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REGENERATION FROM DIFFERENT LEVELS ALONG THE TAIL OF THE GECKONID LIZARD, *BUNOPUS TUBERCULATUS*

By

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تجدد الذيل بعد بتره في مستويات مختلفة في السحلية البرصية *بونوبس تيوبركيولاتس*

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أجري هذا البحث لدراسة تأثير مستوى البتر في ذيل السحلية البرصية ***بونوبس تيوبركيولاتس*** على طول الذيل المتجدد ، معدل التجدد (أو الاستطالة) ، ومعدل الانقسام الميتوزي في الأنسجة المتجددة ، وقد تمت عملية بتر الذيل في ثلاثة مستويات : ذيول بترت لإزالة ثلاثة أرباع الذيل ، وذيول أخرى بترت لإزالة نصف الذيل ، ومجموعة ثالثة بترت لإزالة ربع الذيل .

أظهرت النتائج أن الذيول المبتورة عند جميع مستويات البتر قد مرت بنفس المراحل المورفولوجية والهستولوجية للتجدد وفي نفس الأوقات بعد البتر ، وجددت الذيول نفس التراكيب والأنسجة . وأوضحت النتائج أن الطول النهائي ومعدل الاستطالة للذيل المجدد في حالة البتر في المستويات القريبة أكبر منهما في حالة البتر في المستويات البعيدة وكانت الأطوال الكلية للأجزاء المجددة للذيول متناسبة مع الأطوال التي أذيلت من الذيول بعملية البتر في المستويات المختلفة . عند تعيين معدل الانقسام الخلوي في الأنسجة المجددة في أي وقت أثناء تجدد الذيل ، وجد أن هذا المعدل أعلى بكثير عند مستويات البتر القريبة منه عند مستويات البتر البعيدة ، ولوحظ أن الهبوط التدريجي في ذلك المعدل أثناء تجدد الذيل بعد اليوم الخامس للبتر ، كان أكثر وضوحاً في حالة البتر عند المستوى البعيد.

Key Words: Amputation level, Elongation rate, Mitotic index.

ABSTRACT

Study of the influence or amputation level on regenerate length, elongation rate and mitotic index of the tail of the geckonid lizard, Bunopus tuberculatus revealed that the tails amputated such that three-fourth, or one-half or one-fourth of the tail was removed, passed through the same morphological and histological stages of regeneration at the same times after amputation, and the regenerated structures were also the same. The final length of the regenerate and the elongation rate in tails amputated at more proximal levels were greater than those of regenerates from more distal levels. The total lengths of regenerates from different levels were proportional to the lengths of the tail parts removed by amputation. At any time during tail regeneration, the mitotic index was higher in regenerate tissues from proximal levels than from distal levels. The gradual decrease in mitotic index observed after the fifth post-amputation day, was much more pronounced at the distal amputation levels.

INTRODUCTION

After autotomy of amputation of the lizard tail, the stump initiates a series of events culminating in replacement of the missing part. The stages are: wound-healing (formation of new epidermis above the wound), dedifferentiation (the cells near the amputation surface lose their morphologic characteristics and begin to synthesize DNA), blastema formation (accumulation of dedifferentiated cells distally into a mound), and finally growth and differentiation (see for review: 1, 2, 3, 4, 5, and 6). 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 or amputated, pressure applied at the time of autotomy or amputation, temperature, humidity, hormonal levels and diet (7, 8, 9, 10, 11, 12 and 13).

Earlier studies on regeneration in vertebrates showed that after amputation of different levels along regenerating appendages, the rate of elongation of the regenerate and the amount regenerated is proportional to the amount removed (8, 14, 15, 16 and 17). In the study (18) on tail regeneration of the newt, *Notophthalmus viridescens,* they have shown that the rate of elongation of regenerates from more proximal levels is greater than that of regenerates from more distal levels. The total lengths of regenerates from different levels are proportional to the lengths of tail removed by amputation. Baranowitz *et al.* (19) on their study on lizard and newt tail regeneration have reported that proximal amputations result in longer regenerates than do distal amputations; the proximal amputations elicit greater absolute rates of elongation (in mm/day) than do distal amputations. The present study was carried out to investigate the influence of the amputation level along the tail of the geckonid lizard, *Bunopus tuberculats,* on regenerate length, elongation rate and mitotic index during the different phases of tail regeneration.

MATERIAL AND METHODS

Male and female adult geckos, *Bunopus tuberculats* were obtained from some regions in Doha, Qatar. The body length, from snout to vent, ranged from 35-50 mm and the tail length, from vent to the tip of the tail, ranged from 35-45 mm. The animals were chosen to be nearly of the same size. They were kept in the laboratory in glass museum jars, each containing three to four animals. Heater was placed near the jars to raise their temperature and it was controlled to be 30 ± 2°C, with 8-12 hours of light per day. The animals were hand-fed a standard diet, one meal of meat daily. Water was provided in small dishes.

The tails of the geckos were amputated, with a razor blade, at one of the three different levels: level A, with three-fourths of the tail removed (26.3-33.8 mm, mean 30 mm), level B, with one-half of the tail removed (17.5-22.5 mm, mean 20 mm) and level C, with one-fourth of the tail removed (8.8-11.3 mm, mean 10 mm).

During the first three post-amputation weeks, the regenerate length was measured with an ocular micrometer mounted in one of the eyepieces of a Leitz binocular microscope, and then from the fourth week till the end of the experiments (end of the 18th week after amputation), the lengths measurements were carried out using a millimeter ruler. The elongation or regeneration rate was expressed as the change in regenerate length (in mm/day). To investigate the influence of the amputation level on the mitotic index in the regenerating tails, three tail regenerates from each concerned amputation level (plus a portion of the adjacent stump) were removed and placed in Bouin's fixative at 3, 5, 7, 10 and 14 days intervals. Serial cross sections of 7-8 μm thickness were prepared and stained with borax carmine-modified Azan. Beginning with the distal most complete section, all recognizable mitotic figures from prophase to telophase were counted in the regenerates from each level of amputation. Counts were made in the regenerating tissues (excluding blood and epidermal cells) just above the amputation plane which was clearly defined by the cut ends of the amputated vertebrae and the distal stump scales. The cells of the ependymal vesicle or tube, blastema cells and the differentiating tissue in the regenerate were included in the counting. Counting was carried out in every third section of the regenerate and the mitotic index (%) was expressed as the number of mitotic figures / 100 of total cells. Means of mitotic indices from the three tail regenerates were calculated, at each time interval of fixation, for each amputation level.

To investigate the influence of the amputation level on the histological sequence of the regeneration phases of the tail and the times of these phases after amputation at the different levels, serial longitudinal sections of 7-8 μm thickness, at different time intervals during the first month after amputation, were prepared and stained with borax carmine-modified Azan or haematoxylin-eosin.

A statistical analysis of differences in the regenerate length, elongation rate and mitotic index of tails amputated at different levels was carried out, using the t-test for probability; the difference was considered to be significant if P was less than 0.01. The temperature and light cycle were kept constant during the experiments and all the animals regenerated their tails at the same time, so that these factors did not contribute significantly to differences in the regeneration rates observed in this study.

RESULTS

After tail amputation in *Bunopus,* at any of the three levels (A, B or C), the tail stump initiated a series of morphogenetic activities which restored the missing part. The wound scab was formed by the second day and cellular debris, macrophages, lymphocytes and microglical elements of the spinal cord were accumulated underneath the scab (reviewed in 6), the wound epithelium was formed by the fourth day and the wound-healing stage was achieved. By the 4th-5th day, the ependymal vesicle was formed and stump tissue dedifferentiation was observed in the distal region. The wound epidermis became much thicker forming the apical cap. By the 5th - 7th day, the ependymal vesicle formed the ependymal tube; the stump dedifferentiated tissue cells accumulated to form blastema cells which began to show mitotic activity. By the 7th-9th day, differentiation of blastema cells was observed, promuscles were distinguished and

by the 10th-15th day, some blastema cells were differentiated into procartilage cells surrounding the ependymal tube. These chondroblasts, by the 15th-20th day, formed the cartilaginous tube. From the fourth week after amputation, differentiation and formation of various types of epidermal cells were observed. The inner surface of the epidermis became uneven and dermal papillae were formed, increased in size, pushing the epidermis with scale as the projecting body. By days 30-45, the scales could be extremely distinguished and the scale differentiation proceeded proximodistally until the entire regenerate became covered with scales, nearly 2-2.5 months after amputation.

The investigation of the histological sequence of the regeneration stages have indicated that during the three main phases of regeneration (wound healing and dedifferentiation, blastema formation and growth, and differentiation and morphogenesis), the regeneration tails, from the three levels of amputation, passed through the same stages at approximately the same times after amputation.

Effect of amputation level on the regenerate length and elongation rate

Tails were amputated such that three-fourths, or one-half or one-fourth of the tail was removed in order to examine the influence of the level of amputation on the growth or tail regenerates and their rate of elongation through the stages of tail regeneration. From the first to the third week after amputation, at any of the three concerned levels, i.e. during blastema formation and growth and incipient redifferentiation, the lengths of tail regenerates were nearly comparable. There was a difference in this length between level A and C, but it was insignificant ($P>0.01$, Table 1, Fig. 1). During this period, the regeneration rate for the three amputation levels was almost comparable (Table 2, Fig. 2). By the end of the fourth week post-amputation, beginning of scale appearance from the epidermis took place and the differences in the regenerates lengths were still nonsignificant between levels A and B or B and C ($P>0.01$) but, however, it was significant ($P < 0.01$) between levels A and C. By that time there was a nonsignificants difference in the regeneration rate between the three levels. By the fifth week post-amputation, significant differences in regenerate lengths were observed between levels A and B and between A and C; however, there was still nonsignificant difference between levels B and C. By that time, the elongation rate reached its peak for the three levels, and there were significant differences ($P<0.01$) between them; the proximal levels exhibited higher values than the distal levels. The proximal levels still exhibited longer regenerates (significant differences) than the distal levels till the end of the 10th week after amputation. From Table 1 and Fig. 1, it is indicated that by the 7th, 8th, 9th and 10th weeks after amputation, the regenerates from the proximal level (A) were much more longer than those from the distal level (C), as represented by the highly significant differences ($P<0.001$). A highly significant difference in regenerate length was also observed between levels A and B by the end of the 13th week post-amputation. During the period from the 6th to the 12th week, the difference in elongation rate between levels A and B was insignificant. However, during the 13th week the regenerates from level A showed faster elongation rate than those from level B (significant differences). From the 6th to the 10th week, the regenerates from level A showed higher significant values of elongation rate than those from the level C, whereas a significant difference in this rate was evident between levels B and C only during the period from the 8th to the 10th week.

By the end of the 10th week after amputation, the tail regenerates from level C reached their maximal lengths (mean, 9.7 ± 0.42 mm). Thereafter they showed no increase in length till the end of the 17th week post-amputation, and the regenerating tails appeared to restore nearly the original lengths before amputation. However, tail regenerates from level B were still elongated till the end of the 13th week, by which they had their maximal length (mean 18.4 ± 0.64 mm). The regenerating tails reached nearly their original lengths, and therefore, no more appreciable increase in length was recorded. As for regenerates from proximal level (A), they had continued to lengthen till the end of the 17th week, thereafter, and during the 18th week, no more increase in their lengths was observed.

From tables 1, 2 and Figs. 1, 2, it can be seen that the regenerate length increased gradually and continuously from the 1st till the end of the 10th week after amputation for level C; till the end of the 13th week for level B and till the end of the 17th week for level A. However, the elongation rate increased after amputation at any of the three levels till the end of the fifth week, when it reached its maximal values for all the three levels, and then it steadily decreased by time. The results expressed in such tables and figures also demonstrate that the elongation rate was faster and higher at the proximal levels than at the distal levels; this means that the regenerates from level A lengthened faster than those from level B, and the latter were comparatively faster than those from level C.

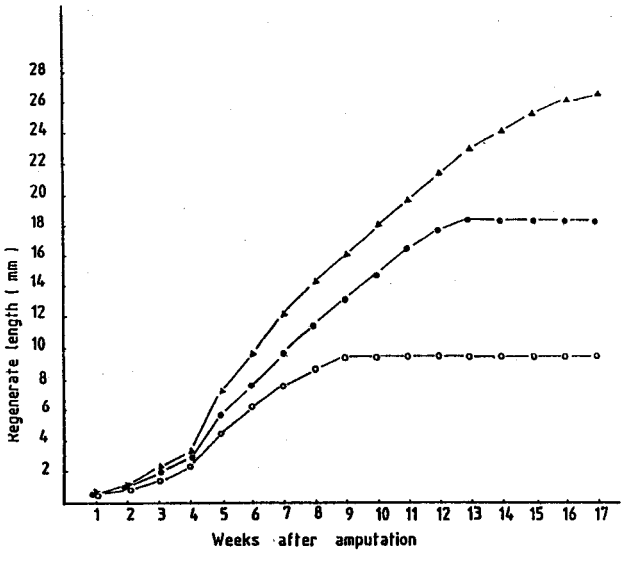

Fig. 1. Mean length of regenerates after amputation at different levels along the tail. Triangles represent regenerates after three-fourths of the tail were removed (level A), closed circles represent regenerates after one-half of the tail was removed (level B), and open circles represent regenerates after one-fourth of the tail was removed (level C

Table 1

Statistical analysis of length differences between regenerates, resulted from amputation levels A, B, and C.
** Regenerates from levels C and B have shown no further increase in their lengths by the end of the 10th and 13th weeks respectively.

Weeks after amputation	Mean Length (mm) ± S.D. of tail regenerates from amputation levels			Probability of mean length differences between tail regenerates from amputation levels		
	A	B	C	A&B	A&C	B&C
1	0.6 ± 0.13	0.5 ± 0.11	0.5 ± 0.11	>0.01	>0.01	>0.01
2	1.3 ± 0.15	1.2 ± 0.13	1.0 ± 0.13	>0.01	>0.01	>0.01
3	2.2 ± 0.18	2.0 ± 0.15	1.6 ± 0.15	>0.01	>0.01	>0.01
4	3.5 ± 0.23	3.0 ± 0.16	2.5 ± 0.18	>0.01	>0.01	>0.01
5	7.2 ± 0.31	5.8 ± 0.26	4.6 ± 0.20	<0.01	<0.01	>0.01
6	9.8 ± 0.37	7.9 ± 0.32	6.4 ± 0.27	<0.01	<0.01	<0.01
7	12.2 ± 0.42	9.8 ± 0.36	7.9 ± 0.34	<0.01	<0.001	<0.01
8	14.3 ± 0.38	11.5 ± 0.28	8.9 ± 0.38	<0.01	<0.001	<0.01
9	16.1 ± 0.54	13.2 ± 0.35	9.5 ± 0.36	<0.01	<0.001	<0.01
10	18.0± 0.70	14.8 ± 0.41	9.7 ± 0.42	<0.01	<0.001	<0.01
11	19.8 ± 0.66	16.5 ± 0.53	**	<0.01	**	**
12	21.5 ± 0.68	17.8 ± 0.52	**	<0.01	**	**
13	23.0 ± 0.72	18.4 ± 0.64	**	<0.001	**	**
14	24.2 ± 0.75	**	**	**	**	**
15	25.4 ± 0.67	**	**	**	**	**
16	26.1 ± 0.74	**	**	**	**	**
17	26.6 ± 0.78	**	**	**	**	**

Table 2

Statistical analysis of elongation rate differences between tail regenerates, resulted from amputation levels A, B, and C.
** Regenerates from levels C and B have stopped to elongate after the 10th and 13th weeks respectively.

Weeks after amputation	Mean elongation rate (mm/day) ± S.D. of tail regenerates from amputation levels			Probability of mean elongation rate differences between tail regenerates from amputation levels		
	A	B	C	A&B	A&C	B&C
1	0.09 ± 0.5	0.07 ± 0.04	0.07 ± 0.02	>0.01	>0.01	>0.01
2	0.10 ± 0.07	0.10 ± 0.06	0.07 ± 0.03	>0.01	>0.01	>0.01
3	0.13 ± 0.10	0.11 ± 0.08	0.09 ± 0.03	>0.01	>0.01	>0.01
4	0.19 ± 0.13	0.17 ± 0.10	0.13 ± 0.07	>0.01	>0.01	>0.01
5	0.53 ± 0.18	0.40 ± 0.14	0.30 ± 0.14	<0.01	<0.01	<0.01
6	0.37 ± 0.21	0.26 ± 0.12	0.26 ± 0.12	>0.01	<0.01	>0.01
7	0.34 ± 0.16	0.27 ± 0.11	0.21 ± 0.13	>0.01	<0.01	>0.01
8	0.30 ± 0.17	0.24 ± 0.10	0.14 ± 0.12	>0.01	<0.01	<0.01
9	0.26 ± 0.22	0.24 ± 0.12	0.09 ± 0.05	>0.01	<0.01	<0.01
10	0.27 ± 0.19	0.23 ± 0.12	0.03 ± 0.02	>0.01	<0.001	<0.001
11	0.26 ± 0.13	0.24 ± 0.15	**	>0.01	**	**
12	0.24 ± 0.14	0.19 ± 0.16	**	>0.01	**	**
13	0.21 ± 0.15	0.09 ± 0.06	**	<0.01	**	**
14	0.17 ± 0.13	**	**	**	**	**
15	0.17 ± 0.12	**	**	**	**	**
16	0.10 ± 0.08	**	**	**	**	**
17	0.07 ± 0.03	**	**	**	**	**

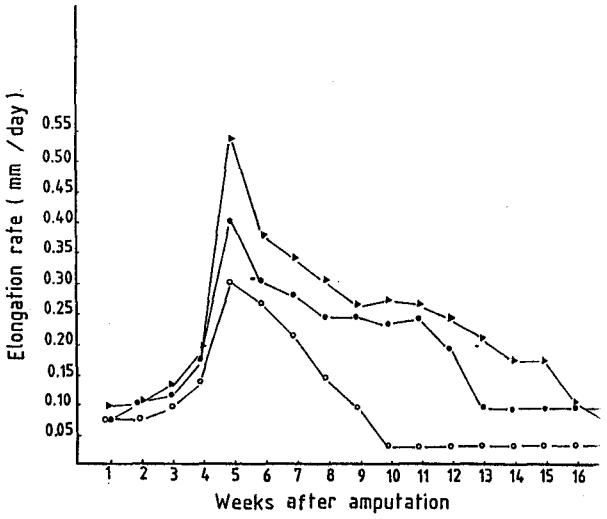

Fig. 2. Mean rate of elongation of regenerates after amputation at different levels. Description as in Fig. 1

Effect of amputation level on mitotic index

During the first three days after amputation at any of the three levels, very few or nearly no mitotic figures were recorded in the distal stump tissue cells underneath the healed wound epithelium. Mitotic activity was first observed by the end of the fourth post-amputation, within the wall of ependymal sac. Some mitotic figures were also observed within the dedifferentiating tissue cells near the amputation site. By the fifth day, the mitotic index was greatly increased at all levels of amputation, but comparatively, it was higher for tails amputated at the proximal level (A) and the middle level (B) than for tails amputated at the most distal level (C), as indicated in Table 3 and Fig. 3. Despite the differences in mitotic indices between levels A or B and C were significant, the values for levels A and B were comparable, by the end of the 7th day no significant change was observed in mitotic index from the state recorded by the fifth day. From the 7th day onwards, the mitotic index steadily decreased at all the three amputation levels. Table 3 and Fig. 3 obviously demonstrate that the drop in mitotic index values, when it decreased from the fifth to the 14th day post-amputation, was much more pronounced at level B (from 2.6 ± 0.8 to 0.7 ± 0.2, i.e. to less than 1/3 the value) and level C (from 1.2 ± 0.7 to 0.2 ± 0.1, i.e. to about 1/6 the value) than at level A (from 2.8 ± 1.1 to 1.2 ± 0.3, i.e. to about 1/2 the value). It was of interest to note that, by the 10th and 14th day, the differences in mitotic index for tail regenerates from the amputation levels A and B or B and C were nonsignificants, while the difference between the most proximal level (A) and the most distal level (C) was significant.

The mitotic activity was thus influenced by the amputation level after redifferentiation had started (during the second week after amputation), and that effect, represented by the decrease in mitotic index value, was much more pronounced at the most distal level of amputation.

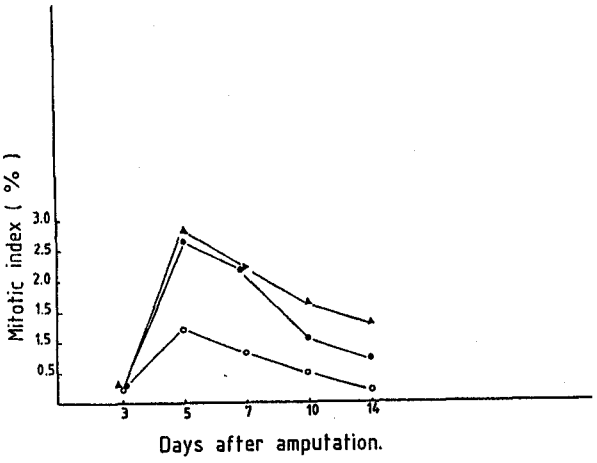

Fig. 3. Mean mitotic index (%) of regenerates after amputation at different levels. Description as in Fig. 1

DISCUSSION

The results reported in the present study on the influence of the level of amputation on the growth of tail regenerates, showed that during the first four to five weeks after amputation, irrespective of the level of amputation, tail regenerates lengthened at nearly the same rate (as indicated by the nonsignificant difference in elongation rate between the three concerned

Table 3

Statistical analysis of mitotic index differences between tail regenerates, resulted from amputation levels A, B, and C.

Days after amputation	Mean mitotic index ± S.D. of tail regenerates from amputation levels			Probability of mean mitotic index differences between tail regenerates from amputation levels		
	A	B	C	A&B	A&C	B&C
3	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	>0.01	>0.01	>0.01
5	2.8 ± 1.1	2.6 ± 0.8	1.2 ± 0.7	>0.01	<0.01	<0.01
7	2.2 ± 0.8	2.2 ± 0.7	0.8 ± 0.2	>0.01	<0.01	<0.01
10	1.6 ± 0.6	1.1 ± 0.5	0.5 ± 0.2	>0.01	<0.01	>0.01
14	1.3 ± 0.3	0.7 ± 0.2	0.2 ± 0.1	>0.01	<0.01	>0.01

amputation levels). This means that during dedifferentiation, blastema cells accumulation and beginning of redifferentiation, the effect of amputation level on the regenerate length and elongation rate of the regenerating tails of the gecko was not evident and unclear. By the end of the 6th week post-amputation, during morphogenesis of tail regenerates, the effect of amputation level on the growth of regenerating tails was pronounced, where significant differences in regenerate lengths were obviously observed between the three levels of amputation. Therefore, regenerates from more proximal levels do not become significantly longer than regenerates from more distal levels until five to six week after amputation. We may conclude that when tail regenerates reached the six week post-amputation, both the regenerate length and its rate of elongation appeared to be depended upon its level along the proximodistal axis of the tail.

It is interesting to note that although the regenerating tails from distal levels reached nearly their original more earlier than those from proximal levels, regeneration from more proximal levels resulted in the production of a significantly longer regenerates than from more distal levels. By 10 weeks after amputation, the mean lengths of regenerates after removal of three -fourths, one-half, and one-fourths of the tail, were respectively 18.0 mm (± S.D. of 0.41), and 9.7 mm (± S.D. of 0.42). With progress of time after amputation, the more proximal level (A) exhibited continuous increase in regenerate length till the end of the 17th week, whereas the more distal level (C) showed no more increase in regenerate length after the end of the 10th week, and the regenerates from the intermediate level (B) had stopped to elongate by the end of the 13th week post-amputation. These observations are in accordance with the results on tadpole tail (reviewed in 14); on newt limb (20) and on newt tail (18). Their studies led to the conclusion that proximal amputations result in longer regenerates than do distal amputations.

The present results clearly demonstrated that the elongation rate was also affected by the level of amputation along the tail. The effect appeared to be evident by the end of the fifth week after amputation when the elongation rate reached its maximal value for the three levels of amputation and then this rate decreased gradually until the end of the rearing period of experimentals. Proximal amputations elicited greater rates of elongation (in mm/day) than did distal amputations. Comparing the regenerate lengths from the different amputation levels with the original lengths of the tail before amputation, it was observed that the percent regenerated of the length is rather constant, regardless of the absolute length regenerated. In other words, the regenerate lengths from the different amputation levels were proportional to the lengths of tail removed by amputation. One of the most interesting features of appendage regeneration in animal is that only the missing parts are replaced; each unique level along the appendage gives rise to an equally unique regenerate. Our results confirm many of the earlier studies on regeneration or elongation rate of vertebrate appendages (Tadpole tail, 14: Lizard Tail, 16; 8; 9; 19; newt tail, 19: fish fins, 16).

A study of the regeneration of adult newt forelimbs from different amputation levels (reviewed in 21) showed that limbs regenerating from proximal stumps lengthened faster that from distal stumps. Recent studies on limb regeneration of larval *Bufo regularis* (reviewed in 22) had revealed significant differences in regenerate length and regeneration rate between regenerating limbs. The more proximal the amputation level, the greater are regenerate length and volume and the greater is the rate of regeneration.

The present study was carried out under standard experimental conditions. None of the animals had previously regenerated its tail, all the animals were maintained at a constant temperature and light. All physiological factors (e.g. age, body weight, nutritional state, etc.) were nearly the same for all animals. This means that most of the parameters governing regeneration were relatively constant for all animals. Therefore, the level of amputation and the resulting cellular proliferation, in the regenerate, which initiate tissue differentiation and morphogenesis may be responsible for the significant differences in regenerate lengths and elongation rates between the three concerned amputation levels. The present study, thus, proposed that there is a proximodistal sequence of positional values along the tail. These results confirm the idea or Baranowitz (19) that the number of cells in the field which initiates proliferation is the factor primarily responsible for the present results that proximal amputations result in longer regenerates and greater elongation rates than did distal amputations. The current observations suggest also that the final regenerate length is directly (positively) dependent on the number of cells initiating regeneration. In serial sections through the gecko's tail (normal or regenerating), the section radius, as well as cross sectional area, decreased rostrocaudally or proximodistally. The animals with greater lengths of the tails removed will have larger stump radii. This would result in greater number of cells initiating proliferation in animals with greater lengths removed, i.e., with proximal amputations, and therefore, longer final regenerates. Thus, a proportionality between amount removed and amount regenerated would be maintained. From the present investigations, it may be suggested that some relations might be occurred between stump radius and final length of the regenerate. To understand such relationship, two conditions are considered. The first condition involved the cell types, which actually dedifferentiate and accumulate into a mound. If the amounts of those tissue cells decrease with decreasing stump radius, then stump radius would be expected to show some relationship with the final regenerate length. The second condition involves those factors which influence the processes involved in blastema formation. Such processes may include wound-healing, removal of cellular debris, dedifferentiation, proliferation of dedifferentiated cells to contribute to the mound, and migration of dedifferentiated cells to the mound. If those influencing factors also decrease distally with stump radius, then the stump radius and final regenerate length would be expected to show some relationship. Tail regenerates of the newt, from different levels (18) do not appear to have similar masses during regeneration. The length and shape of such tail regenerates are similar, but the cross-sectional area of the base of the blastema varies with the level along the tail, due to the taper of the tail. They have suggested that this difference in mass between tail blastemas from different levels is a significant factor in determining the rate of elongation and the eventual length of tail regenerates. These results were in contrast with the earlier observations of Tassava and Goss (16)

who studied the effect of stump radius on rate of regeneration in *Anolis* tail, which tapers greatly, and the gourami *(Trichogaster* sp.) fin, which tapers only slightly. They concluded that no relationship between stump radius and either the length of the regenerate or the rate of regeneration was apparent in either species. A closer correlation was shown to exit between the amount of the appendage removed and the elongation rate than between the diameter of the stump and the rate of lengthening. Recent studies on limb regeneration in *Xenopus laevis* and *Bufo regularis* (23) have established that the blastema volume (calculated from sectional areas of blastema) relative to stump volume was higher at the early larval stage 53 than at late larval stage 57, indicating higher regenerative ability at stage 53, which regenerates the whole lost part of the limb with most of toes.

The influence of amputation level along the tail may also include, in addition to the number of cells initiating regeneration, the mitotic activity of distal stump tissues. The results reported in this study have shown that the mitotic activity was actually recorded by the fourth post-amputation day and then, it reached its maximal value by the fifth day at the three levels of amputation. During that time, blastema cells were accumulated and then proliferated to form cone-shaped blastema. The mitotic index was determined (number of dividing cells/ 100 of total cells) in three tail regenerates at each amputation level, and it was found that mitotic divisions started in most animals at approximately the same time after amputation, confirming the results of the autoradiographic studies (4, 24, 25, 26 and 27). It has been stated that in a given species kept under standardized conditions, mitosis begins in most animals at nearly the same time after amputation. In tail regenerates of *Bunopus,* the mitotic index was observed to decrease by the 7th day after amputation, and then it gradually decreased during the second week after amputation. There are reports in the literature that the mitotic index shows a continuous, gradual increase from the first time mitotic figures are visible in the stump, and then it levels off (25, 26 and 27). Mitotic activity was said to start about seven to eight days post-amputation in lizard tail regeneration (*Lygosoma laterale,* 4) and five to six days post-amputation in amphibians (*Rana pipiens* tail, 28; *Notophthalmus viridescens* limb, 27; *Ambystoma meximanum* limb, 25 and 26).

In the regenerating tissues of the tail in *Bunopus*, it was determined that at any time the mitotic index was calculated, the percent of dividing cells was much higher at the proximal amputation levels (A) than at the distal amputation levels (C), indicated by the significant differences in mitotic index between them (see Table 3, Fig. 3). The higher value of mitotic index in the regenerates resulted from proximal amputations is supported by the larger number of the proliferating cells, initiating differentiation and growth of the regenerates, which are found to be located in the larger cross-sectional areas of the stump and in the larger stump radii at the proximal amputation levels, and this would lead to larger final structures. Donaldson and Wilson (29) have reported that at four days after amputation of the newt tail, the mitotic index seen in adjacent stump tissues of tails amputated through the proximal region of caudal vertebra 7 or 8 is significantly lower than through the distal region of the same vertebra in the adult newt, *Notophthalmus viridescens.* The situation is contrary to the principle we clearly confirmed that during tail regeneration of the adult gecko, elongation rate is generally faster from proximal than distal levels.

From the present work it is suggested that the influence of the level of amputation on growth of tail regenerates in the gecko is essentially comparable to that reported for tail regenerates from different levels in the newt (18) and this indicate that similar mechanisms may be involved in the control of their growth. However, the results on newt (18) showed that the level of amputation affects not only the overall rate of elongation and the final length of the regenerate, but also the structures regenerated in the tail. In the regenerating tail of the gecko, *Bunomus,* the length, elongation rate as well as mitotic index were affected by amputation level, while the regenerated structures were the same for the three levels of amputation. When the morphological and histological events of tail regeneration in the gecko were investigated, it was found that throughout all the regeneration phases the general appearance and temporal sequence of the stages of tail regeneration were the same irrespective of the level of amputation. Therefore, neither the amount of tail to be regenerated nor the rate of elongation appeared to influence the overall development sequence of morphological and histological events seen in tail regeneration.

The present investigations revealed that despite the mitotic index decreased during the second week after amputation at all the three concerned levels, the regenerate length and elongation rate increased. During the fifth week, the elongation rate was greatly increased; there was a progress in the development of the scales proximo-distally, differentiation of various types of epidermal cells, elongation of ependymal and cartilaginous tubes. Therefore, in addition to differences in regenerate length, elongation rate and mitotic index in the tail regenerates from different levels, an assessment needs to be made of the extent to which increase in cell size and intercellular matrix production contribute to the observed growth rates and the eventual lengths of tail regenerates. Thus, the study is extended to speculate that such factors apart from amputation level might also play an important role in influencing the growth of the regenerating tails.

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REFERENCES

[1] **White, C. P., 1925.** Regeneration of the lizard's tail J. Path. Bact., 28: 63-68.

[2] **Hughes, A.** and **D. New, 1959.** Tail regeneration in the geckonid lizard, *Sphaerodactylus.* J. Embryol. Exp. Morph., 7: 281-302..

[3] **Singer, M.** and **M. M. Salpeter, 1961.** Regeneration in vertebrates: the role of the wound epithelium. In Growth in

living System (Proceedings of an international symposium on growth, Ed. M. X. Zarrow). New York: Basic Books.

[4] **Simpson, S. B., Jr., 1965.** Regeneration of the lizard tail. In: Regeneration in Animals and Related Problems. V. Kiortsis and H.A.L. Trampusch, eds. North-Holland Pub. Co., Amsterdam, pp. 431-443..

[5] **Magon, D. K., 1977.** Effects of temperature on growth in the regenerating tail of the scincid lizard, *Mabuya striata.* Zoologica Africana, 12(2): 383-388.

[6] **Abd El-Karim, A.E.** and **M. I. Michael, 1992.** Tail regeneration after autotomy in the geckonid lizard, *Bunopus tuberculatus.* Qatar Univ. Sci. J., 13(2): 3-9..

[7] **Moffat, L.A.** and **A.D'A. Bellairs, 1964.** The regenerative capacity of the tail in embryonic and post-natal lizards (*Lacerta vivipara* Jacquin). J. Embryol. exp. Morph., 12: 769-786.

[8] **Bryant, S. V.** and **A.D'A. Bellairs, 1967.** Tail regeneration in the lizards *Anguis fragilis* and *Lacerta dugestii.* J. Linn. Soc. (Zool.), 46: 297-305.

[9] **Maderson, P.F.A.** and **P. Licht, 1968.** Factors influencing rates of tail regeneration in the lizard *Anolis carolinensis.* Experientia, 24: 1083-1086.

[10] **Shah, R.V.** and **T.V. Chakko, 1968.** Preliminary observations on the changes in the thyroid activity in the house lizard, *Hemidactylus flaviviridis.* J. Biol. Sci. 11: 41-44.

[11] **Balinsky, B. I., 1970.** An introduction to Embryology, Philadelphia: W.B. Saunders.

[12] **Magon, D.K., 1975a.** Rate of growth in the regenerating tail of the house lizard, *Hemidactylus flavivirdis* during the different seasons of the year. Broteria, 44: 135-140.

[13] **Magon, D.K., 1975b.** Changes in thyroid activity in the house lizard, *Hemidactylus flavivirdis* during the different phases of regeneration in the different seasons of the year. Broteria, 44: 113-120.

[14] **Ellis, M.M., 1990.** The relation of the amount of tail regenerated to the amount removed in tadpoles of *Rana clamitans.* J. Exp. Zool., 7: 421-455.

[15] **Morgan, T.H., 1906.** The physiology of regeneration. J. Exp. Zool., 3: 457-500.

[16] **Tassava, R.A.** and **R. J. Gass, 1966.** Regeneration rate and amputation level in fish fins and lizard tail. Growth, 30: 9-21.

[17] **Zeleny, C., 1916.** Studies on the factors controlling the rate of regeneration. Illinois Biological Monographs, Vol. 3, No. 1. University of Illinois Press, Urbana.

[18] **Iten, L.E.** and **S.V. Bryant, 1976.** Regeneration from different levels along the tail of the newt, *Notophthalmus Viridescens.* J. Exp. Zool., 196: 293-306.

[19] **Baranowitz, S.A., P.F.A. Maderson** and **T.G. Connelly, 1979.** Lizard and newt tail regeneration: A quantitative study. J. Exp. Zool., 210: 17-28.

[20] **Iten, L.E.** and **S.V. Bryant, 1973.** Forelimb regeneration from different levels of amputation in the newt, *Notophthalmus viridescens:* Length, rate and stages. Roux Archiv., 173: 263-282.

[21] **Goss, R.J., 1969.** Principles of regeneration. New York: Academic Press.

[22] **Abd El-Karim, A.E., 1989.** Amputation level and hind limb regeneration in larvae of the Egyptian Toad *Bufo regularis* Reuss: Length, Volume and rate. Folia Morphol., 37: 238-248.

[23] **Abd El-Karim, A.E., 1990.** Blastema volume and nuclear size in the regenerating hind limbs in larvae of *Xenopus laevis* and *Bufo regularis.* Qatar Univ. Sci. Bull., 10: 227-238.

[24] **Cox, P.G., 1969.** Some aspects of tail regeneration in the lizard, *Anolis carolinensis.* II. A description based on histology and autoradiography. J. Exp. Zool., 17: 127-150.

[25] **Kelly, D.J.** and **R.A. Tassava, 1973.** Cell division and ribonucleic acid synthesis during the initiation of limb regeneration in larval axolotls *(Ambystoma mexicanum).* J. Exp. Zool., 185: 45-54.

[26] **Tassava, R.A., L.L. Bennett** and **G.D. Zitnik, 1974.** DNA synthesis without mitosis in amputated denervated forelimbs of larval axolotls. J. Exp. Zool., 190: 111-116.

[27] **Mescher, A.L.** and **R.A. Tassava, .** Denervation effects on DNA replication and mitosis during initiation of limb regeneration in adult newts. Dev. Biol., 44: 187-197..

[28] **Bieber, S.** and **G.H. Hitchings, 1959.** Effects of growth - inhibitors on amphilbian tail blastema. Cancer Research, 19: 112-115.

[29] **Donaldson, D.J.** and **J.L. Wilson, 1975.** Dedifferentiation and mitotic activity in amputated newt tails locally deprived of the spinal cord (including a note on effects of amputation level on mitosis). J. Exp. Zool., 191: 9-24.