

HISTOLOGICAL OBSERVATIONS ON THE CHANGES IN
THYROID ACTIVITY IN THE GECKONID LIZARD,
BUNOPUS TUBERCULATUS, DURING TAIL REGENERATION

By

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دراسة هستولوجية عن التغيرات التي تطرأ على نشاط الغدة
الدرقية في السحلية البرصية بونوبوس تيويركيولاتس
أثناء تجدد الذيل

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أشارت نتائج الدراسة الهستولوجية على نشاط الغدة الدرقية في السحلية البرصية بونوبوس تيويركيولاتس أثناء المراحل المختلفة لتجديد الذيل أن هناك إنخفاضاً ملحوظاً في نشاط الغدة أثناء مرحلة التئام الجرح . وفي أثناء عملية التجدد يزداد نشاط الغدة الدرقية ليصل إلى أقصى معدل له في مرحلة تكوين البلاستيم حيث ثبت ذلك بوجود إرتفاع في قيم أطوال خلايا حويصلات الغدة وكذلك في إنخفاض كمية الإفراز الغروي الموجود في تجاويف حويصلات الغدة .

وكشفت الدراسة أنه في أثناء تجدد الذيل فإن الحيوان يحتاج إلى مستوى أفضل من لثيروكسين في الدم غالباً في مرحلة تكوين البلاستيم . وفي مراحل التميز والنمو أثناء التجدد كان هناك إنخفاض واضح في نشاط الغدة . وعند اكتمال تجدد الذيل فإن نشاط الغدة كان تقريباً مماثلاً لما هو عليه في الذبول الضابطة (العادية) أي التي لم تبتر .

Key Words: Thyroid, Regeneration, Follicle, Cell height.

ABSTRACT

Histological observations on the thyroid activity during different phases of tail regeneration in the geckonid lizard, *Bunopus tuberculatus*, have indicated a decreased thyroid activity during wound-healing phase. During regeneration, the thyroid activity increased reaching its maximal level during blastema formation phase, as indicated by the increase of follicle cell height as well as the reduction in colloid amount from the follicle lumen. An optimal concentration of thyroxine might be required almost during blastema phase. During differentiation and growth phases a noticeable reduction in thyroid activity was indicated. In the fully regenerated tail, the thyroid activity was nearly comparable to that of the control (normal) tail.

INTRODUCTION

In all vertebrates, the thyroid gland originates as an outgrowth from the floor of the pharynx at the level of the first and second gill pouches [1]. The position and gross form of the gland in adult animals vary considerably. The microscopic anatomy of the thyroid gland is remarkably similar in all vertebrates: its cells are arranged into hollow vesicles known as follicles, the cavities of which, in most vertebrates, are filled with a viscous secretion known as colloid. At stage 46-47 of *Xenopus laevis* larvae [2 &

3], the paired thyroid lobes each consists of a small mass of cells about 20 µm long. At stage 49-50, there are 13-16 follicles per lobe. The development of the thyroid gland was studied in three Iraqi anurans: *Bufo viridis viridis* Laurenti, *Hyla arborea savignyi* Audouin and *Rana ridibunda ridibunda* Pallas [4], and it was similar to that of other anurans; during anuran larval development, the thyroid steadily increases in overall size.

The time sequence of events in thyroid histogenesis is similar in both reptiles and birds [5, 6]. In the viviparous lizard, *Xantusia*

vigilis [7], the thyroid gland develops and persists in the adult as a unilobular structure. At the time of birth (22 mm stage) of that lizard, there seemed to be a decrease in thyroid activity as evidenced by lower follicular epithelium and denser colloid. Etkin [8, 9] stated that the increase in height of the follicular cells, at the onset of the metamorphic period, is an index of increased thyroidal functional activity.

The thyroid gland is known to influence either directly or indirectly the process of regeneration. Hyperthyroidism in amphibians is known to inhibit regeneration, whereas hypothyroidism favours the process [10, 11]. Schmidt [10] also suggested that the healing process such as cellular migration, proliferation and accumulation of epithelial cells covering the amputation wound in newts take less time in the hypothyroid newt, *Triturus viridescens*, than in euthyroid ones. A hormonal dependence of tail regeneration was suggested by the effect of prolactin in the lizard, *Anolis carolinensis* [12]. The changes in the thyroid activity with regard to tail regeneration in the house lizard, *Hemidactylus flaviviridis* [13] was found to depend upon the season and the phase of regeneration. The observations on thyroid activity during tail regeneration in *Mabuya striata* [14] revealed a consistent change in the thyroid activity during blastema phase. Prolactin in combination with thyroxine was reported to be effective in restoring regenerative ability to newts that were hypophysectomized prior to forelimb amputation [15, 16, 17].

MATERIAL AND METHODS

Adults of the geckonid lizard, *Bunopus tuberculatus* were collected from different areas in and around Doha, Qatar. The body length, from snout to vent, ranged from 35-50 mm and the tail length, from vent to the tail tip, ranged from 35-45 mm. The animals were chosen to be nearly of the same size and weight and with original tails, i.e. their tails were not previously regenerated. The animals were kept in the laboratory in glass museum jars, and the tails were amputated, with a razor blade, at the middle level of the tail so that one-half of the tail (17.5-22.5 mm, mean 20 mm) was removed. Heater was placed near the jars to raise their temperature, and it was controlled to be $30 \pm 1^\circ\text{C}$, with 8-12 hours of light per day. The animals were hand-fed a standard diet, one meal of meat daily, and water was provided in small dishes.

Animals were dissected and the thyroid gland was removed and fixed in Bouin's fluid at different phases of tail regeneration (see regeneration phases in *Bunopus* in 18): wound-healing (2-3 post-amputation days), dedifferentiation (3-5 days), blastema formations (5-8 days), differentiation and growth (8-40 days), as well as at the end of regeneration period (fully regenerated tail, 4-55 days), when the tail attains its original length. The gland was embedded in paraplast, cross sections of 5 μm thickness were obtained and stained in borax carmine-modified Azan or in haematoxylin-eosin for histological observations. Thyroid measurements were calculated from five animals at each phase of tail regeneration. The thyroid parameters used were: the height of epithelial cells of follicle (μm), the inner diameter of follicle,

D, (μm), the number of cells in a follicle (N), and the ratio between follicle diameter and cell number (D/N ratio). For measurements, the slides were scanned with a dissecting microscope to identify the section with the greatest number of follicles, usually 25-30. Using the clock face as a point of reference, the four follicles at 12, 3, 6 and 9 o'clock were selected for examination. Within each follicle the same frame of reference was used to select four epithelial cells for height measurements. Epithelial cell heights within each follicle were averaged, and the mean of each of the four follicles was determined. Each of the four follicles was also evaluated on a zero (none) to four (full) basis for the amount of colloid present. Colloid data are presented as an average of the four values per gland. Data were taken at each concerned regeneration phase and data were also taken from animals with normal or unamputated tails as controls.

RESULTS

Structure of thyroid gland in *Bunopus*

The thyroid gland in *Bunopus* is located posterior to the pharyngeal area, near the trachea and adjacent to the carotid arteries or their major branches. The thyroid appears as a unilobular structure, with two sides, located anterior to the heart (Fig. 1). The gland consists of a cluster of rounded follicles, and each follicle is lined by a single layer of cells which are usually cuboidal, or sometimes columnar when highly active (Fig. 2). The viscous secretion product, called colloid, which fills the follicles (Fig. 3) stores thyroglobulin. The thyroid has an exceedingly rich blood supply for its size (Fig. 4).

Changes in thyroid activity during regeneration

During the different phases of tail regeneration of *Bunopus*, the thyroid activity was profoundly changed. The data on thyroid activity are summarized in table 1. The height of the follicle cell decreased during the wound-healing phase, and the D/N ratio increased, revealing a decrease in thyroid activity. During dedifferentiation, the activity increased, as indicated by the increase of follicle cell height and the decrease of D/N ratio. The Thyroid activity reached its maximal level during blastema formation. The increased secretion of thyroxine during blastema phase was also revealed by the reduction in colloid from the lumen of the follicle (Fig. 4, Table 1). Thereafter, during differentiation and growth phase, the follicle cell height decreased and the D/N ratio increased, indicating a decrease in thyroid activity. This activity showed a continuous decrease till the end of regeneration when the regenerating tail restored its normal length before amputation. In the fully regenerated tail, the height of follicle cells and D/N ratio, as well as the colloid amount in follicle were almost similar to those of the normal (control) tail. This may mark the completion of the process of tail regeneration where active morphological and metabolic activities have more or less settled at a normal condition. Thyroid glands from males and females were investigated and the results have indicated no difference, between male and female, in the structure as well as the changes in thyroid activity during regeneration.

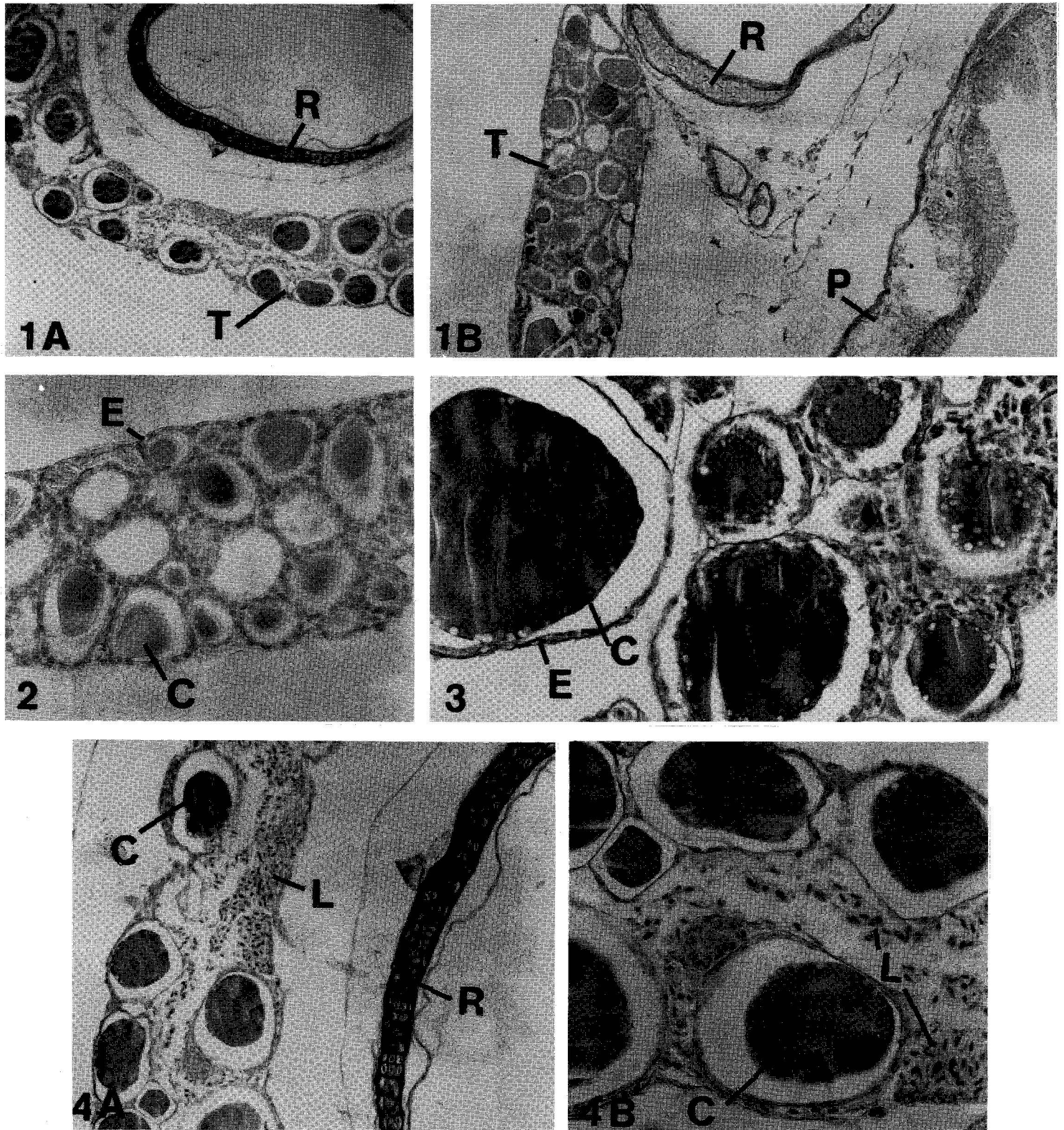


Fig. 1A: Photomicrograph of thyroid gland (T) which appears as a unilobular structure located near the trachea (R). X 180.
 Fig. 1B: Photomicrograph of thyroid gland (T) which is located posterior to the pharynx (P) and close to the trachea (R). X 180.
 Fig. 2: Photomicrograph of thyroid gland during blastema phase of the regenerating tail. Note the gland is formed of rounded follicles lined with cuboidal epithelium (E). Some follicles contain no colloid (C). X 260.
 Fig. 3: Photomicrograph of thyroid follicles filled with colloid (C). Note the follicles are lined with lower or squamous epithelium (E) revealing an inactive state of the thyroid. X 320.
 Fig. 4A: Photomicrograph of thyroid follicles the areas between them are occupied by a well vascularized network of connective tissue (L). C, colloid; R, trachea. X 260.
 Fig. 4B: Photomicrograph of thyroid follicles, showing the blood supply (L). C, colloid. X 320.

Table 1
Thyroid measurements during different phases of tail regeneration of *Bunopus tuberculatus*
*Colloid amount was scored on a scale of 0-4: 0=empty; 1=1/4 full; 2=1/2 full; 3=3/4 full; and 4= full.

Thyroid parameters	Control	Wound-healing	Dedifferentiation	Blastema Formation	Differentiation & Growth	Fully Regenerated
Diameter of follicle	75.5 ± 5.39	64.2 ± 3.54	60.5 ± 3.18	65.7 ± 4.78	68.5 ± 4.86	72.4 ± 5.80
Height of follicle cell	2.4 ± 0.04	1.9 ± 0.02	2.7 ± 0.08	3.5 ± 0.19	2.9 ± 0.15	2.1 ± 0.03
No of cells in a follicle	25.0 ± 2.30	22.0 ± 2.66	24.0 ± 2.75	32.0 ± 3.36	30.0 ± 3.28	27.0 ± 3.18
D/N ratio	3.02 ± 0.03	2.92 ± 0.02	2.52 ± 0.01	2.05 ± 0.02	2.28 ± 0.03	2.68 ± 0.02
Amount of colloid per follicle*	3.5 ± 0.1	4.0 ± 0.2	3.0 ± 0.1	2.0 ± 0.07	2.5 ± 0.1	3.0 ± 0.1

DISCUSSION

The present investigations revealed obvious changes in thyroid activity during tail regeneration in the geckonid lizard, *Bunopus tuberculatus*. The thyroid gland, a major endocrine gland is a 'standard equipment' in all vertebrates. More than any other endocrine gland, the thyroid has great capacity for storage of its secretion. This is reflected in its microscopical anatomy. The structural and functional unit of the thyroid is the follicle, present in large numbers within the gland. Follicles are cystlike structures featuring a secretory epithelium consisting of a single layer of cells. These cells are ordinarily cuboidal in shape, but may be flattened or columnar in dependence of function (see Fig. 4). The areas between follicles are occupied by a well vascularized network of connective tissue. In consequence of the secretory activity of the cells of the follicular epithelium, a sizeable volume of colloid is deposited within the follicular lumen. The principal component of the colloid is glycoprotein known as thyroglobulin. This is believed to be the storage form of the thyroid hormone. The size of the thyroid cells reflects the state of activity of the thyroid [1, 9, 19-22]. In the inactive state, the cells regress to a low squamous state, whereas in stimulated glands the cells enlarge to a cuboidal or columnar state. Miller [7], in his study on the lizard *Xantusia vigilis* considered the lower follicular epithelium and denser colloid to be an indicator to the decrease in the thyroid activity at the time of birth (22mm stage). The increase in height of the follicle cells indicates an active state of thyroid secretion [23]. Since increased height of follicle cells and their number indicate an active state of thyroid secretion, it could be concluded, from the present results, that a higher thyroxine production might be taking place during blastema formation. This sudden burst of thyroid secretion during blastema phase may be in connection with the initiation of differentiation and once this is achieved, a higher level of thyroid hormone may not be required. Therefore, during differentiation and growth phases, there was a noticeable reduction in thyroid activity as indicated by the decrease of height

of cells as well as their number. A reduction in the colloid secretion from the lumen of the follicles confirmed the increased secretion of thyroxine during blastema formation. From the current investigations, one may speculate that a decreased thyroid activity during wound-healing process, favours the initiation of that process, and the optimal concentration of thyroxine is required almost during blastema formation of the regenerating tail. The present study concurs with the observations of Magon [14] that during wound-healing phase of the regenerating tail in the scincid lizard, *Mabuya striata*, there was a decrease in thyroid activity while there was an increase at the end of blastema phase.

Earlier studies on regeneration [24, 25 & 26] showed that thyroidectomy, treatment with thiourea, or increased doses of thyroxine hinder regeneration in amphibians. Nevertheless, prolactin when present with thyroxine [27 & 28] promotes limb regeneration. Thyroxine inhibits tail regeneration of *Rana catesbeiana* tadpoles and induces tissue resorption in the tail regenerates as tadpoles approach late metamorphic stages [29]. These reports suggested some possible relationship of thyroxine to metamorphic changes and capacity for regeneration in the amphibians. However, it was reported [30] that regeneration is essentially independent of thyroid activity and thyroxine concentration remains constant throughout regeneration. Experimental studies employing administration of prolactin and thyroxine and analysis of serum tyrosine level in the blood are currently conducted in our laboratory. These experiments may provide further information on the role of prolactin and thyroxine during tail regeneration in geckos.

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REFERENCES

- [1] Kluge, A.G., 1977. In Chordate structure and function: The endocrine glands of vertebrates. Macmillan Publishing Co., Inc. 2nd Ed. 527-540, New York.
- [2] Fox, H., 1981. In Metamorphosis: A problem in developmental Biology (eds. L.I. Gilbert and E. Frieden). 2nd Ed. 327-362, Plenum Press. New York.
- [3] Fox, H., 1984. In amphibian morphogenesis: The thyroid gland 56-66, The Humana Press Inc. Clifton, New Jersey.
- [4] Michael, M.I. and M.A. Al Adhami, 1974. The development of the thyroid glands in anuran amphibians of Iraq. J. Zool. Lond., 174: 315-323.
- [5] Pischinger, A., 1937. Kiemenanlagen und ihre Schicksale bei Amnioten. Schilddrüse und epitheliale Organe der Pharynxwände bei Tetrapoden. "Handbuch der vergleichende Anatomie der Wirbeltiere, III" (L. Bolk, E. Goppert, E. Kallius, and W. Lubosch, eds.) pp. 299-310. Urban und Schwarzenberg, Berlin.
- [6] Dawson, A.B., 1953. Histochemical evidence of early differentiation of the suprarenal gland of the chick, J. Morphol., 92: 579-595.
- [7] Miller, M. R., 1963. The histogenesis of the endocrine organs of the viviparous lizard, *Xantusia vigilis*, Gen. Comp. Endocrinol., 3: 579-605.
- [8] Etkin, W., 1936. The phenomena of anuran metamorphosis. III. The development of the thyroid gland, J. Morph., 59: 69-89.
- [9] Etkin, W., 1955. Metamorphosis. In analysis of development: 631-663. Willier, B.H., Weiss, P.A. & Hamburger, V. (Eds). Philadelphia: Saunders Co.
- [10] Schmidt, A.J., 1968. "Cellular Biology of Vertebrate Regeneration and Repair". The University of Chicago Press. Chicago and London.
- [11] Michael, M.I. and F.K. Aziz, 1976. Effect of sodium perchlorate on the restoration of the limb regenerative ability in a metamorphic stage of *Bufo regularis* Reuss. Folia Biol. (Krakow), 24: 59-65.
- [12] Licht, P. and N.R. Howe, 1969. Hormonal dependence of tail regeneration in the lizard, *Anolis carolinensis*, J. exp. Zool. 171: 75-84.
- [13] Magon, D.K., 1975. Changes in the thyroid activity in the house lizard, *Hemidactylus flaviviridis*, during different phases of regeneration in the different seasons of the year. Broteria (Ciencias Naturais), XLIV: 113-120.
- [14] Magon, D.K., 1977. Histological observations on the changes in thyroid activity in the scincid lizard, *Mabuya striata*, during different phases of tail regeneration. Zoologica Africana, 12 (2): 389-392.
- [15] Connelly, T.G., R.A. Tassava and C.S. Thornton, 1968. Limb regeneration and survival of prolactin-treated hypophysectomized adult newts. J. Morphol., 126: 365-371.
- [16] Hessler, A.C. and R. Landesman, 1981a. An investigation of the prolactin-thyroxine synergism in newt limb regeneration. J. Morphol., 167: 103-108.
- [17] Landesman, R. and A.C. Hessler, 1985. Temporal analysis of the role of growth hormone in the initiation and maintenance of limb regeneration in the hypophysectomized newt, *Notophthalmus viridescens*. J. Morphol., 183: 301-310.
- [18] 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): 293-300.
- [19] Etkin, W., 1964. Metamorphosis. In physiology of the Amphibia: 227-268. Moore, J.A. (Ed.) New York: Academic Press.
- [20] Etkin, W., 1968. Hormonal control of amphibian metamorphosis. In Metamorphosis: a problem in developmental biology: 313-348. Etkin, W. & Gilbert, L.I. (Eds.). Iowa: Meredith Co.
- [21] Kollros, J.J., 1959. Thyroid gland function in developing cold-blooded vertebrates. In symposium on comparative endocrinology: 340-350. Gorbman, A. (ED.) New York: John Wiley and Sons.
- [22] Kollros, J.J., 1961. Mechanism of amphibian metamorphosis: Hormones. Am. Zool., 1: 107-114.
- [23] Shah, R.V. and T.V. Chakko, 1968. Preliminary observations on the changes in the thyroid activity in the house lizard, *Hemidactylus flaviviridis*, during the different phases of tail regeneration. J. Biol. Sci., 11: 41-44.
- [24] Pawlowsky, E.N., 1933. Regeneration of the intestine in *Rana clamitans* larvae, J. exp. Zool., 141: 449-475.
- [25] Peadon, A.M., 1953. The effects of thiourea on limb regeneration in the tadpole, Growth, 17: 21-44.
- [26] Schotte, O.E. and W.W. Washburn, 1954. Effects of thyroidectomy on the regeneration of the forelimbs in *Triturus viridescens*, Anat. Rec., 120: 156.
- [27] Tassava, R.A., 1969. Hormonal and nutritional requirements of limb regeneration and survival of the adult newts, J. exp. Zool., 170: 33-54.
- [28] Bromley, S.C. and C.S. Thornton, 1974. Effect of a highly purified growth hormone on the limb regeneration in the hypophysectomized newt. *Notophthalmus viridescens*. J. exp. Zool. 190: 143-154.
- [29] Li, C.W. and H.A. Bern, 1976. Effects of hormones on tail regeneration and regression in *Rana catesbeiana* tadpoles. Gen. Comp. Endocrinol., 376-382.
- [30] Liversage, R.A. and R.G. Korneluk, 1978. Serum levels of thyroid hormone during forelimb regeneration in the adult newt, *Notophthalmus viridescens*, J. exp. Zool. 206: 223-227.