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ANATOMICAL, HISTOCHEMICAL AND ULTRASTRUCTURAL ADAPTATIONS OF THE ALIMENTARY CANAL OF THE *UROMASTYX AEGYPTIUS* AND THE *SPALEROSOPHIS DIADEMA* TO THEIR FOOD HABITS

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ABSTRACT. This study deals with anatomical, histochemical and ultrastructural adaptations of the alimentary canal of the *Uromastyx aegyptius* and the *Spalerosophis diadema* to their food habits. Proteins and nucleic acids are highly pronounced in the alimentary tract mucosal cells of the studied two species. A variable distribution of proteins and nucleic acids was observed in the different regions of the alimentary tract mucosa of the studied two species. The activity of alkaline phosphatase showed obvious variations not only among different organs, but also between the two species. At the ultrastructural level, the oesophageal mucosal cells contained oval shaped euchromatic nucleus with condensed chromatin and the perinuclear cytoplasm contained some electron-light vesicles. The gastric mucosal cells contained oval shaped euchromatic nucleus with condensed chromatin and the cytoplasm contained many rough endoplasmic reticulum, also many tonofilaments formed thick bundles which converged at the adherence junction in the lateral membranes. The small and large mucosal intestinal cells contained oval euchromatic nuclei and their cytoplasm contained few electron-light vesicles, also their lateral membranes showed many interdigitations. In spite of their difference in taxonomy, habitat, mode of feeding and their vital activities, they show more or less a similarity in the histochemical and ultrastructural patterns of their alimentary tract mucosa. This study can be applied to distinguish between different species of reptiles and for establishment of natural reserves.

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Key words: Histochemistry; Lacertilian; *Uromastyx aegyptius*; Ophidia - *Spalerosophis diadema*; Electron microscopy; Alimentary tract mucosa.

INTRODUCTION

The histochemistry of the reptilian alimentary tract attracts attention of several authors (Suganuma *et al*., 1981; Taib and Jarrar, 1983; Zaher *et al*., 1990; El-Dawoody *et al*., 1991; Abdel Aziz, 2010; Hamdy *et al.,* 2014). However the comparative studies on the histochemical configuration of the reptilian gut mucosa was only considered recently by few authors (Zaher *et al*., 1991 a, b; Abdeen and Zaher, 1991; Kenawy *et al*., 1991). These investigations tried to cast light on the rate of correlation between the histochemical phenotype of the reptilian gut mucosa on one side and the taxonomic position, mode of feeding and habitat on the other side. However, the fine structure of the reptilian gut has been described in only few species of snakes, lizards and tortoises (Ferri *et al*., 1974; Giraud *et al*., 1978; Perez-Tomas *et al.,* 1990; Abdel Aziz, 2010). The present study is a new addition to the field of the comparative histochemical and ultrastructural studies on the reptilian gut mucosa. The present study surveys the distribution of proteins, nucleic acids, alkaline phosphatase in the gut mucosa and ultrastructural study of the gut mucosa of the Agamid lizard *Uromastyx aegyptius* and the snake *Spalerosophis diadema.*

MATERIALS AND METHODS

The material used in the present work was the alimentary tract of the Agamid lizard *Uromastyx aegyptius* and the snake *Spalerosophis diadema*. The animals were caught fom Ashira in Makkah, KSA. Animals were dissected and oesophagus, stomach, small and large intestines were obtained. Total proteins were detected by the application of the mercuric bromophenol blue method (Mazi *et al*., 1953), where proteins were stained in a deep clear blue color. The methyl green-pyronin method of Kurnick (1955) was applied to detect DNA & RNA. The cytoplasm and the nucleoli were stained in a pink-red color indicating the presence of RNA, while chromatin was stained blue green.

For the histochemical demonstration of alkaline phosphatase, fresh specimens of the oesophagus, stomach, small and large intestines were directly frozen in carbon dioxide and sectioned on the cryostat at thickness of 10 µm. The activity of enzyme was demonstrated using Singh and Sulochana method (1996).

Samples for electron microscopy were fixed in 3.5% glutaraldehyd at pH 7.4 in 0.1M sodium cacodylate solution. The samples were post fixed for 1 h in buffered 1% osmium tetroxide, dehydrated and embedded in Epon resin. Ultrathin sections were double-stained with uranyle acetate and lead citrate (Reynolds, 1963). The methyl green-pyronin method of Kurnick (1955) was applied to detect RNA and DNA.

RESULTS

Total proteins

The application of mercuric bromophenol blue method on the different regions of the gut mucosa of the agamid lizard *U. aegyptiaca* revealed a positive reaction. The oesophageal columnar epithelial cells were found to be charged with a considerable amount of proteonic elements (*Fig. 1*). In the stomach the superficial columnar cells were found to be heavily charged with proteonic content (*Fig. 2*). In the small and large intestines a large amount of proteins was detected in the columnar cells (*Figs. 3, 4*). The application of mercuric bromophenol blue method on the different regions of the gut mucosa of the snake *S. diadema* revealed a positive reaction. The mucosal epithelium cells of the oesophagus contained moderate amount of proteins (*Fig. 5*). In the stomach a protein content higher than that of the oesophagus was observed (*Fig. 6*). In the mucosal epithelium of the small and large intestines a large amount of proteins was detected in the columnar cells (*Figs. 7, 8*).

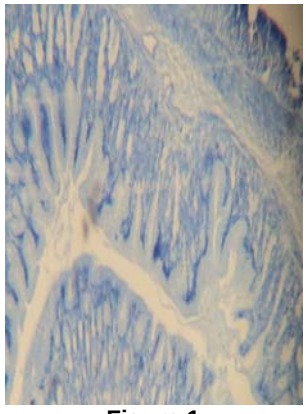


Figure 1

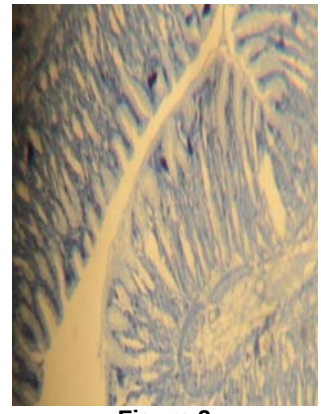


Figure 2

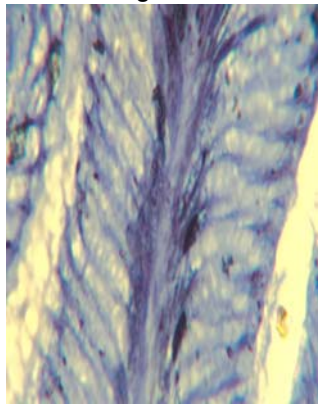
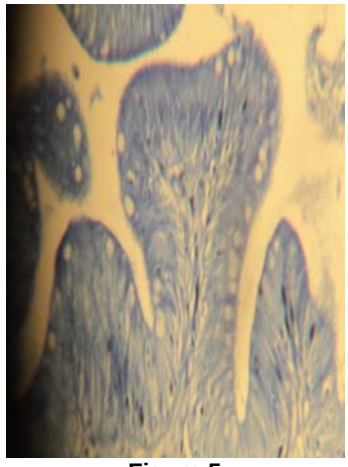


Figure 3

Figure 4



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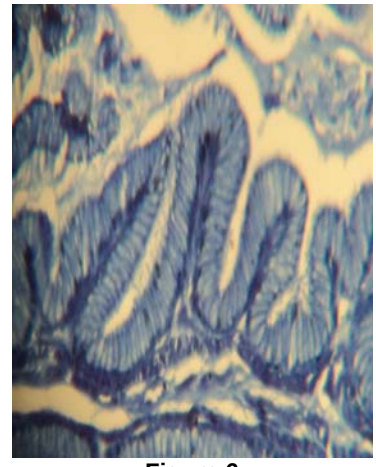


Figure 6

Figure 7

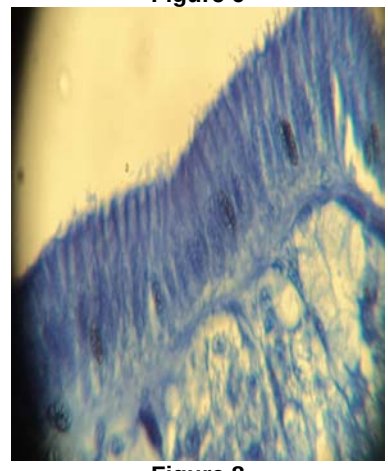
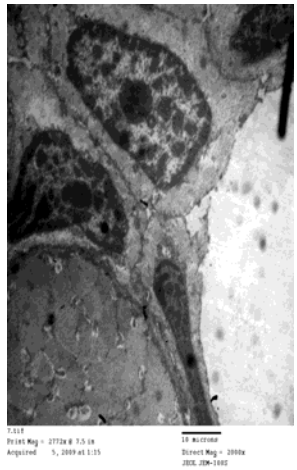


Figure 8

Ultrastructure observations

The ultrastructure studies for the different regions of the gut mucosa of the agamid lizard *U. aegyptiaca* revealed that the oesophageal mucosal cells contained oval shaped euchromatic nucleus with condensed chromatin and the perinuclear cytoplasm contained some electron-light vesicles (*Fig. 9*).The gastric mucosal cells contained oval shaped euchromatic nucleus with condensed chromatin and the cytoplasm contained many rough endoplasmic reticulum, also many tonofilaments formed thick bundles which converged at the adherence junction in the lateral membranes (*Fig. 10*). The small and large mucosal intestinal cells contained oval nuclei and their cytoplasm contained many electron-light vesicles, also their lateral membranes showed many interdigitations (*Figs. 11, 12*).

The ultrastructure studies for the different regions of the gut mucosa of the snake *S. diadema* revealed that the oesophageal mucosal cells contained oval shaped euchromatic nucleus with condensed chromatin (*Fig. 13*). The gastric mucosal cells contained oval shaped euchromatic nucleus with condensed chromatin and the cytoplasm contained a variable number of zymogen granules and vesicles (*Fig. 14*). The small and large mucosal intestinal cells contained oval euchromatic nuclei and their cytoplasm contained few electron-light vesicles, also their lateral membranes showed many interdigitations (*Figs. 15, 16*).



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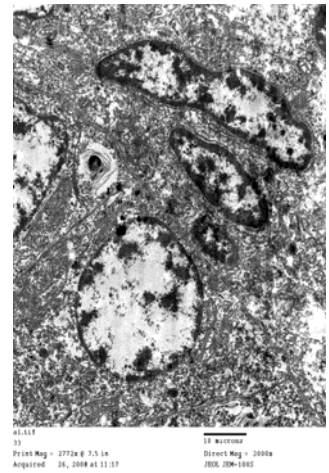

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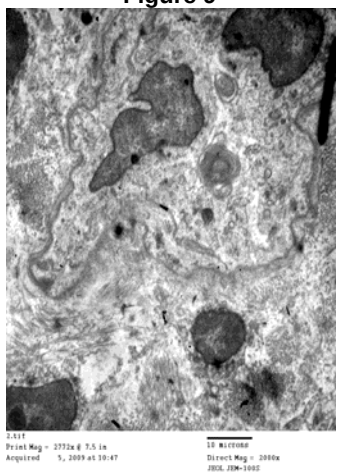
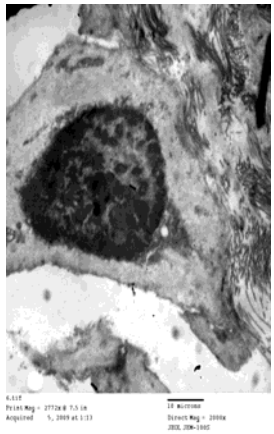


Figure 11

Figure 12



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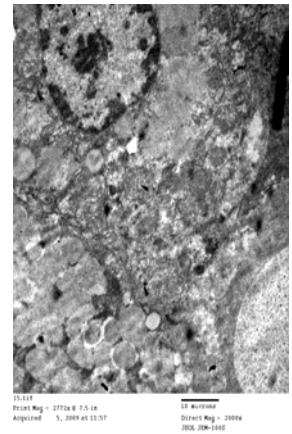


Figure 14

Figure 15

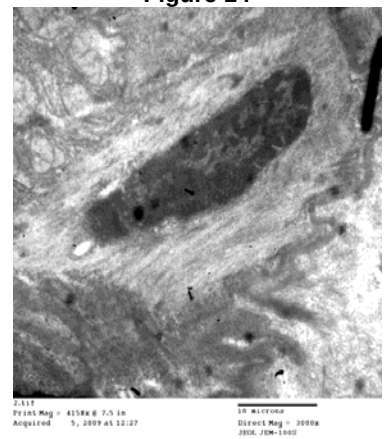
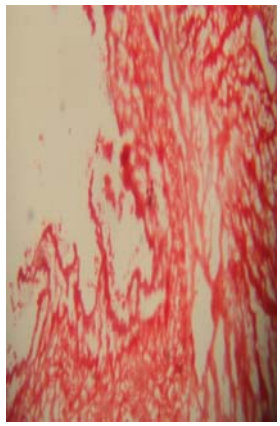


Figure 16

Alkaline phosphatase

In *U. aegyptiaca* and *S. diadema*, the oesophageal epithelial cells showed a moderate alkaline phosphatase reaction (*Figs. 17, 21*). A strong activity of alkaline phosphatase was demonstrated in the gastric mucosa (*Figs. 18, 22*). In the small intestine of both species, alkaline phosphatase activity was highly demonstrated in the apical borders of the mucosal epithelium (*Figs. 19, 23*). In the large intestine of the two examined species, the mucosal epithelium was recorded a strong activity for the enzyme (*Figs. 20, 24*).



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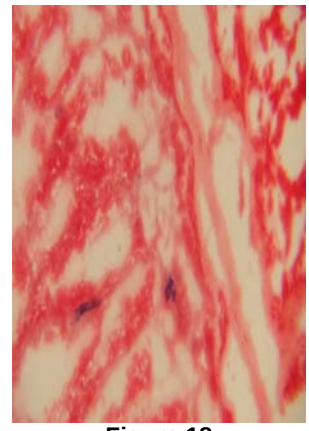

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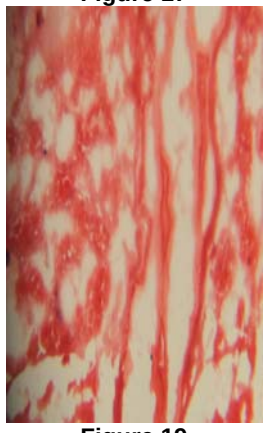

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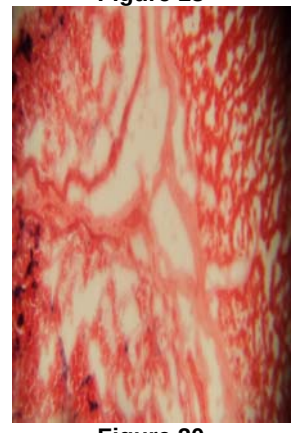

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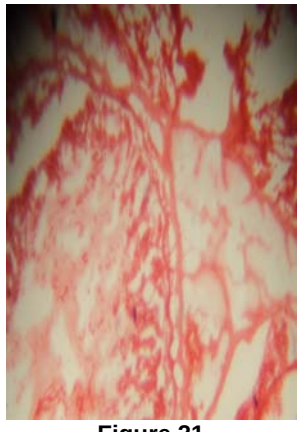

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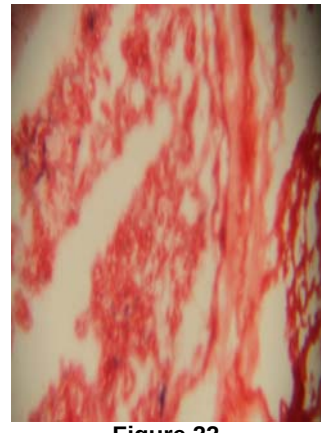

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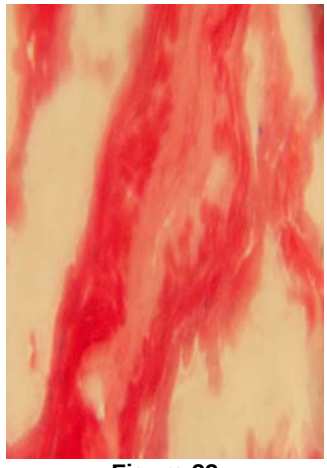

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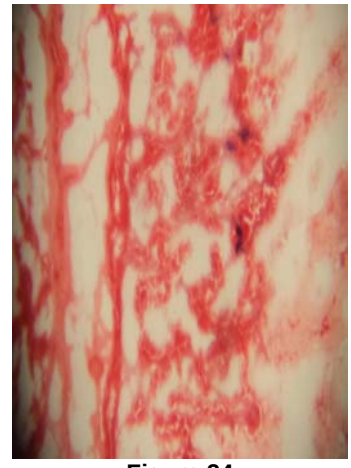
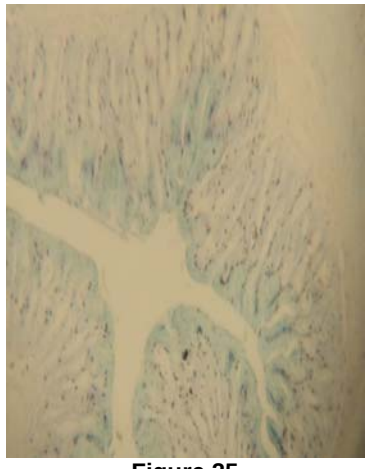


Figure 24

Nucleic acids

In *U. aegyptiaca* and *S. diadema*, the application of the methyl green-pyronin staining technique revealed the presence of DNA-positive bodies only in the nuclei.

The nuclei of the oesophageal mucosa were faintly stained (*Figs. 25, 29*). In case of the stomach (*Figs. 26, 30)*, the nuclei of the lining columnar mucosa were moderately stained. The most strong reaction was in the nuclei of the small and large intestinal mucosae (*Figs. 27, 28, 31, 32*). On the other hand, a positive reaction for RNA was observed in the cytoplasm on the mucosal epithelial cells. The reaction product was low in the oesophagus (*Figs. 25, 29*), moderate in the stomach (*Figs. 26, 30*) and higher in the small and large intestine (*Figs. 27, 28, 31, 32*).



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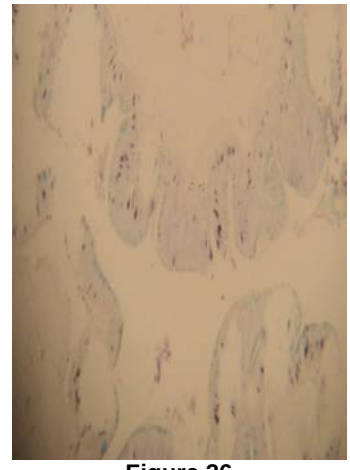

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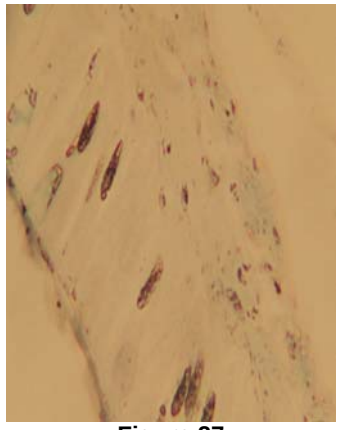

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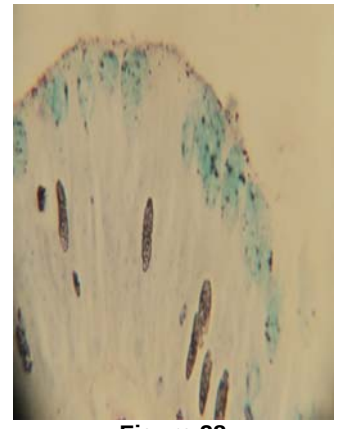

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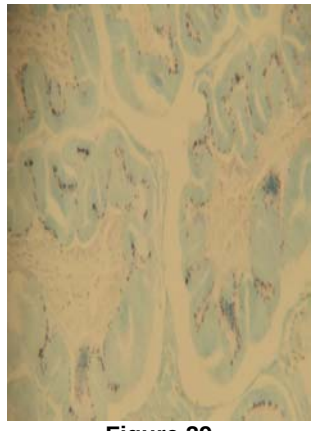

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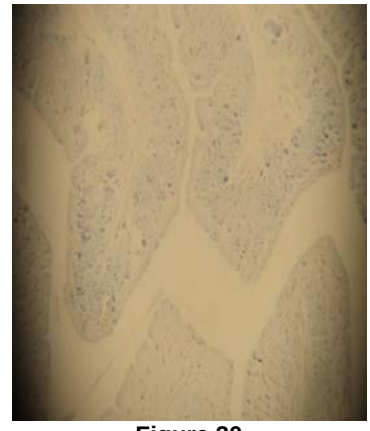

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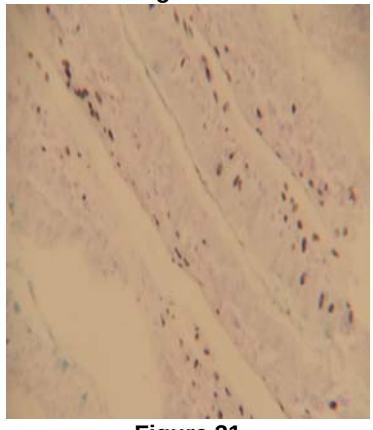

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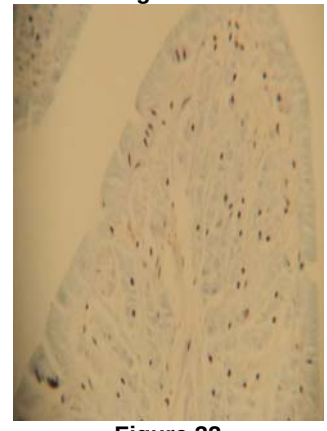

Figure 32

DISCUSSION

In the present study the application of the mercuric bromophenol blue method proved that the proteinic substances are considerable amount in the oesophageal columnar epithelial cells and a large amount in the stomach and intestinal mucosal epithelial cells of the two described species. This feature was similar with slight variation to the condition of the previously described squamate species (Dehlawi *et. al.,* 1988; Abo-Taira *et al.,* 1989; Abdeen *et al.,* 1990; Zaher *et al.,* 1990; Abdel Aziz, 2010; Hamdy *et al.,* 2014).This slight variation in protein content between the studied two species seem to be dependent on the locality of the animal, mode of feeding and the taxonomic position.

In the present species *U. aegyptiaca* and *S. diadema* , as commonly found in lizards and snakes, the nuclei of the gut mucosal cells are rich in DNA indicating the presence of chromatin granules, while RNA is present sufficiently in the cytoplasm (Amer *et al*., 1987; Zaher *et al*., 1990; Abdel Aziz, 2010). Meanwhile, the intensity of RNA along the alimentary tract mucosa is reported to be proportional to the amount of proteins in these regions, since RNA represents the major source of the protein production. This confirmed the findings of Amer *et al. (*1990) and Abdel Aziz (2010).

Few studies have used transmission electron microscope to examine the fine structure of the reptilian gut mucosal cells (Ferri *et al*., 1974; Giraud *et al.*, 1979; Perez-Tomas *et al*., 1990; Abdel Aziz, 2010). In *U. aegyptiaca* the oesophageal mucosal cells contained oval shaped euchromatic nucleus with condensed chromatin and the perinuclear cytoplasm contained some electron-light vesicles, while in *S. diadema* the oesophageal mucosal cells contained oval shaped euchromatic nucleus with condensed chromatin, these findings to that previously described by Guyton (1988). In *U. aegyptiaca* and *S. diadema* the gastric mucosal cells contained oval shaped euchromatic nucleus with condensed chromatin and the cytoplasm contained a variable number of zymogen granules and vesicles, similar to those reported by Giraud *et al*. (1979) and Abdel Aziz (2010). In *U. aegyptiaca* and *S. diadema* the small and large mucosal intestinal cells contained oval nuclei and their cytoplasm contained many electron-light vesicles, also their lateral membranes showed many interdigitations confirm earlier findings of Takahata (1981), Banan Khojasteh *et al.* (2009) and Abdel Aziz (2010).

The high alkaline phosphatase activity detected in the stomach and intestinal mucosal cells of the studied two species due to the fact that alkaline phosphatase might play an important role in the absorption of fats (Zaher *et al*., 1995; Abdel Aziz, 2010).

Positive reaction for DNA in the nuclei and for RNA in the cytoplasm of the cells lining all the alimentary canal regions of the studied two species were clearly visible in the

present investigation. The mucosa lining the small and large intestine displayed a stronger reaction than that lining the oesophagus and stomach. The present findings support the observations of Abdel Aziz (2010).The present result support the observations of Amer *et al.* (1987, 1988), Zaher *et al.* (1991 a, b) and Abdel Aziz (2010), that the detected amounts of RNA are proportional to the demonstrated proteins.

In spite of their difference in taxonomy, habitat, mode of feeding and their vital activities, they show more or less a similarity in the histochemical and ultrastructural patterns of their alimentary tract mucosa. This study can be applied to distinguish between different species of reptiles and for establishment of natural reserves.

CONCLUSION

In spite of their difference in taxonomy, habitat, mode of feeding and their vital activities, the Agamid lizard *Uromastyx aegyptius* and the snake *Spalerosophis diadema* show more or less a similarity in the histochemical and ultrastructural patterns of their alimentary tract mucosa. This study can be applied to distinguish between different species of reptiles and for establishment of natural reserves.

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