ABSTRACT:
The ontogenetic development of the gastrointestinal tract (GIT) of the Egyptian toad, *Bufo regularis* was described and illustrated using light microscopy from the embryonic stage up to the adult one. Seven developmental stages were mainly considered including larval i.e. 42, premetamorphic; 50, prometamorphic; 55, metamorphic; 60, postmetamorphic; 66, juvenile and adult stages. Morphometric parameters were employed for tracing the developmental changes in the GIT morphology and histology including the length, diameter, wall thickness and height of mucosal folds. Morphologically, the length of the GIT had the same attitude as the body length and exhibited progressive increase until stage 55, decreased sharply at stages 60 and 66, then increased progressively at juvenile and adult stages. The anterior portion of the small intestine exhibited more shortening than the posterior, being 89.89% and 85.97% at stage 63, respectively, after which the switchback point disappeared. At stage 66 the small and large intestinal shortenings were 91.86% and 68.42%, respectively. Histologically, the histomorphometric analysis revealed that the diameter of the GIT increased progressively until reaching stage 55, decreased sharply at stages 60 and 66, then increased progressively at juvenile and adult stages. The wall thickness and the height of the mucosal folds of the GIT increased progressively in all the investigated developmental stages with the exception of stage 42, where the values of the height of mucosal folds were higher than stage 50 owing to the presence of the yolk. The stomach exhibited a far less remodelling trend in terms of the investigated parameters.

KEY WORDS:
Ontogenetic development, Histology, Morphometry, Gastrointestinal tract, *Bufo regularis*

INTRODUCTION:
The metamorphosis of a tadpole into a frog is one of the most spectacular events during which the body of the tadpole undergoes remodelling from the larval to the adult form to adapt the transition from the aquatic to the terrestrial life (Shi, 1999). This remodelling is a developmental system that is entirely controlled by the thyroid hormone (Kikuyama *et al.*, 1993; Ishizuya-Oka *et al.*, 2009), and thus offered a unique model system for the study of organ remodelling (Shi, 1999). In this remodelling process, cell death occurs widely in larval-specific organs such as the tail, the kidney, and the gill and also in other organs that exist in both tadpoles and frogs (Ishizuya-Oka *et al.*, 2010). The obvious example of the latter organs is the gastrointestinal tract (GIT) which undergoes remarkable remodelling during metamorphosis. The GIT remodelling transforms a simple tubular organ into a complex multi-folded structure similar to that in higher vertebrates (Smith *et al.*, 2000).

This process of transformation involves both macroscopic changes via shortening and narrowing and microscopic changes at the histological level. The latter includes degeneration of the primary larval epithelium through apoptosis and concurrent proliferation and differentiation of secondary adult cell
types (Ishizuya-Oka and Uda, 1996; Ishizuya-Oka et al., 2010). Intestinal remodelling at metamorphosis transforms the larval herbivorous intestine to the adult carnivorous gut via prodigious amounts of cell death and cell proliferation (Heimeier et al., 2010). During this time larval cells are completely replaced by adult progenitor cells, (Shi and Ishizuya-Oka, 1996). During metamorphosis, the larval epithelial cells undergo apoptosis and are replaced by a multiple-folded adult epithelium with elaboration of connective tissue and muscles (Shi et al., 2001, Schreiber et al., 2005 & 2009).

Previous studies revealed that there is an evident correlation between feeding strategies and the remodelling of the GIT. During metamorphic climax in anurans, the tadpole stops feeding while the intestine shortens considerably (Ishizuya-Oka et al., 2010). Intestinal remodelling is not restricted to amphibians but can also be found in other vertebrates including mammals. In the latter, intestinal remodelling is essential for adaptation of infants to their new environment upon birth and for the development of the complex adult GIT, which begins as they start to eat solid food (Smith et al., 2000). To adapt to its changing dietary environment, the GIT is extensively remodeled from the embryo to the adult during vertebrate development. Morphologically, tadpole intestine is comparable to the mammalian embryonic intestine as both are simple tubular structures mainly consisting of a single layer of primary epithelium (Shi and Ishizuya-Oka, 1996). In amphibians, as in many other taxa, intestinal length is often an indicator of diet (Kramer and Bryant, 1995). Therefore there is a general relationship between the length of the intestine and feeding habits. During development, herbivorous feeding of the anuran larva is superseded by carnivorous feeding in the post-metamorphic froglet and adult. These functional changes are paralleled by significant GIT histo-morphological alternations and remodelling and the intestine has a special situation in this regard where it exhibits adaptive plasticity to variation in digestive demand (Burggren and Just, 1992).

The larval-to-adult epithelial transformation can be divided into two processes at the cellular level: (1) apoptosis of larval epithelial cells (Ishizuya-Oka and Ueda, 1996), and (2) active proliferation and subsequent differentiation of adult epithelial cells (Hourdry and Dauca, 1977; Ishizuya-Oka and Ueda, 1996). Concomitant with epithelial remodelling is the sudden development of the connective tissue, which before metamorphosis is immature and mostly localized in a single longitudinal fold, the typhlosole (Marshall and Dixon, 1978; Ishizuya-Oka and Shimozawa, 1987). The cellular mechanisms responsible for this remodelling have been studied extensively, and tissue–tissue interactions are thought to play important roles in GIT morphogenesis during embryogenesis (Chalmers and Slack, 1998) and at metamorphosis (Hourdry and Dauca, 1977; Dauca et al., 1990). In vitro experiments showed that the mesenchyme influences the transition from a larval to an adult epithelium (Ishizuya-Oka and Shimozawa, 1992).

It has been previously reported that in Xenopus leavis, within 5 days at climax the intestinal tract shortens by about 75% along its entire length and forms a typical vertebrate stomach and small intestine (McAvoy and Dixon, 1978; Ishizuya-Oka and Shi, 2005). The shortening happens rapidly in several days and heaps the epithelium into a thick temporary multicellular lining. The tadpole single cell epithelium becomes temporarily heaped into many layers by the shortening of the intestine and constriction of intestinal diameter (Schreiber et al., 2005). Another study by Ishizuya-Oka and Shi (2005) showed that the phenomenon of shortening and narrowing during intestinal remodelling involves constriction of longitudinal muscle fibers and contraction and death of the radial muscle. During this time, epithelial cells facing the lumen undergo cell death. An evident effect of these size changes is transient heaping of the epithelium into many layers.

Phases in the Egyptian toad ontogeny have been established according to the external features. However, the embryonic, larval and different metamorphic periods involve extensive developmental changes in the internal organs. Among the latter, the GIT lacks a detailed investigation in terms of both morphological and histological changes.

The purposes of this study therefore aimed to:

1- Describe the ontogenetic development of the GIT of the Egyptian toad Bufo regularis in terms of both morphological and histological features.

2- Clarify quantitatively, the remodelling process of the GIT in different selected developmental stages.

3- Determine the structural changes of the GIT before, during and after spontaneous metamorphosis.

4- Gain more and better understanding and provide knowledge in terms of the ontogenetic development of the anuran GIT.

MATERIAL AND METHODS:
Animals and Husbandry:

All the experimental aspects of this work were conducted in compliance with the institutional guidelines for the care and use of animals. Several ribbons of fertilized eggs or
sometimes newly hatched spawns of the toad, *Bufo regularis* were brought into the laboratory from the fields of Shebeen El-Koom districts during the breeding season which lasts from March to September. Developing eggs were collected in a mesh-collecting basket and transferred in plastic bags filled with de-chlorinated tap water. The ribbons were divided into small bunches and kept in either white enamel-coated pans of 30 x 20 x 3.5 cm.; provided with two litres of de-chlorinated tap water or glass aquaria with sufficient supply of de-chlorinated tap water so that the water’ depth remained equal in both cases. De-chlorination was carried out by keeping the ordinary tap water in large uncovered vials in an aerated place, at least overnight. On reaching the feeding stage (44), the tadpoles were gently extruded and freed to the saline. Early developmental stages from stage 10 up to stage 39 were fixed wholly whereas the GIT of the developmental stages starting from stage 40 to stage 66 were dissected and fixed either wholly or divided into their different anatomical parts. In addition to these developmental stages, the GIT of both juvenile and adult stages (10 specimens per each stage) were also included to the morphological and histological investigation. After this preliminary investigation, it was evident and appropriate to start dealing with the GIT from stage 42 onwards. Therefore, the study was based mainly on stages 42, 50, 55, 60, 66, juvenile and adult. When necessary, some other developmental stages were included. A total of 620 animals were used throughout the study.

Isolation of the GIT has been achieved by dissecting the specimens using binocular dissection microscope. A small Petri dish filled with solid paraffin wax was used to fix the tadpole during surgical procedure. The tadpole was placed in the centre of the dish with abdomen up and held in position by an entomological pin passing vertically through the tail just near the cloacal aperture. Irredectomy scissor was carefully used to make a longitudinal opening in the abdominal wall with saving the underlying gut from injury by lifting up the body wall with the help of fine tipped forceps. The outer membranes surrounding the gut were removed using fine pointed forceps. The exposed gut was then excised with an irredectomy scissor, washed in saline and the required morphometric measurements were quickly recorded before the immediate fixation.

**Morphological parameters:**

Starting from stage 42 and after discarding any abnormal individuals, specimens were collected randomly from each tank, anesthetized in 250 mg/l MS222 (tricaine methane sulphonate, Sigma, St. Louis, Mo-USA) and staged. Ten individuals from each selected developmental stage had a prolonged anesthetization and were measured for total body length, i.e. snout-vent, and then dissected to isolate their GIT using binocular dissection microscope. The total lengths of the whole outstretched GIT, as well as the lengths of the four separated parts of the GIT (i.e. stomach, anterior part of the small intestine, posterior part of the small intestine and large intestine) were measured using linear eye piece micrometer in case of the early stages and a ruler in the more advanced developmental stages. The switchback point was appointed to determine the end and start of the anterior and posterior portions of the small intestine in a cranio-caudal direction respectively using the study of Pretty et al. (1995) who reported that the centre of the double coil is the switchback point i.e. the point at which the spiral
reverses itself. The whole specimens, as well as their GIT of the selected developmental stages were photographed using digital camera.

**Histological preparation and parameters:**

For light microscopical investigation, different isolated parts of the GIT were fixed via immersion for 24 hours at room temperature in either 10% neutral formalin or Bouin’s fluid. Smith fixative was also used for the same duration in case of whole specimens (i.e. developmental stages 10-44) to overcome the difficulty of sectioning the early embryonic stages with large amount of yolk. Fixation in 10% neutral formalin and Smith fluid was followed by washing the specimens in running tap water for 12 hours and storing in 70% ethanol until further processing. However, specimens fixed in Bouin’s fluid subsequently received three changes of 70% ethanol and stored in another change of 70% ethanol until further processing.

To overcome the problem of transparency of the GIT segments that belong to the early developmental stages, borax carmine was used to stain these segments in toto for 10 minutes before dehydration and embedding. At the beginning of the histological processing and after storing in 70% ethanol, the specimens were transferred to 80% ethanol and then dehydrated in an ascending series of a mixture composed of both ethanol and pure butanol as follows:

1- 80% ethanol + butanol (3:1)……for 30 min
2- 95% ethanol + butanol (2:2)……for 30 min
3- Absolute ethanol + butanol (1:3)…for 30 min
4- Butanol………………………………for 30 min

Thereafter, the specimens were transferred to the oven at 60°C to substitute the butanol by pre-melted paraffin wax as follows:

1- Paraffin wax + butanol (1:1)………… 15 min
2- Paraffin wax I………………………… 15 min
3- Paraffin wax II………………………… 15 min
4- Paraffin wax III ……………………… 15 min

The specimens were then oriented and blocked out in fresh paraffin wax in order to produce transverse sections and in some cases longitudinal sections. 5μm thick serial sections were cut using a rotatory microtome. Serial sections were mounted on albumin-coated slides. Histological staining was performed with Ehrlich’s hematoxylin and counter-stained with aqueous eosin.

Different morphometric parameters including diameter, wall thickness and height of mucosal folds of the sectioned GIT were measured using linear eye piece micrometer previously calibrated for different magnifications used with a stage micrometer. Some histological sections were photographed using Olympus microscope.

**Statistical analysis:**

All data sets were expressed as mean ± standard error of the mean (SEM). The data were analyzed statistically for normal distribution (Student ‘t’ test) and homogeneity of variances (Levene test) using statistical program of social sciences (SPSS) software for windows, version 11. Differences were considered significant at P<0.05.

**RESULTS:**

The morphological appearance:

The morphological appearance of the seven investigated developmental stages each with its own GIT is presented in Fig. 1 A-G. The graphs show that obvious morphological developmental changes occurred within the GIT of the investigated stages. More pronounced and evident variations between the developmental stages under consideration can be seen from the morphometric parameters.
Fig. 1. Photographs showing developmental changes in the morphology of the investigated stages, each with its own gastrointestinal tract.

A- stage 42
B- stage 50
C- stage 55
D- stage 60
E- stage 66
F- juvenile stage
G- Adult stage.

Abbreviations: Anterior (An), Anterior Portion of Small Intestine (APSI), Duodenum (D), Ileum (I), Large Intestine (LI), Liver (LV), Posterior (Po), Posterior Portion of Small Intestine (PPSI), Small intestine (SI), Stomach (St), Switch back Point (SBP). Arrows indicate the start and end of different GIT parts.

The morphometric parameters:

Developmental changes in the lengths, diameters, wall thicknesses and height of mucosal folds, are summarized in figure 2 A-J. These developmental changes can be described separately as following:

The length:

Figure 2 A shows that the lengths of the body and the GIT exhibited an evident and concurrent relationship in their trend of longevity and shortening during development. The lengths of the GIT were obviously higher than those of the body lengths except at the metamorphic climax stage 60 and the postmetamorphic stage 66 where there were a severe shortening and both had a somewhat similar measurements (19 ± 0.26, 18.45 ± 0.28 for stage 60 and 10.35 ± 0.20, 7.7 ± 0.15 for stage 66 for body and GIT lengths, respectively). The gastric length displayed a progressive increase in the investigated developmental stages and this increase was slow at early stages but dramatic at juvenile and adult stages (Fig. 2B). Another evident and concurrent length relationship was found between the two parts of the small intestine starting from stage 45 where the switchback point appeared until the developmental stage 63 after which the switchback point disappeared (Fig. 2C). The percentage of shortening of the anterior and posterior portions of the intestine exhibited slight
variations being 89.89%, 85.97% for both, respectively compared to its maximal length at stage 55.

Despite the obvious difference in the recorded measurements for both, there was an evident concurrent relationship between lengths of small and large intestines of the investigated developmental stages (Fig. 2D). By the end of metamorphosis; at stage 66, the small and large intestines shortened by 91.86% and 68.42%, respectively compared to its maximal length at stage 55.

The diameter of the GIT:

Both gastric and intestinal diameters gradually increased until reaching the larval stage 55 (Fig. 2 E&F) where the highest values, compared with other larval, metamorphic and postmetamorphic stages, i.e. 42, 50, 60 & 66, were recorded (430 ± 7.75, 320 ± 11.25, 711.66 ± 6.54) for stomach, small and large intestines, respectively. Thereafter, there was a decline in the GIT diameter until stage 66 which had the lowest values along the GIT (218.33 ± 4.01, 110.66 ± 4.77, 340.66 ± 15.20) for stomach, small intestine and large intestine, respectively.

This evident narrowing of the intestinal diameter of the developmental stages 60 and 66 occurred presumably as a consequence of reduction in length. On the other hand, the juvenile stage displayed an evident increase in terms of the GIT diameter (Fig. 2 E&F), but with less values than the adult stage which exhibited the absolute highest values (1690.66 ± 8.82, 1068.33 ± 21.63, 2528.33 ± 8.33) for stomach, small and large intestines, respectively. The highest value recorded for the large intestinal diameter reflects the evident reduction in the height of the mucosal folds.

The wall thickness of the GIT:

The GIT of the investigated developmental stages displayed a progressive increase in its wall thickness (Fig. 2 G&H). This increase went gradually lower until stage 55, and then was moderate at the developmental stages 60 & 66 after which the increase was dramatically evident until the adult stage. The evident increase in the wall thickness of the GIT of the investigated developmental stages 60 and 66 compared with that of the previous larval stages occurred presumably as a consequence of reduction in length.

The height of mucosal folds:

In a similar trend as the increase in the wall thickness, the height of mucosal folds displayed a progressive but slowly gradual increase until the postmetamorphic stage 66 after which the increase was dramatically evident in both juvenile and adult stages (Fig. 2 I&J).
The histological features:

The earliest histological events witnessed the formation of the archenteron as a more or less circular-shaped cavity. Concurrent with the significant elongation of the embryo; the gut was clearly subdivided into fore-gut, mid-gut and hind-gut and the histological differentiation was very slow until reaching the larval stage 41.

Stage 42, i.e. two stages before the external feeding stage, marked the first indication for the presence of a distinct stomach compartment concurrently with the appearance of the hepatocytes (H) and the progressive migration of the splanchnic mesoderm in a proximo-distal (PD) direction (Fig. 3 A).

During the endogenous feeding phase, cellular differentiation of the developing GIT was accompanied with the progressive intercellular consumption of the yolk in a PD direction. The yolk mass showed a complete absorption anteriorly at the gastric region, and thus the stomach possessed a pronounced lumen (Gl) compared to the rest of the intestinal regions (Fig. 3 A). At stage 50, while the intestinal wall was flat, some mucosal folds (Mf) started to appear in the gastric wall as a result of the rapid epithelial cell proliferation. The invagination of the mesodermal cells into the mucosal folds was clear at this stage and resulted in the construction of the lamina propria submucosa (Lps) (Fig. 3 B). The migration of the mesodermal cells - which differentiated to loose connective tissue (Ct) under the gastric epithelial cells - went concurrently with the epithelial cell proliferation starting from stage 42 and became more evident at stage 55 where the stomach was consisted of a submucosa (Sm), a muscularis of circular fibres (C) and a serosa (S) (Fig. 3 C). Due to continuous epithelial cell proliferation, the gastric as well as the anterior intestinal mucosal folds increased in number and modified into zigzag pattern. The continuous histogenesis along the developing GIT was evident in a PD direction and the mutual interaction between the mesoderm and endoderm was evident and expressed by their concomitant cellular behaviour. At the metamorphic climax stage 60, the four gastric layers became more evident and the more notable event was the appearance of some cell debris within the gastric lumen (Gl) (Fig. 3 D). These cell debris became less prominent at the post-metamorphic stage 66 which displayed the typical histology of the vertebrate stomach (Fig. 3 E). The stomach of the juvenile stage (Fig. 3 F) exhibited more advanced histological features than the post-metamorphic stage. However, the general architecture was relatively lesser than that of the adult stage (Fig. 3 G).

The two intestinal regions displayed less degree of histogenesis than the gastric one. The small intestine of stage 42 was composed of a single layer of larval epithelial cells that has only a single epithelial fold - the typhlosole (Tf) - running longitudinally in the anterior portion of the small intestine (Fig. 3 H). When investigated at high magnification, the typhlosole appeared consisted of fibroblasts loosely dispersed in an extensive extracellular matrix between the epithelium and muscle layers. The layer of connective tissue was prominent in the typhlosole, while that in the rest of small intestine remained very thin. The small intestine displayed the early signs of folding at stage 50. The epithelial cells were proliferated rapidly to
form the mucosal folds; there was a concurrent change in the mesenchymal cells underneath. The connective tissue started to enter the thickened points of the epithelium as a sign of intestinal mucosal folds formation (Fig. 3 I). At stage 55 the intestinal epithelial cells had columnar shape and a distinct brush border, while the connective tissue became thicker than the previous stage (Fig. 3 J). The mesenchyme cells showed segregation into two main distinct areas. There were cells found at the periphery of the mesenchyme oriented circularly giving rise to the muscle layers through myogenesis and a subset of mesenchymal cells became close to the epithelium and placed parallel to it giving rise to the connective tissue (Ct).
Fig. 3. Photomicrographs showing developmental changes in the histology of the GIT of the seven investigated stages.

A-G: Transverse sections through the stomach of stages 42, 50, 55, 60, 66, juvenile and adult respectively.

H-J: Transverse sections through the small intestine of stages 42, 50 and 55 respectively.

The metamorphic stage 60 witnessed the presence of intestinal cellular debris in the lumen and the more condensation of the connective tissue underneath the epithelial cells (Fig. 4 A). The rapidly growing cell nests had converged at many points, almost completely replacing the original tadpole epithelium which had sloughed into the lumen. At the post-metamorphic stage 66, the intestinal region displayed less degree of histogenesis than the gastric one and consequently, while the epithelial cells were proliferated rapidly to form the mucosal folds, there was a concurrent change in the mesenchymal cells underneath. The connective tissue started to enter the thickened points of the epithelium as a sign of intestinal mucosal folds formation. The circular muscle layer (C) exhibited earlier differentiation than the longitudinal one (L) (Fig. 4 B). At this post-metamorphic stage, the intestine is configured once again as a single cell-thick epithelium, but it is now highly folded into ridges and troughs that more closely resemble the anatomy of a typical adult vertebrate intestine. The small intestinal musculature had become considerably thickened presumably as a consequence of reduction in length.

Both juvenile and adult stages (Fig. 4 C&D) exhibited a progressive increase in the lumen of the small intestine (Sil), mucosal folds (Mf) and connective tissue (Ct). The underlying connective tissue layer, more developed than in the previous stages, was surrounded by a circular layer (C) of striated muscle cells and a longitudinal muscle layer (L) in the regions of the stomach and small intestine. The epithelium together with the underlying lamina propria constitutes the mucosa (M), the other layers of the intestine are: submucosa (Sm), muscularis (longitudinal and circular) and serosa (S).

As shown in figure 4 E, the large intestinal cells of stage 42 had an evident endodermal yolk mass (Eym) compared to both the gastric and small intestinal ones. At stage 55, the large intestinal epithelial cells started to show the columnar shape compared with stage 50 (Fig. 4 F). Besides the progressive developmental changes in both the epithelial and connective tissues, the most prominent feature of stage 60 was the presence of some cellular debris in the large intestinal region (Fig. 4 G). At stage 66, the epithelium of the large intestine consisted of a single layer of taller columnar mucous cells organized into shallow folds (Fig. 4 H). The muscles were arranged in two layers, inner circular (C) and outer longitudinal (L), and correspond to the muscularis externa of mammals. A thin serosa (S) enveloped the gut wall (Fig. 4 I).
Fig. 4. Photomicrographs showing developmental changes in the histology of the GIT of the seven investigated stages.
DISCUSSION:

The present descriptive and analytical investigation for the development of the GIT of the Egyptian toad *Bufo regularis* started from the developmental stage 42 and it should be noted that some other inter-developmental embryonic and larval stages were also investigated but their description was discarded for the sake of avoiding unnecessary repetition.

It is well documented that the embryonic origin of the GIT is similar in all vertebrates although species differences do exist (Grapin-Botton and Melton, 2000; Smith et al., 2000; Heimeier et al., 2010; Lalremsanga and Hooroo, 2012). According to these previous studies, the development of the GIT resulted from the association of intrinsic genetic factors, endogenous regulatory mechanisms and environmental influences. Also, during the early histological development, the vertebrate GIT is established as an endodermal structure surrounded by mesenchyme (Grapin-Botton and Melton, 2000). As observed in the present study and in accordance to the work of Chalmers and Slack (1998) on *Xenopus*, the GIT of the developing tadpole was composed of an outer smooth muscle layer derived from the mesoderm, an inner epithelial layer derived from the endoderm and connective tissue in between. Since the pioneer work of Yasugi and Mizuno (1990), it has been well established that the morphogenetic processes within the vertebrate GIT are dependent on interactions and mutual cooperation between the epithelium and mesenchyme. Other investigations (Yasugi, 1993; Shi and Ishizuya-Oka 1996; Roberts et al., 1998; Wells and Melton, 1999) have revealed two main interesting points. First, patterning of the gut involves reciprocal inductive signaling between the endoderm and mesoderm during embryonic development. Second, the mesoderm plays a permissive role in the morphogenesis and cytodifferentiation of the gut endoderm (Henry et al., 1996) as for example Ishizuya-Oka and Shimozawa (1994) were able to demonstrate that the connective tissue which is localized in the typhlocole of the larval small intestine is essential for the development of adult epithelium but not for cell death of larval epithelium. Horb and Slack (2001) examined the role of mesodermal endodermal interactions during gut formation in *Xenopus* using endodermal and mesodermal molecular markers. Their study revealed that endoderm formation is autonomous but regional specification and subsequent differentiation occurs only when there is concurrent formation of mesoderm. Equally similar, the differentiation of the mesoderm into smooth muscle is dependent on signals from the adjacent endoderm (Grapin-Botton and Melton, 2000). Based on the mentioned studies, it is well established that epithelial –connective tissue interactions are necessary for the development of the GIT.

The present study revealed a gradual arrangement of the endodermal epithelial cells and concurrent migration of the mesoderm in a proximodistal direction in order to establish the wall of the developing GIT. This manner of behaviour exhibited by the endodermal and mesodermal cells during the histogenesis of the GIT provided indirect evidence for their mutual interaction. The morphogenetic movements displayed by both the mesodermal and endodermal derivatives during the histogenesis of the GIT in the toad *Bufo regularis* suggested a probable reciprocal relationship between the two components. The stratified endoderm formed of undifferentiated epithelial cells underwent a gradual proximo-distal differentiation and at the same time the mesodermal cells exhibited a concurrent spontaneous arrangement underneath the epithelium in order to establish the GIT wall. However, investigating the role of splanchnic mesoderm as well as the specific mesodermal endodermal interaction during the morphogenesis of the GIT needs special techniques and is therefore beyond the scope of the present study. Although it still remains uncertain what kind of interactions occur during the development of GIT, it is strongly suggested, at least from structural point of view, that the increasing cells of the connective tissue play some roles in the rearrangement of the GIT epithelium (Ishizuya-Oka and Shimozawa, 1987; Louvard et al., 1992; Henry et al., 1996).

Before the start of exogenous feeding, the developing GIT of most fishes and amphibians is not fully differentiated (Verreth et al., 1992; Sarasquete et al., 1995; Chalmers and Slack, 1998; Morrison and Wright, 1999; Gisbert et al., 2004; Kamaci et al., 2009; Heimeier et al., 2010). However they must bring their GIT to a differentiated and functional state by the time they start the external feeding even if its anatomical structure is not fully developed (Henning, 1986; Ribeiero et al., 1999). During the endogenous feeding phase of fish, gut differentiation proceeds from the distal to the proximal part (Gisbert et al., 1998; Elbal et al., 2004). This is in contrast to the present observation which agrees with previous
observation on anurans in which the differentiation of the GIT follows the PD direction (Hourdy et al., 1996). It was also evident from the present investigation that the yolk mass was utilized in differentiation and organogenesis rather than in growth as body lengths were not altered significantly when passing from stage 42 to the external feeding stage 44.

According to the morphological findings of this study, there was an evident correlation between the lengths of the body and that of the GIT. Similar correlation between body length and gut length has been reported in the toad Bufo stomaticus by Khan (2008) and Lalremsanga and Hooroo (2012) in the toad Microhyla berdmorei. The anterior portion of the small intestine exhibited more percentage of shortening than the posterior, one being 89.89% and 85.97% at stage 63 after which the shortening is very few until stage 59 and then rapidly increase in number at stage 60. The larval epithelial cells were totally removed through apoptosis by stage 63, while the adult epithelial cells replacing the larval ones finally differentiated by the end of metamorphosis (Ishizuya-Oka and Ueda, 1996; Ishizuya-Oka et al., 2010). In general, programmed cell death is one of the most essential phenomena underlying the anuran remodelling from the larval to adult form (Brown and Cai, 2007).

Compared with anurans, urodeles are known to have gradual GIT development with a very limited degree of remodelling (Harris, 1967; Badawy, 2003). This situation is probably due to genetic factors and feeding strategy as they are carnivorous during their entire life (Schreiber et al., 2005). It therefore, seems evident that the GIT of the toad Bufo regularis undergoes extensive remodelling as it proceeds from larval to adult forming the adaptation to terrestrial carnivorous life. Besides anuran amphibians, many fish species reflects the impact of the feeding strategy on the development of the GIT (Albrecht et al., 2001; Abdulhadi, 2005, German and Horn, 2006). In accordance to the work of Chalmers and Slack (1998) on Xenopus and Lalremsanga and Hooroo (2012) on Microhyla berdmorei, the present results indicated a dramatic changes of both connective tissue and epithelial cells at the metamorphic climax stage 60. Similar to the observations of Ishizuya-Oka and Shimozawa (1987) on Xenopus laevis, in contrast to the larval stages, the metamorphic and post-metamorphic stages develop a thick connective tissue layer. At the juvenile and adult stages, the mesenchyme and muscle layers became thicker, and the epithelium folds into the apical crypts and villi that characterized all adult vertebrate intestines. As in all vertebrates examined (Grapin-Botton and Melton, 2000) and in Xenopus (Chalmers and Slack, 1998), the intestine showed many mucosal folds which smooth muscles were found in two layers of muscle fibres, i.e. an inner circular layer and outer longitudinal layer. The outermost layer was the serosa, composed of a thin layer of epithelial tissue.

The major part of the anuran intestinal shortening is caused by DNA fragmentation (Ishizuya-Oka et al., 2010; Heimeier et al., 2010 and Badawy et al. in preparation) and the remodelling process depends mainly on apoptosis (Roberts, 2000; Nakajima et al., 2005). Indeed, cellular debris was clearly noticed within the histological sections after stage 55. The increased cell proliferation is the major contributor to the temporary thickening of the epithelium at metamorphic climax. Other factors like muscular contraction cause some of the fragmented cells to force together in the lumen (Chalmers and Slack, 1998). In the Xenopus laevis small intestine, apoptotic cells were very few until stage 59 and then rapidly increase in number at stage 60. The larval epithelial cells were totally removed through apoptosis by stage 63, while the adult epithelial cells replacing the larval ones finally differentiated by the end of metamorphosis (Ishizuya-Oka and Ueda, 1996; Ishizuya-Oka et al., 2010). In general, programmed cell death is one of the most essential phenomena underlying the anuran remodelling from the larval to adult form (Brown and Cai, 2007).

The histological findings of the present study demonstrated that the fully developed GIT of the toad showed a general histological pattern similar to that of other vertebrate species including fishes (Suucmez and Ulus, 2005; Khojasteh et al., 2009). Therefore, it exhibited a 4-layer structure: the innermost layer is the mucosa, which is composed of the epithelial lining of the gut and the adjacent overlying mesoderm, including the muscularis mucosa, a thin layer of smooth muscles. The next layer is the submucosa, characterized by undifferentiated connective tissue and vascular tissue. The next layer the muscularis layer, composed of layers of smooth muscle. The
gradually decreased in height in a PD direction. The posterior end of the large intestine was consisted of somewhat straight part with a thickened epithelial wall.

It has been reported that the anurans stomach enlarges and develops mature glands, whereas the intestine undergoes shortening and histological remodelling (Chalmers and Slack, 1998). The progressive increase in the gastric length- despite the presence of some cellular debris within the gastric lumen- indicates that the rate of cell proliferation in the gastric region is relatively higher than that in the intestinal region. This deduction is in accordance with the work of Rovira et al. (1995) on the frog Rana temporaria where metamorphic changes in the stomach witnessed apoptotic features with no signs of shortening. According to the present study (see figure 1 A-G), the developing stomach of the toad Bufo regularis displayed a clear and gradual signs of rotation exhibited by different tetrapod stomach including humans (Nebot-Cegarra et al., 1999).

In conclusion, the ontogeny of the GIT followed the same general pattern that most anurans species described to date follow. However, species specific differences were noted (Chalmers and Slack, 1998; Khan, 2008). The present work represents a comprehensive anatomical and histological guide to the developing GIT in the toad Bufo regularis. The described morphological and histological ontogenetic changes in the GIT of the toad Bufo regularis indicate the presence of both ultrastructural and molecular alterations which are currently under investigation in our lab.

REFERENCES:


المج homem:  
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التعديل الشكلي والسيجي للقناة المعدية المعوية في الصعدة المصرية الرفطاء، يوفر حيولاً سناً صارم معروف حين طارق الريح،  

جسم علم الحيوان، كلية العلوم، جامعة المنوفية

تم وصف وبيان نمو القناة المعدية المعوية في الصعدة المصرية الرفطاء (النوبة) حيولاً سناً صارم معروف حين طارق الريح، وتشمل النمو في مرحلة 42 في الريح،  

وطفل المغذية الساقية بنين النتوءات الإدمانية في شكل وتسمح القناة المعوية وتشمل قطاع وسمك الريح والاندام، وتقع النمو في مرحلة 42 في الريح،  

وطفل المغذية الساقية بنين النتوءات الإدمانية في شكل وتسمح القناة المعوية وتشمل قطاع وسمك الريح والاندام، وتقع النمو في مرحلة 42 في الريح،