied by the formation and elongation of the cartilage tube, differentiation of various types of epidermal cells and the beginning of scale differentiation. During the proceeding development of regenerate scales, .accompanied by the formation of keratin lamellae and the epidermis became no

longer much thicker as in the early stages (from the 6th to the 9th week), the regeneration rate was decreased.

Table 1

Lenghts of regenerates (mm) and regeneration rates (mm/day) of the regenerating tails of the gecko, *Bunopus tuberculatus*. Values are means ± S.D. (20 animals were used).

Days after tail autotomy	Regenerate length (mm)	Regeneration rate (mm/day)
7	0.5 ± 0.12	0.07 ± 0.01
14	1.0 ± 0.14	0.07 ± 0.02
21	2.0 ± 0.18	0.14 ± 0.07
28	3.5 ± 0.20	0.21 ± 0.08
35	6.0 ± 0.19	0.36 ± 0.12
42	7.0 ± 0.22	0.14 ± 0.10
49	7.8 ± 0.36	0.11 ± 0.06
56	8.5 ± 0.41	0.10 ± 0.09
63	9.1 ± 0.28	0.08 ± 0.05

Non-regenerating tails:

Out of the forty tails autotomized, 31 showed normal regeneration (according to histological investigation); they formed thickened epithelium, and there was dedifferentiation of distal stump tissues, blastema formation, redifferentiation and growth of the new tissues in the regenerate. Six animals died during rearing and were excluded from the study. In the remaining three animals, the tails failed to restore the autotomized segments. In such operated cases there formed wound epithelium which was not thickened as in the normally regenerating tails and stump tissue dedifferentiation was not observed (Figs. 11, 12 & 13). At the centre of the new epidermis, an inward prolongation appeared in the form of a papilla (Fig. 13). Melanocytes were numerous within the central papilla. The ependymal tube and the cartilage tube were never formed. However, a mass of cartilage cells was found at the level of autotomy near the cut border of the vertebra (Fig. 12). There were also observed some aggregates of promuscles. The regenerate never exceeded 0.5 mm in length (ranging from 0.2-0.5 mm), occupied mostly by connective tissue (Fig. 12), with rare mitotic figures. The regenerate part was covered by developing scales; below the epidermis of such scales, the connective tissue of the dermis formed dermal papilla containing some scattered melanocytes just underneath the epidermis. At the distal part of the regenerate part there appeared a dense and compact layer of fibrous connective tissue forming a connective tissue scar.
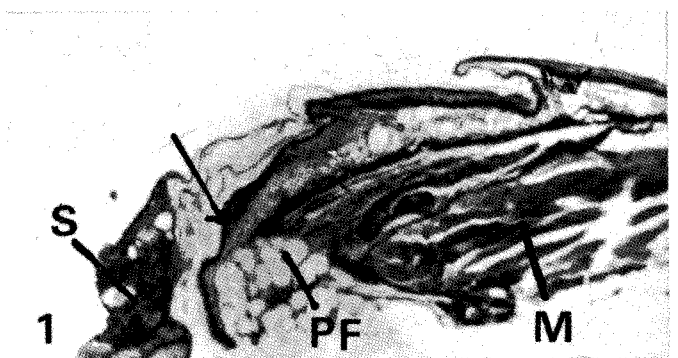

Fig. 1: Longitudinal section through regenerating tail, three days after autotomy. The arrow indicates the wound epithelium which migrated as a sheet under the scab. M, muscle; S, scab; PF, perivertebral fat. X 80.
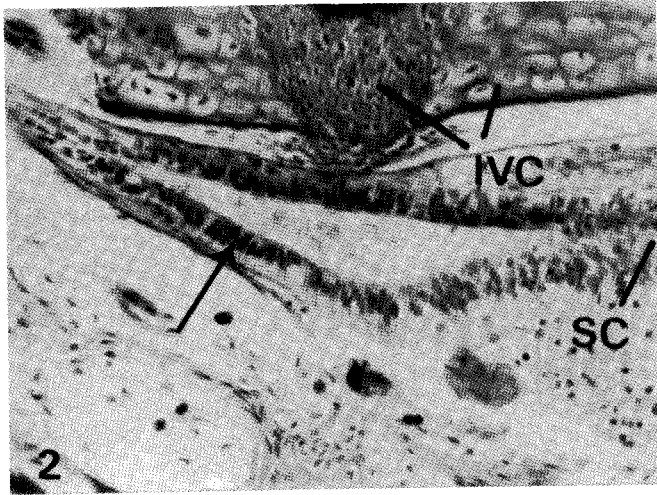
Fig. 2: Part of longitudinal section through the distal region of regenerating tail, 6 days after autotomy. The arrow indicates the ependymal tube. IVC, interventebral cartilage; Sc, Spinal Cord. X 340.
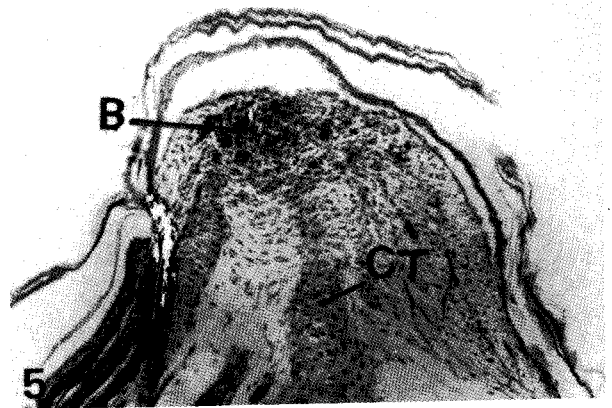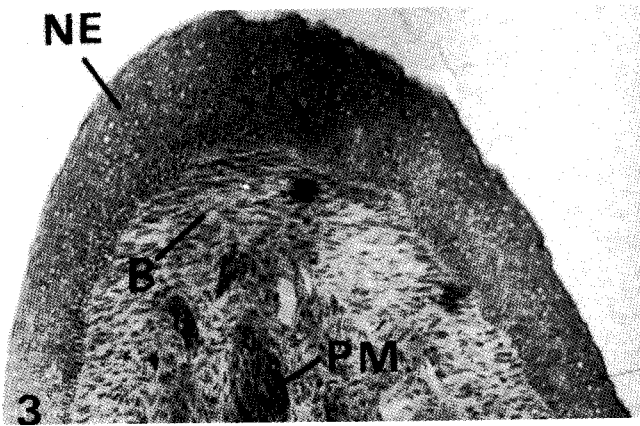

Fig. 3: Longitudinal section through the distal part of tail regenerate, 11 days after autotomy. Note the thickness of the regenerated epidermis (NE) which formed the apical Cap. B, blastema cells; PM, promuscles. X 185.

Fig. 4: Longitudinal section through tail regenerate, 13 days after autotomy. ET, ependymal tube; PC, procartilage cells that formed the cartilaginous tube. X 185.

Fig. 5: Longitudinal section through regenerating tail, 15 days after autotomy. Note the cartilaginous tube (CT) appeared open. B, blastema cells that contained many melanocytes. X 80.
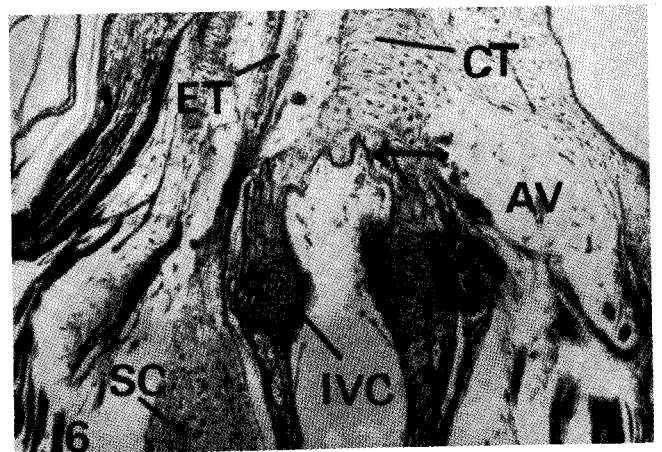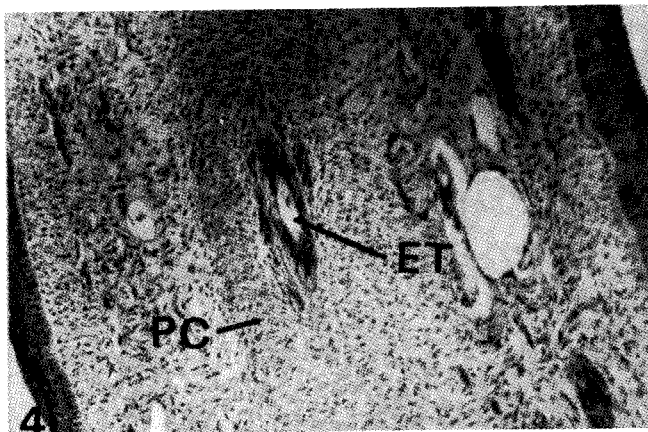

Fig. 6: Longitudinal section through regenerating tail, 16 days after autotomy. The arrow indicates the broken border of the vertebra at the autotomy level, just distal to the intervertebral cartilage (IVC). AV, autotomy cavity; CT, cartilaginous tube; ET, ependymal tube; SC, spinal cord. X 160.
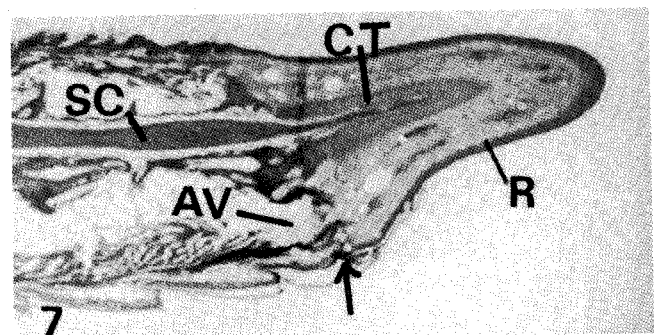

Fig. 7: Longitudinal section through regenerating tail, 18 days after autotomy. The regenerate (R) became 1 mm length. Note the autotomy level (arrow) between the regenerate and the stump. AV, autotomy cavity; CT, cartilaginous tube; SC, spinal cord of stump. X 42.
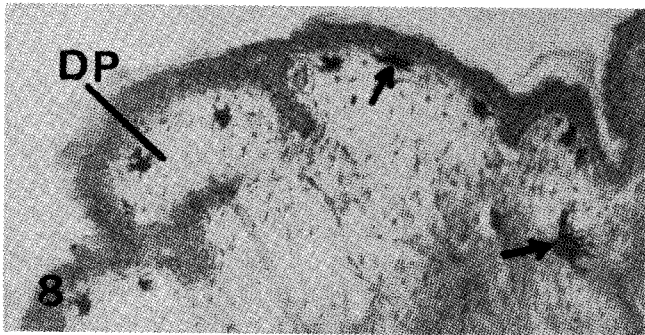
Fig. 8: Part of longitudinal section through the distal region of a 2mm regenerate, 22 days after autotomy. Note the beginning of formation of dermal papilla (DP) in the future scales. The arrows indicate the melanocytes. X 340.

Fig. 9: Part of longitudinal section through a 6 mm regenerate, 35 days after autotomy. CT, cartilaginous tube; ET, ependymal tube; PF, perivertebral fat. X 220.

Fig. 10: Part of longitudinal section through a 9 mm regenerate, 60 days after autotomy. CT, cartilaginous tube; DS, differentiating scales; RM, regenerated muscles. X 185.
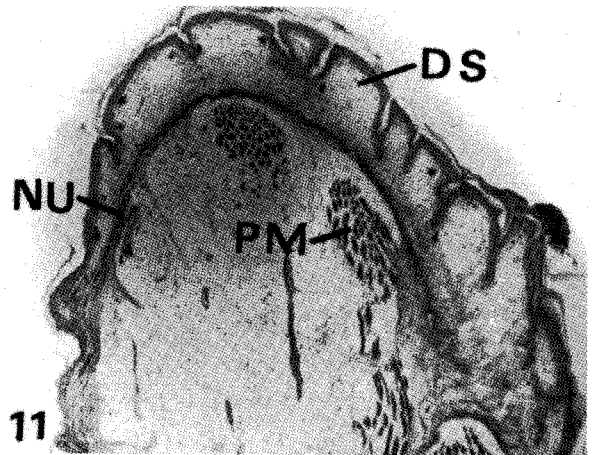

Fig. 11: Longitudinal section through the distal part of a non-regenerating tail, 35 days after autotomy. DS, developing scales; PM, promuscle aggregates; NU, connective tissue scar. X 80.
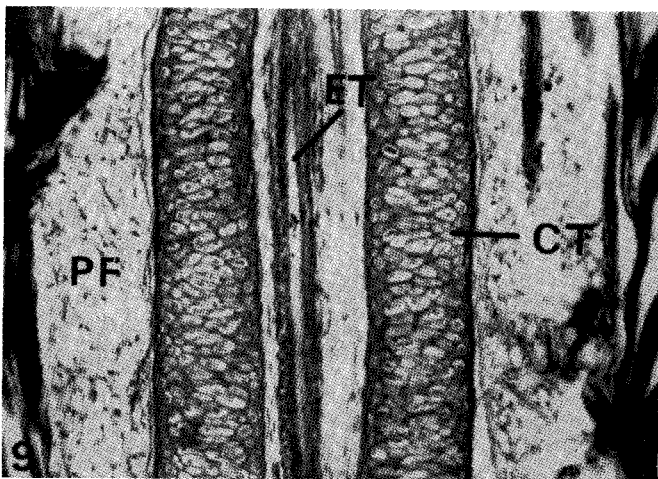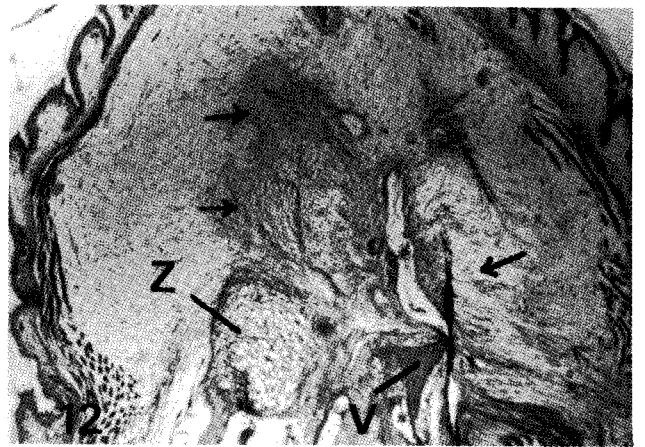

Fig. 12: Longitudinal section through the distal part of a non-regenerating tail, 50 days after autotomy. The arrows indicate the connective tissue which occupies the regenerate. Z, mass of cartilage cells; V, vertebra. X 80.
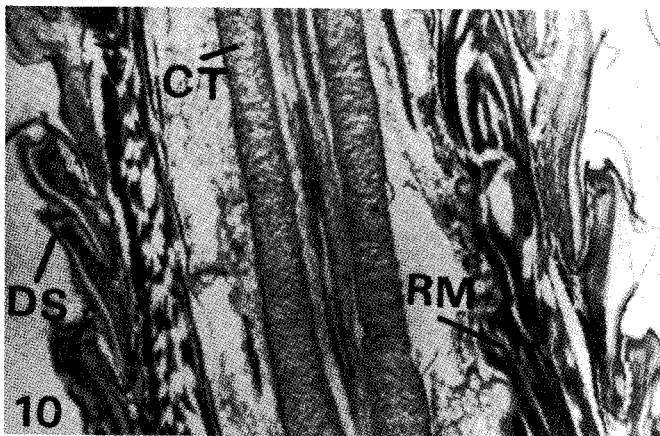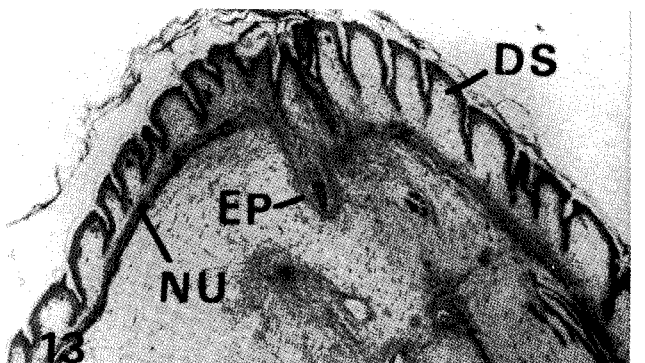

Fig. 13: Longitudinal section through the distal part of a non-regenerating tail, 40 days after autotomy. DS, developing scales; EP, epidermal papilla; NU, connective tissue scar. X 80.

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DISCUSSION

The ability of many lacertilians to autotomize their tails is a widely known phenomenon. This ability was pronounced in the regeneration of tails in *Bunopus tuberculatus;* among the autotomized tails, 78% regenerated their lost pieces. Although all these regenerating tails restored the autotomized parts, the regeneration rate varied greatly among them. Since all animals studied were of the same species, nearly of the same size, were collected within a known restricted geographical range, and were maintained under identical laboratory controlled conditions, we suggest that the variation in regeneration rate is not primarily a function of population differences. It is also suggested that variation is not associated with either age group or with environmental conditions. Maderson and Licht (1968) reported that the rate and final extent of tail regeneration varied greatly among 60 adult male *Anolis carolinensis* maintained under closely controlled experimental conditions. In the experiments of Maderson and Salthe (1971) on *Anolis carolinensis,* great variations were observed during tail regeneration. Their observations demonstrated that variability is not associated with population or individual genetic make-up, age, or environment. We may suggest an argument that since lacertilian reptiles, like other ectotherms, live under variable environmental conditions (notably with regard to temperature), maintaining them under constant conditions for experimental purposes may produce such variations in the regeneration rates and may also produce the non regenerating tails. Zweifel and Lowe (1966) suggested that the ability for tail autotomy and subsequent regeneration in *Xantusia vigilis* is an important factor in the survival of this species in which individuals characteristically become sexually mature after four years, and have a low fecundity. In contrast, Salthe and Maderson (1969) have reported that high coefficients of variability are associated with traits that are not important for the survival of individuals. The present observation confirmed that conclusion; the adult animals of *Bunopus* collected were nearly of similar sexual maturity and positively responded for tail regeneration after tail autotomy.

In the present investigations, the maximum rate of regeneration was 0.36 mm per day. This low rate indicates a slow elongation of the regenerating tails in *Bunopus,* and it is much slower than in other studied lacertilians. The maximum rate pf growth of a normal regeneration in *Sphaerodactylus* (Hughes and New, 1959) was about 0.47 mm per day. In *Anolis carolinensis,* Kamrin and Singer (1955) measured rates of growth of 0.4 mm per day. In *Hemidactylus flavivirdis* a rate of 1.12 mm per day could be deduced from Woodlands (1920) drawings. In *Lacerta,* Hooker (1912) recorded more rapid elongation, namely 1.36 mm per day. The rapid growth of the regenerate from day 14 to day 36 after autotomy in *Bunopus,* seems to be the result of cell proliferation both in blastema and in the differentiating tissues (as in promuscles) and of expansion of the differentiating elements. The growth phase of regeneration (Magon, 1977) is characterized by a gradual attainment of structural and functional maturity of the fully differentiated cell types of the fail regenerate.

The period taken by a regenerating tail to achieve its original length varies from species to species as well as from individual to individual. Such variation in the rate of growth of regenerates seems to be correlated with several factors, viz, the amount of tail autotomized, pressure applied at the time of autotomy, temperature, humidity, hormonal levels and diet (Moffat and Bellairs, 1964; Bryant and Bellairs, 1967; Maderson and Licht, 1968; Shah and Chakko, 1968; Balinsky, 1970; Magon, 1975a, 1975b).

In the present work, there were three autotomized tails, out of the forty experimental animals, failed to regenerate or restore their lost parts. Even so, they showed some growth beyond the level of autotomy, and this limited regenerate was soon covered by developing scales (see Figs. 11, 12 & 13). The stumps of these non-regenerating tails were occupied by connective tissue elements with some regenerating promuscles, and at the apical region just beneath the dermal papillae of the developing scales, there was observed scar connective tissue which appeared to be nearly similar (but less in thickness) to that observed in the non-regenerating hind limbs of late larval stages in the toad, *Xenopus laevis* (Anton *et al.,* 1988). This sealing tissue appeared to be an indication to the failure of regeneration, and as it is formed, the tail stops to grow. In the stumps of the non-regenerating tails, tissue dediffereentiation was not observed and there was no sign of blastema cell occumulation. Mitotic figures were rarely observed within the fibrocytes of the connective tissue invading the regenerate part. The limited potentiality of these fibrocytes and the absence of tissue dedifferentiation as well as the failure to form any mesenchyme cell accumulation may attribute to the failure of tail regeneration in some cases.

The present observations have demonstrated clearly that tissue dedifferentiation, in the regenerating tails, occurred in the distal region of the tail stump and resulted in the formation and liberation of mesenchyme-like cells which accumulated in the apical part underneath the wound epidermis forming the blastema. There were many melanocytes observed within this blastema. By 1 mm long regenerate, two-types of cells could be recognized in the blastema, spindle-shaped cells and stellate cells, some of these cells differentiated into procartilage cells which formed later the cartilaginous tube surrounding the ependymal tube (see Figs. 5, 6, 7 & 9). The present results are in accordance with the earlier conclusions of Singer and Salpeter (1961) who ahve reported that the blastema arises by dedifferentiation 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 early observations of White (1925) on lizard tail autotomy described how beneth 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. Quattrini (1954) considered that cells emerge from the intermuscular septa of the stump and from the muscles of the regenerate, and that the periosteum of the broken vertebra contributes formative cells for the new skeletal tube. The present results do not prove that latter consideration; it was clearly demonstrated that cells from blastema were arranged with their axes perpendicular to that of the ependyma, and these cells differentiated into the cartilaginous tube surrounding the ependymal tube. This cartilaginous tube will form the skeletal element of the regenerate. Woodland (1920) described the blastema in the regenerating tail of the gecko, *Hemidactylus flaviviridis* as being composed of quasiembryonic spindle-shaped cells. Hughes and New (1959), in *Sphaerodactylus,* mentioned that it is not a homogeneous accumulation of cells but rather, 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 content. Simpson (1965) reported an accumulation blastema that is made up of various sizes. In the present investigations two cell types were clearly pronounced within blastema, spindle-shaped (fibroblast-like) cells and stellate (mesenchyme-like) cells.

The damaged muscle fibres remaining in the tail stump, observed by Cox (1969) in *Anolis carolinensis,* undergo rapid

degeneration with only limited muscle regeneration of the repair type described for other vertebrates (Betz et al., 1966). The present observations on *Bunopus* have revealed muscle dedifferentiation in the tail stump, resulting in contribution to blastema formation. Muscle redifferentiation was also pronounced, represented by many aggregates of promuscles in the regenerate. These promuscles, within which mitotic figures were evident, formed the muscles of the regenerating tail. Observations of Spiedel (1938) and Niazi (1965) demonstrated the migration of dedifferentiated myoblasts into the regenerate to give rise to muscle. Holzer (1956) reported that, in salamander larvae, muscle and perhaps other tissues of the tail differentiate to give rise to a non-apical accumulation blastema located ventral and just anterior to the regenerating ependymal tip. This blastema, in turn, gives rise to the cartilage rod and the connective tissue elements, while the muscle, the tip of which is some distance rostral to the blastema, probably arises from the base directly from muscle. The present study on *Bunopus*, confirming those earlier observations, are in contrast with the conclusions of cox (1969) who stated that although damaged muscle is present in *Anolis carolinensis*, there is no evidence that it contributes to the formation of the regenerate, and the apical blastema is never formed. His histological and autoradiographic studies (using tritiated thymidine) suggested that the connective tissue cells, particularly the myoseptum, the periosteum and the dermis, are the primary source of cells for initiation of regeneration. Studies on *Demognathus fuscus* (Mufti, 1969; Mufti and Simpson, 1972) and on *Plethodon cinereus* (Dinsmore, 1977a) have shown that the tail autotomy mechanism precludes muscle participation in the formation of the blastema by means of a physical barrier, the myoseptum. We can not confirm the idea of Cox (1969) that denied the blastema formation. The stump tissues are well differentiated and therefore how can they contribute to the formation of regenerate tissues? These tissues near the site of autotomy had dedifferentiated liberating mesenchyme-like cells which form blastema. Therefore, we may state that the blastema formed in the regenerating tail of *Bunopus*, after normal autotomy is, as has been established in various studies on tail regeneration cited in literature involved an accumulation of mesenchyme-like cells capable of proliferation and of differentiation into various mesodermal structures.

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