

## EFFECT OF THE APICAL ECTODERMAL RIDGE ON MITOTIC INDEX IN HIND LIMB BUD MESENCHYME IN *BUFO REGULARIS*

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### ABSTRACT

The apical ectodermal ridge (AER) was formed at stage 51 of *Bufo regularis*, it persisted at stage 52 (limb bud acquiring paddle-shaped outline), then it disappeared by stage 53 (first indication of fourth and fifth toes). Mitotic index (MI) in the AER (stage 51) was higher (40%) than that of the apical ectoderm at stage 50, then it decreased (15%) by stage 52. By apical ectoderm removal at stage 50, the wound epithelium formed an AER by stage 51. The operated bud (80% of cases) showed a decrease in its volume and there was no significant difference in mesenchyme MI. By AER removal at stage 51, it reformed showing normal appearance (70% of cases), and a pronounced decrease in limb size was also observed. Mesenchyme MI (stage 51) was decreased 30% distally and 20% proximally, then distal mesenchyme revealed another decrease (15%), and by stage 52, MI increased distally (20%). Proximal mesenchyme exhibited a pronounced decrease (40%). It is suggested that AER removal reduced the mesenchyme mitotic activity and caused delaying of limb development in early stages, and might play an important role in morphogenetic pattern and in maintaining outgrowth.

### INTRODUCTION

The presence of the apical ectodermal ridge (AER) was earlier reported in the developing limbs of *Xenopus laevis* (Tschumi, 1957). Balinsky (1965) stated that there are no apical ridges on amphibian limb buds, then Tschumi (see Dober and Tschumi, 1969) denied its presence. This structure was then established in *Xenopus laevis* by Tarin and Sturde (1971) and by Abdel-Karim (1985). Many investigators have demonstrated the formation of the AER in the chick limb bud (see Saunders, 1948; Janners and Searls, 1971; Summberell, 1974, 1977). However, the results of

Sturdee and Connock (1975) and of Tank *et al.* (1977) confirmed the absence of AER in limbs of embryonic urodeles. It has been earlier described in other classes of vertebrates (mammals, Milaire, 1956; man, O'Rahilly *et al.*, 1956 and reptiles, 1956). The apical epidermis at stage 53 of *Bufo viridis* was distinctly thickened, being composed of three layers of cells (Michael and Al-Adhami, 1983). A series of 15 stages of development for the mouse limb bud have been defined by Wanek *et al.* (1989). At stage 3, an AER is present as a distinct thickening of the distal epidermis (3-4 cells); and at stage 7, the AER is becoming reduced and it has flattened at stage 8.

When the ectodermal cap of limb buds in *Rana fusca* and *Triturus taeniatus* was injured by cautery (Steiner, 1928), limb development stopped until the cap regenerated. Balinsky's (1931) ideas about co-operative relations between ectoderm and mesoderm were based on experiments in which ectoderm of limb buds, following rotation of 180°, seemed to influence the position of the limbs.

Gasseling and Saunders (1961) postulated that there is an inductive effect of AER on limb outgrowth, part of which might involve some effects of vascularization. Milaire and Mulnard (1969) have presented evidence for inductive chemical factors produced by the ectoderm that are necessary for growth and chondrogenesis of mouse limb bud mesenchyme. Amprino (1965) and Coworkers agree that the ectoderm is indispensable for development of the chick limb bud, but did not believe that an inductive influence of the ridge on mesodermal outgrowth has been definitively shown. Studies of Rubin and Saurders (1972) indicated that the ectoderm is necessary for outgrowth of the limb, but does not act as a level-specific inductor of its spatial patterns of differentiation. Summerbell *et al.* (1973) suggested that the function of the AER is to maintain the mesenchyme at the distal tip in a state of developmental liability, and called this region the "progress zone." Removal of the AER (Summerbell, 1977) caused a reduction in the rate of outgrowth of wing-bud and the loss of distal parts. It caused a decrease in the rate of cell proliferation. The purpose of the present study was to investigate the formation of the AER in the developing hind limbs in *Bufo regularis* and to follow up the changes in the mitotic activity in limb mesenchyme after removal of AER.

## MATERIALS AND METHODS

Embryonic and early larval stages of *Bufo regularis* were collected from some ponds in El-Zobara Farm in Doha, Qatar and reared in the laboratory at 24± 1°C. Larval stages 44, 47, 48, 49, 50, 51, 52 and 53 (according to the Normal Table of Sedra and Michael, 1961, for the same species) were used in the study. Limb buds/limbs were fixed, dehydrated and embedded in paraffin. Serial longitudinal sections (5 u thick) were prepared and stained with Borax carmine — Modified

thickness, still observed only on the most distal margin of the paddle and it then disappeared by stage 53. At that stage, the terminal part is flattened medio-laterally to form the foot paddle which shows the first indications of the fourth and fifth toes (see Sedra and Michael, 1961).

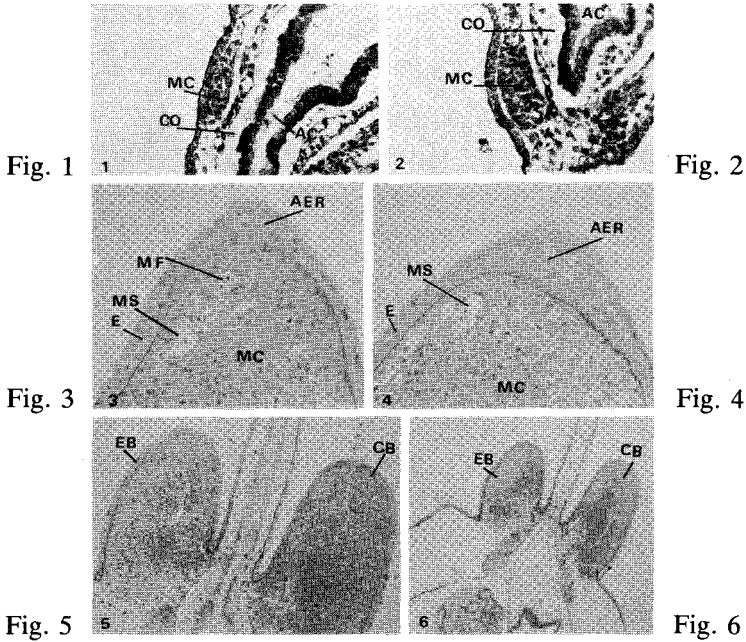


Fig. 1: Longitudinal section of stage 44 tadpole to show the earliest visible features of the hind limb. Note the mesenchymal condensation (MC) close to the anal canal (AC). CO, Coelom. X 400. Fig. 2: Longitudinal section of stage 47 tadpole to show the increasing number and mass of the mesenchyme cells (MC). AC, anal canal; CO, Coelom. X 400. Fig. 3: Longitudinal section of early limb bud at stage 51. Note the apical ectodermal ridge (AER) is restricted to the distal end and tapers down to the thin pre- and post-axial ectoderm. E, epidermis; MC, mesenchyme cells; MS, marginal sinus; MF, mitotic figure, X 280. Fig. 4: Longitudinal section of early limb bud at stage 51. Note the apical ectodermal ridge (AER) is formed of four layers of cells. The marginal sinus (MS) is very close to the distal tip of the limb bud. E, epidermis; MC, mesenchyme cells. X 350. Fig. 5: Longitudinal section of early limb buds, showing the experimental limb bud (EB) and the control one (CB). The AER was removed at stage 50, and the limb buds were fixed two days post-removal. X 190. Fig. 6: Longitudinal section of early limb buds, showing the experimental limb bud (EB) and the control one (CB). The AER was removed at stage 50, and the limb buds were fixed three days post-removal. X 112.

Table 1 shows that the MI in the apical epidermis increased continuously from stage 48 onwards reaching its maximal value by stage 51, by which the MI of the AER was seven times that of the apical epidermis at stage 48, and the increase was about 40% from stage 50 to 51. When the AER diminishes in thickness by stage 52, the MI decreased by 15%. The proximal epidermis showed an increase in mitotic activity reaching its maximal value by stage 50, before the formation of the AER. However, MI decreased by about 30% when the AER was formed at stage 51 and then there was an increase by stage 52.

**Table 1**

Mean mitotic index (MI)  $\pm$  SD of the apical epidermis or apical ectodermal ridge and proximal epidermis of the limb buds of early larval stages.

Stage at Fixation	MI (%) of limb epidermis	
	Apical epidermis	Proximal epidermis
48	0.30 $\pm$ 0.21	0.24 $\pm$ 0.51
49	1.12 $\pm$ 0.40	0.32 $\pm$ 0.36
50	1.50 $\pm$ 0.20	1.80 $\pm$ 0.34
51	2.10 $\pm$ 0.32	1.26 $\pm$ 0.40
52	1.78 $\pm$ 0.26	1.64 $\pm$ 0.28
53	0.85 $\pm$ 0.53	1.58 $\pm$ 0.30

#### Effect of the AER removal:

When the apical ectoderm was removed at stage 50, it regenerated by the next day and it appeared as the normal ectoderm of the other parts of the bud. By the 2<sup>nd</sup> and 3<sup>rd</sup> days following removal, limb bud was slightly elongated without any change in its apical ectoderm (Figs. 5, 6). Then by the 4<sup>th</sup> day when the larva was at stage 51, the apical ectoderm (in all cases) became thickened forming an AER with normal appearance. These limb buds (80% of cases) showed a significant reduction in size compared with controls (Table 2); as the control limb reached morphologically that of stage 54 (the limb is differentiated into thigh, shank and foot, and the third, fourth and fifth toes are differentiated), the operated limb was still morphologically at stage 52 (the limb is paddle-shaped with flattened terminal region).

When the AER (stage 51) was removed, it regenerated by the first day post-operation (in 70% of cases) and became more thickened by the 2<sup>nd</sup> day showing its normal structure. The AER decreased gradually in thickness through the following three days (stage 52) and then it disappeared by the 6<sup>th</sup> day (stage 53). The operated limbs were significantly smaller in size than the control ones till the

end of the eighth day post-removal of AER. However, reaching the end of metamorphosis (stage 66), the difference in size between the operated and control limbs, in both operated stages 50 and 51, was insignificant. In 20% of cases operated at stage 51, an apical cap was formed and the distal limb area was occupied by blood cells, primarily phagocytes, crowding into the area below the wound epithelium. These phagocytes are involved with removal of detritus resulting from the degeneration of the mesenchyme cells. During the operation for these cases, the AER was probably removed together with the most distal part of the mesenchyme. The rest of the operated cases (10%) exhibited no thickening and instead, the limbs formed normal epidermis.

When the apical epidermis was removed at stage 50, the mesenchyme MI was slightly decreased in both distal and proximal areas, during the first two post-operation days. Then it increased by the 3<sup>rd</sup> day especially in the distal region and by the 4<sup>th</sup> day it reached nearly the control value when the AER was formed by stage 51. The results, thus, revealed an insignificant change from the control side. However, the removal of the AER by stage 51 caused a sharp reduction in mesenchyme MI in both distal (30%) and proximal (20%) regions. The distal mesenchyme revealed another decrease in MI (15%) compared to the control, then the MI increased distally (20%) by the 3<sup>rd</sup> post-operation day and it continually increased by the 4<sup>th</sup> day but still lesser than the control value. The proximal mesenchyme MI showed a continuous decrease during the first four post-operation days, exhibiting, by the fourth day, less than half the value of the distal mesenchyme. The difference in mesenchyme MI between the operated and control limbs was significant.

## DISCUSSION

The formation of the AER in the limb buds of *Bufo regularis* was established at stage 51 and it was well distinguished from the proximal epidermis which is formed of one layer of cuboidal cells. These results confirm the observations of Tarin and Sturdee (1971) and Abd El-Karim (1985) on *Xenopus laevis*. In *Xenopus*, the ridge appeared at stage 50, reached its maximal size at stage 51, and subsequently disappeared by stage 53. At stage 50 of *Bufo v. viridis* (Michael and Al-Adhami, 1983), the apical epidermis is thickened, composed of deep cuboidal cells, then at stage 53, it is distinctly thicker, being composed of three layers of cells. However, at stage 53 of *Hyla arborea* and *Rana r. ridibunda*, the epidermis becomes three-layered all over the surface of the limb (Michael and Al-Adhami, vide ibid). The authors related this epidermal stratification to the activating mitotic effect of thyroxine (McGarry and Vanable, 1969). The ridge as seen with the scanning electron microscope (Tarin and Sturdee, 1971) appeared as a single elevation in the limb buds indicating that the ridge was a genuine structure and not an artificial

**Table 2**

Mean limb volume of the experimental (E) and control (C) limbs  $\pm$  SD, during the first eight days after removal of apical epideamias (stage 50) or AER (stage 51). Five animals were used for each post-operation day, at each stage.

Post-operation days	Operated stage 50				Operated stage 51			
	Stage at Fixation		Limb volume ( $\mu^3 \times 10^6$ )		Stage at Fixation		Limb volume ( $\mu^3 \times 10^6$ )	
	E	C	E	C	E	C	E	C
1	50	50	11.10 $\pm 3.07$	12.31 $\pm 2.08$	51	51	20.91 $\pm 2.08$	23.72 $\pm 3.06$
2	50	50	14.40 $\pm 3.70$	16.22 $\pm 1.40$	51	52	24.58 $\pm 4.06$	31.97 $\pm 4.15$
3	50	51	16.82 $\pm 5.22$	19.14 $\pm 3.37$	52	52	31.80 $\pm 4.16$	45.30 $\pm 5.62$
4	51	51	21.97 $\pm 3.66$	26.81 $\pm 5.04$	52	53	43.37 $\pm 8.40$	59.85 $\pm 6.18$
5	51	52	25.31 $\pm 4.80$	35.51 $\pm 3.16$	52	54	54.20 $\pm 6.32$	81.56 $\pm 6.28$
6	52	52	33.40 $\pm 4.53$	48.29 $\pm 4.24$	53	55	63.38 $\pm 7.12$	102.30 $\pm 11.70$
7	52	53	40.73 $\pm 3.08$	64.42 $\pm 3.74$	53	55	71.46 $\pm 6.82$	141.55 $\pm 18.67$
8	52	54	51.02 $\pm 5.02$	85.91 $\pm 6.03$	54	56	86.44 $\pm 8.15$	193.86 $\pm 15.20$

crease produced by drying. In the chick embryo (Saunders, 1948) and in reptiles (Milaire, 1957), the cells of this ridge (in cross section) take the form of a nipple-like extension of the epidermis and appear to be a pseudo-stratified columnar epithelium. In *Bufo regularis*, the ridge appears as a thickened cap on the conical outgrowth and is formed of stratified columnar epithelium. The AER has not been found in limb buds of larval urodeles (Sturdee and Connock, 1975). The results of Tank *et al.* (1977) on axolotl revealed a slight increase in thickness of

**Table 3**

Mean mitotic index (MI)  $\pm$  SD of limb bud mesenchyme in its distal (D) and proximal (P) regions of experimental and control limbs during the first four days after removal of apical epidermis (stage 50) or AER (stage 51). Five animals were used for each post-operation day at each stage.

Post-operation days	Mesenchyme MI (%)							
	Operated stage 50				Operated stage 51			
	Experimental		Control		Experimental		Control	
	D	P	D	P	D	P	D	P
1	0.60 $\pm$ 0.14	0.73 $\pm$ 0.17	0.66 $\pm$ 0.21	0.75 $\pm$ 0.20	0.84 $\pm$ 0.31	0.64 $\pm$ 0.20	1.20 $\pm$ 0.30	0.80 $\pm$ 0.22
2	0.78 $\pm$ 0.21	0.80 $\pm$ 0.20	0.83 $\pm$ 0.35	0.84 $\pm$ 0.26	0.68 $\pm$ 0.32	0.72 $\pm$ 0.34	1.85 $\pm$ 0.51	0.64 $\pm$ 0.32
3	0.40 $\pm$ 0.41	0.84 $\pm$ 0.20	0.96 $\pm$ 0.42	0.86 $\pm$ 0.25	0.82 $\pm$ 0.37	0.43 $\pm$ 0.25	2.14 $\pm$ 0.62	0.75 $\pm$ 0.30
4	1.21 $\pm$ 0.44	0.83 $\pm$ 0.31	1.32 $\pm$ 0.40	0.82 $\pm$ 0.27	0.96 $\pm$ 0.40	0.41 $\pm$ 0.26	1.74 $\pm$ 0.46	0.58 $\pm$ 0.27

some of the cells of the outer layer at stage 37; and in some limbs at stage 39; the superficial epidermal cells on the dorsal surface of the limb bud are thicker (approaching a cuboidal shape) than the squamous cells that cover the ventral part of the limb bud. However, their results confirmed the absence of AER in limbs of embryonic axolotls. In the present results, the AER was decreased in thickness by stage 52 and gradually disappeared by stage 53. During that time, degenerating cells were seen in the AER, especially at the pre-and post-axial sides which were becoming flat. Apparently degeneration of cells is one feature of ridge regression and beginning of toes differentiation.

During the formation of the AER (from stage 50 to 51), the MI (percent of mitotic figures to the total cells) of the apical epidermis increased (40%). The proximal epidermis exhibited its highest value of MI at stage 50 (1.80%) and then it decreased by 30%; but, it increased once again by stage 52 (about 30%). This means that the decrease in MI in the proximal epidermis, was observed during the AER formation. This may indicate a contribution of the dividing cells of the proximal epidermis to the formation of the AER, more dividing cells being added proximodistally. The epidermal cells move proximodistally relatively faster than the mesenchyme cells and consequently the former cells pile up at the marginal

ridge and spread along it. The ridge is thus made up, in part, of cells which were formerly part of the proximal (lateral) epidermis. However, the increasing MI of the apical epidermis during AER formation leads us to suggest that some cells of the ridge come from local mitotic activity. This result confirms the earlier observations of Amprino and Camosso (1955a) on chick limbs and of Tschumi (1955, 1957) who applied carbon marks to limb buds of various stages of *Xenopus*.

Amprino (1965) has shown, by means of  $H^3$ -thymidine labelling studies, that the limb ectoderm continuously slides in a proximodistal direction from the base toward the apex of the bud.

The present observations revealed the AER regeneration after its removal at stage 51, and its formation when the distal ectoderm was removed at stage 50. In *Xenopus*, the AER (Tschumi, 1955, 1956, 1957) may regenerate after it is damaged or removed, and some undifferentiated non-limb ectoderm may, when in contact with the apical region of the limb bud mesoderm, form a ridge. Removal of the apical epidermis (at stage 50) or AER (at stage 51) caused a significant reduction in the limb size during the first eight days post-operation. Afterwards the difference in size between the operated and control limbs become gradually less until there was nearly no difference between them at the end of metamorphosis (about 25-30 days post-operation). This may reveal the important role played by the AER in the outgrowth of the limb during early limb development, and such an ecto-mesodermal interaction may extend for some days beyond the time of AER disappearance. The AER may actively induce the mesenchymal outgrowth, and as a result of the expanding and distal sliding of the whole covering ectoderm, the outgrowing mesenchyme is shaped. We suggest that the AER promotes the proximodistal limb outgrowth, confirming the results of Saunders and Gasseling (1968), Zwilling (1961) and Globus and V-Globus (1976). The present observations are also in accordance with the results of Rubin and Saunders (1972) on chick limb bud. Amprino (1965) and Coworkers have stated that the ectoderm plays a biomechanical role by molding the overall shape of the bud. By AER removal at stage 51, the MI of the distal mesenchyme was greatly decreased during the first two post-operative days, and the mesenchyme cells were much densely packed than in the control. However, by removal of apical epidermis at stage 50 (before the AER is discernible), an insignificant decrease in mesenchyme MI was observed. The AER exerts, therefore, its effect on limb bud mesenchyme in two ways: (1) inducing the limb outgrowth through its expanding distally and providing a space at the apex of the limb into which the mesenchyme can be lodged distally and, (2) increasing the mitotic activity of the distal mesenchyme. As the AER diminishes in thickness and disappears, the MI of the distal mesenchyme increases, revealing an intrinsic factor of mesenchymal proliferation.

In the experimental limbs (stage 51), the MI of the distal mesenchyme is lower than in control during the first three post-operative days, and by the fourth day it



Azan. In such series, the formation of the apical ectodermal ridge (AER) was observed in the developing limb buds.

#### **Removal of the AER:**

After treatment of the limb buds of larval stages 50 and 51 with EDTA solution (ethylenediamine tetra-acetic acid) for about 10 minutes, the apical epidermis or AER was removed with watchmaker's forceps. The operated limb buds/limbs were fixed daily for 8 days after the operation, and processed as described above. All recognizable mitotic figures from prophase to telophase were counted in the apical as well as proximal epidermis of the normal developing limb buds/limbs from stage 48 to 53. Counting was also performed for the distal or subridge mesenchyme as well as the proximal mesenchyme in the operated (Stages 50, 51) and control limbs during the first four days, after epidermal removal. Counting was carried out in every third section of the limb and the mitotic index MI(%) was calculated as  $(X/Y) \times 100$ ; X, number of mitotic figures; Y, total numbers of epidermal or mesenchymal cells counted in each case.

Every third section of the experimental series (operated at stages 50, 51) and of the controls was drawn with the aid of a camera lucida. The area of each drawing was determined by means of Graphics tablet (Apple Computer Inc.), and the total volume of the bud/limb was computed by means of computer programme (Apple IIe). A statistical analysis of differences in mesenchyme MI between the experimental and control limbs was carried out, using the t-test for probability; the difference was considered to be significant if P was less than 0.01.

A total of about 270-280 limb buds were used in the study.

## RESULTS

#### **Formation of the AER:**

At stage 44 of *Bufo regularis*, mesenchyme cell condensation was observed beneath the flank epidermis and close to the anal canal and the caudal region of the coelomic cavity (Fig. 1). By stage 47, mesenchymal cells increased in number (Fig. 2). From stage 48 onwards, the limb bud increased dramatically in size and length. By stage 51, the limb bud was continuously elongated and nerve fibres were seen entering the limb bud. A prominent ectodermal ridge was observed, formed of 3-4 layers the inner one of which is the stratum germinativum (Figs. 3, 4). The ridge is restricted to the distal end and tapers down to the thin pre- and post-axial ectoderm. This means that the ridge itself is higher (thicker) in the mid-portion of the bud than at either side. By stage 52, the limb bud was flattened at its terminal region and is acquiring paddle-shaped outline. The AER was diminished in

increased to come closer to the control state. The reduction in MI maybe indicative of a change in the overall rate of proliferation, and this is consistent with the results of Summerbell (1977) who had concluded that there may be an increase in the length of the cell cycle or some cells are pulling out of the division cycle. The present results cannot, therefore, support the conclusions of Mazia (1961), Cunningham *et al.* (1967) and Grosset and Odartchenk (1975) that the reduction in MI is due to an increase in the length of mitosis itself. The depression in MI and the consequent slow rate of outgrowth of the limb in the first few days, after AER removal, may be dependent on a temporary change in the density of packing of the cells in the distal tip of the limb. This suggestion is concordant with the results of Summerbell (1974) who had concluded that the increase in density in the distal tip of the limb is caused by a simple wound-healing response; the cells in the vicinity of the wound adhere firmly together to form a dense aggregate. The local contraction results in a local increase in cell density (the cells are packed into a smaller volume). This in turn affects the rate of cell division by a negative feedback mechanism (Summerbell and Wolpert, 1972; Amprino, 1974) so that the cells divide less frequently, and there is a reduction in the rate of limb elongation. The new results of Solursh and Reiter (1988) suggested that the chick limb ectoderm has dual effects on cartilage differentiation, depending on the stage of the mesenchyme. One effect involves an early mesenchymal dependence on the ectoderm. This effect requires contact between the ectoderm and mesoderm (G. Pinot, 1980). On the other hand the ectoderm can act without direct contact between the ectoderm and mesoderm, to inhibit chondrogenesis over some distance (Solush *et al.* 1981).

## REFERENCES

- Abdel-Karim, A.E. 1985.** Quantitative investigations on limb regeneration of larval and metamorphic stages of the Egyptian Toad, *Bufo regularis* Reuss and the clawed toad, *Xenopus laevis* Daudin, Ph.D. Thesis, Alex. Univ., Egypt. pp. 101-104.
- Amprino, R. 1965.** Aspects of limb morphogenesis in the chicken. In: Organogenesis (ed. R.L. DeHaan and H. Ursprung), pp. 255-281. New York: Holt, Rinehart.
- Amprino, R. 1974.** Cell density as a factor of negative control of tissue proliferation in the early development of the chick embryo limb. In: Recherches Biologiques Contemporaines, pp. 245-250. Nancy: Imp. Vagner.
- Amprino, R. and M. Camosso, 1955a.** Ricerchesperimentali sulfa morfogenesi degli arti nel polo. J. exp. Zool., 129: 453-493.

- Balinsky, B.I. 1931.** Zur Dynamik der Extremitätenknospenbildung. Roux Arch., 123: 565-648.
- Balinsky, B.I. 1965.** An Introduction to Embryology, pp. 404-415. Philadelphia and London.
- Cunningham, L., H. Cottier, E.P. Cronkite, R.K.R. Janse, S. Safier, and H.P. Wagner. 1967.** Studies on lymphocytes. VIII. Short in vivo mitotic time of basophilic lymphoid cells in the thoracic duct of calves after simulated or effective extracorporeal irradiation of the circulating blood. Expl. Cell. Res., 47: 479-488.
- Dober, E. and P.A. Tschumi. 1969.** Entwickeln sich die Extremitäten von *Xenopus laevis* Daud. ohne Epidermisleiste. Revue suisse Zool., 76: 1046-1055.
- Gasseling, M.T. and J.W. Saunders. 1961.** Effects of the apical ectodermal ridge on growth of the versene-stripped chick limb bud. Develop. Biol., 3: 1-25.
- Globus, M. and V. Globus. 1976.** An in vitro analogue of early chick limb bud outgrowth. Differentiation 6: 91-96.
- G-Pinot, M. 1980.** Ectoderm and mesoderm interactions in the limb bud of the chick embryo studied by transfilter cultures: Cartilage differentiation and ultrastructural observations. J. Embryol. Exp. Morphol., 51: 157-173.
- Grosset, L. and N. Odartchenko. 1975.** Duration of mitosis and separate mitotic phases compared to nuclear DNA content in erythroblasts of four vertebrates. Cell Tissue Kinet., 8: 91-96.
- Janners, M.Y. and R.L. Searls 1971.** Effect of removal of the apical ectodermal ridge on the rate of cell division in the subridge mesenchyme of the embryonic chick wing. Develop. Biol. 24: 465-475.
- McGarry, M.P. and J.W. Vanable. 1969.** The role of thyroxine in the formation of gland rudiment in the skin of *Xenopus laevis*. Develop. Biol. 20: 426-434.
- Mazia, D. 1961.** Mitosis and the physiology of cell division. In: The Cell (ed. J. Brachet and A.E. Mirsky), pp. 77-412. Academic Press.
- Michael, M.I. and M.A. Al-Adhami. 1983.** The development of the hind limbs in anuran amphibians of Iraq. Folia Morph., 31: 270-279.
- Milaire, J. 1956.** Contribution a l'étude morphologique et cytochimique des bourgeons de membres chez le Rat. Arch. Biol., 67: 297-391.
- Milaire, J. 1957.** Contribution a la connaissance morphologique et cytochimique des bourgeons de membres chez quelques Reptiles. Arch. Biol., 68: 429-512.

- Milaire, J. and Mulnard. 1969.** Le role de l'épiblaste dans la chondrogenese du bourgeon de membre chez la souris. *J. Embryol. Exp. Morphol.*, 20: 215-236.
- O'Rahilly, R., E. Gardner and D.J. Gray. 1956.** The ectodermal thickening and ridge in the limbs of staged human embryos. *J. Embryol. Exp. Morph.*, 4: 254-264.
- Rubin, L. and J.W. Saunders. 1972.** Ectodermal-mesodermal interactions in the growth of limb buds in the chick embryo: Constancy and temporal limits of the ectodermal induction. *Devel. Biol.*, 28: 94-112.
- Saunders, J.W. 1948.** The proximo-distal sequence of origin of the parts of the chick wing and the role of the ectoderm. *J. Exp. Zool.*, 108: 363-403.
- Saunders, J.W. and M.T. Gasseling. 1968.** Ectodermal mesenchymal interactions in the origin of limb symmetry. In *Epithelial-Mesenchymal Interactions* (ed. Fleischmajer and Billingham), pp. 78-97. Baltimore: Williams and Wilkins.
- Sedra, S.N. and M.I. Michael. 1961.** Normal table of the Egyptian toad, *Bufo regularis* Reuss, with an addendum on the standardization of the stages considered in previous publications. *Cesk. Morf.*, 9: 333-351.
- Solursh, M. and R.S. Reiter. 1988.** Inhibitory and stimulatory effects of limb ectoderm on invitro chondrogenesis. *J. Exp. Zool.*, 248: 147-154.
- Solursh, M., C.T. Singley, and R.S. Reiter. 1981.** The influence of epithelia on cartilage and loose connective tissue formation by limb mesenchyme cultures. *Develop. Biol.*, 86: 471-482.
- Steiner, K. 1928.** Entwicklungsmechanische Untersuchungen uber die Bedeutung des ektodermalen Epithels der Extremitätenknospe von Amphibienlarven. *Roux Arch. Entw. Mech. Organ.*, 113: 1-11.
- Sturdee, A. and M. Connock. 1975.** The embryonic limb bud of urodele. *Morphological studies of the apex. Differentiation*, 3: 43-49.
- Summerbell, D. 1974.** A quantitative analysis of the effect of excision of the AER from the chick limb bud. *J. Embryol. Exp. Morph.*, 32: 651-660.
- Summerbell, D. 1977.** Reduction of the rate of outgrowth, cell density and cell division following removal of the apical ectodermal ridge of the chick limb bud. *J. Embryol. Exp. Morph.*, 40: 1-21.
- Summerbell, D. and L. Wolpert. 1972.** Cell density and cell division in the early morphogenesis of the chick limb. *Nature New Biol., Lond.*, 238: 24-26.

- Summerbell, D., J.H. Lewis and L. Wolpert. 1973.** Positional information in chick limb morphogenesis. *Nature*, 244: 492-496.
- Tank, P., B. Carlson and T. Connelly. 1977.** A scanning electron microscope comparison of the development of embryonic and regenerating limbs in the axolotl. *J. Exp. Zool.*, 201: 417-430.
- Tarin, D. and A.P. Sturdee. 1971.** Early limb development of *Xenopus laevis*. *J. Embryol. Exp. Morph.*, 26(2): 169-179.
- Tschumi, P.A. 1955.** Versuche uber die Wachstumsweise von Hinterbeinknospen von *Xenopus laevis* Daud. und die Bedeutung der Epidermis. *Rev. Suisse Zool.* 62: 281-288.
- Tschumi, P.A. 1956.** Die Bedeutung der Epidermisleiste fur die Entwicklung der Beine von *Xenopus laevis* Daud. *Rev. Suisse Zool.* 63: 707-716.
- Tschumi, P.A. 1957.** The growth of the hind limb bud of *Xenopus laevis* and its dependence upon the epidermis. *J. Anat.*, 91: 149-173.
- Wanek, N., K. Muneoka, and S.V. Bryant. 1989.** Evidence for regulation following amputation and tissue grafting in the developing mouse limb. *J. Exp. Zool.*, 249: 55-61.
- Zwilling, E. 1961.** Limb morphogenesis, *Advan., Morphogenesis*, 1: 301-330.

## تأثير الحرف الاكتودرمي القمي على معدل الانقسام في ميزنشيمية برعم الطرف الخلفي في بوفورجيو لارس

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أظهرت هذه الدراسة أن الحرف الاكتودرمي القمي يتكون في برعم الطرف الخلفي للمرحلة ٥١ في الضفدعة المصرية بوفورجيو لارس ، ويستمر وجوده في المرحلة ٥٢ حيث يكتسب الطرف شكلاً مجدافياً ، ثم يختفي في المرحلة ٥٣ والتي فيها تظهر أول علامات للإصبعين الرابع والخامس . وقد أوضحت الدراسة أن معدل الانقسام في خلايا الحرف الاكتودرمي ( مرحلة ٥١ ) يفوق ( ٤٠٪ ) ذلك الموجود في الاكتودرم القمي في مرحلة ٥٠ ، ولكنه ينخفض ( ١٥٪ ) في المرحلة ٥٢ . عند إزالة الاكتودرم القمي في المرحلة ٥٠ نجد أن طلائية الجرح تنجح في تكوين حرفاً اکتودرمياً قميّاً في المرحلة ٥١ . وقد وجد أن ٨٠٪ من براعم الأطراف التجريبية أظهرت اختزلاً في حجمها ، ولكنها لم تظهر تغيراً معنياً في معدل الانقسام للخلايا الميزنشيمية الموجودة بالبراعم . أما في حالة إزالة الحرف الاكتودرمي القمي في المرحلة ٥١ فقد أظهرت ٧٠٪ من الحالات تجديداً لهذا الحرف بتركيبه الأصلي ، كما لوحظ أيضاً اختزال في حجم الطرف . أما معدل الانقسام ( مرحلة ٥١ ) فقد انخفض ٣٠٪ في المنطقة البعيدة (الطرفية) و ٢٠٪ في المنطقة القريبة ، ثم أظهرت الخلايا الميزنشيمية في المنطقة البعيدة انخفاضاً آخر في معدل الانقسام ( ١٥٪ ) . وفي المرحلة ٥٢ ارتفع معدل الانقسام في المنطقة البعيدة ( ٢٠٪ ) ، أما المنطقة القريبة فقد أظهرت انخفاضاً كبيراً ( ٤٠٪ ) . وقد برهنت نتائج البحث على أن إزالة الحرف الاكتودرمي القمي يؤدي إلى انخفاض النشاط الانقسامي في ميزنشيمية برعم الطرف ، كما يسبب تأخر نمو الطرف في المراحل المبكرة ، وربما يلعب هذا الحرف دوراً هاماً في التكوين المورفولوجي للطرف وفي توجيه نموه للخارج .