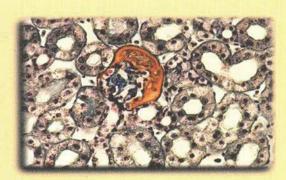




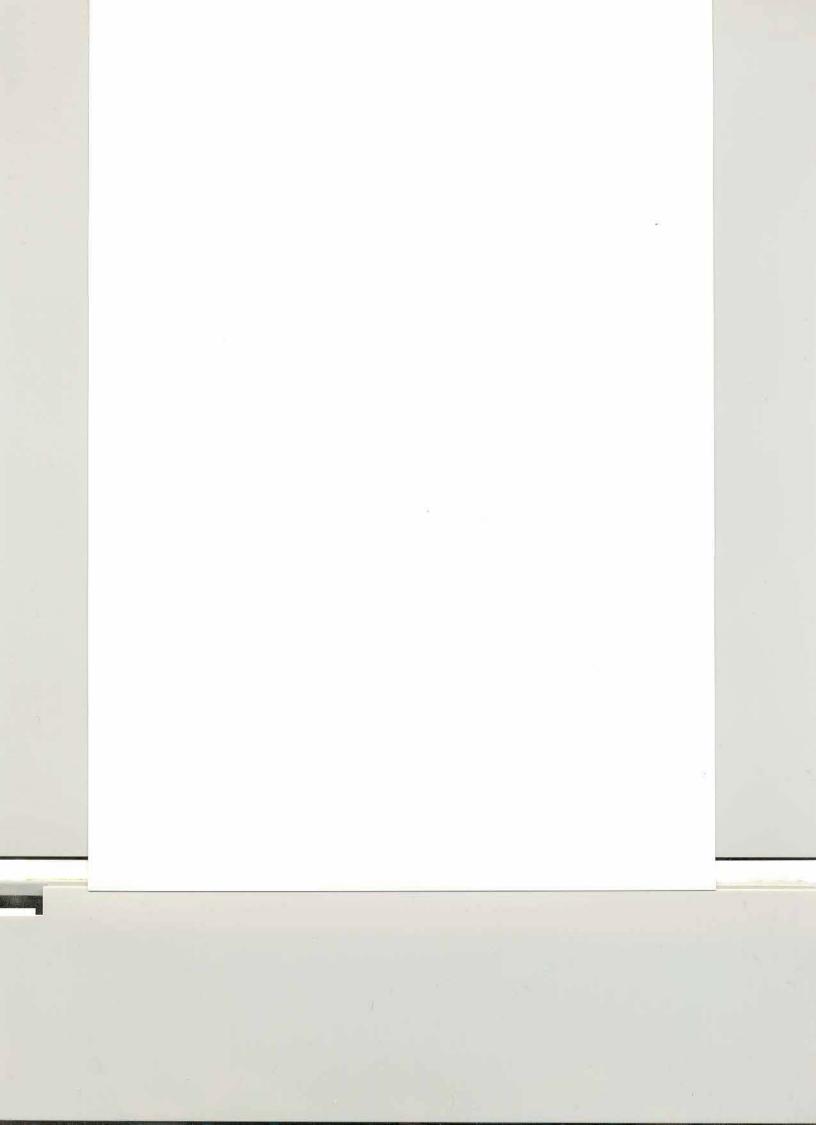
AND ENZYME
HISTOCHEMICAL
DEMONSTRATIONS OF
QATARI REPTILES IN
RELATION TO SEASONAL
AND ENVIRONMENTAL
VARIATIONS
PART II
(HISTOLOGICAL REVIEW)

Dr. Aisha Saud Al-Thani Dr. Gamal El-Sherif





Scientific and Applied Research Center (SARC)
Univiersity of Qatar
2002



RECORD, HISTOLOGICAL AND ENZYME HISTOCHEMICAL DEMONSTRATIONS OF QATARI REPTILES IN RELATION TO SEASONAL AND ENVIRONMENTAL VARIATIONS

Project No. (HE/45/95)

PART 2 (HISTOLOGICAL REVIEW)

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SUMMARY

This part concerning the Histology and Histo-chemistry of some common Qatari reptiles which are investigated as an extension of a thorough study has began in 1996 and completed as part (1) in year 2000. Anatomy of worm like lizard *Diplomotopon zarudnyi*, which is common in Qatar environment, was described with special reference to the arrangement and shape of the internal organs. The left lung was found larger in size with about four folds compared to the right lung. Gut was found generally straight or slightly folded. Transition from oesophagus, stomach, small and large intestine and cloaca was in a form of just swelling or narrowing. Liver and kidney of D. zarudnvi were studied through H&E and PAS techniques. Unlike mammals, no lobules were observed in liver, it was found to be composed of hepatocytes surrounding blood sinusoids. The walls among sinusoids are of two cells thick and the adjacent hepatocytes are held together by the tiny bile channels. Liver capsule, ductules and cell surfaces lining sinusoids exhibited a moderate polysaccharide content. The metanephric kidney was typically formed of renal capsules and nephric tubules which are divided into proximal, intermediate and distal tubules without distinct cortex and medulla like mammals. Proximal tubules were of brush-border cuboidal or low columnar cells while the intermediate segment was of thin ciliated cells and again cells lining the distal tubules were cuboidal with few or without microvilli. The parietal walls of renal capsules were of simple Squamous epithelium. Proximal tubules showed a light polysaccharide content while the visceral walls of the renal corpuscles exhibited more content. Anatomy of D. zarudnyi exhibits a classical mode of almost a straight reptilian gut. Structure of liver and kidney of D. zarudnyi confirms the metabolic and water conservation mechanisms of reptiles.

The structure of the digestive tract of *Diplometopon zarudnyi* was studied by means of light microscopic histology and PAS reaction. The oesophagus is lined with stratified columnar epithelium while the stomach is lined with mucous columnar epithelium. The small intestine is lined with brush-border absorptive columnar cells and goblet cells. While the large intestine is lined with mucous pyramidal cells and larger goblet cells with an extreme layer of mucin lining. The oesophageal and gastric mucosae are uniformly PAS positive. In both the large and small intestine, goblet cells were normal with large amounts of mucin granules. The muscular layer varies in structure being only longitudinal in the oesophagus, thin longitudinal and circular in stomach and more thick in the large intestine.

The histological structure of the gastrointestinal tract, liver and kidney of the non-horned snake, Cerastes vipera and the pattern of the PAS positive materials as well, were described, both the histological findings and also the mucin content in the GIT of C. vipera was found to be related to the nature of the food stuffs and the feeding mechanism of snakes. The digestive action of the high enzyme content of the snake saliva reflects the clear differences between snakes and other animals. Mucins play a critical role in digestion and excretion in the snakes. The internal structure of the liver and kidney was also reflects the adaptations of this snake to the desert environment.

PREFACE

Inspite, reptiles have been attracted the attention of many investigators mostly as surveying studies, no parallel studies on histological structures have been established. Histology of reptiles as of lower animals is relatively primitive and reflects the simplicity of anatomical characteristics of this group. Histology and anatomy of the gastro-intestinal tract (GIT) of reptilian species has attracted the attention of some investigators. It is well known that the anatomy and histology of GIT plays a vital role in feeding habits of reptiles which is considered as an important and critical factor in any ecosystem specially in desert ecosystem of \P atar.

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RESULTS AND DISCUSSIONS

Part I

Histological and Histochemical study of the digestive tract of the worm-like reptile

Diplometopon zarudnyi (Squamata)

HISTOLOGICAL AND HISTOCHEMICAL STUDY OF THE DIGESTIVE TRACT OF THE WORM-LIKE REPTILE Diplometopon zarudnyi (SQUAMATA)

ABSTRACT

The structure of the digestive tract of Diplometopon zarudnyi was studied by means of light microscopic histology and PAS reaction. The oesophagus is lined with stratified columnar epithelium while the stomach is lined with mucous columnar epithelium. The small intestine is lined with brush-border absorptive columnar cells and goblet cells. While the large intestine is lined with mucous pyramidal cells and larger goblet cells with an extreme layer of mucin lining. The oesophageal and gastric mucosae are uniformly PAS positive. In both the large and small intestine, goblet cells were normal with large amounts of mucin granules. The muscular layer varies in structure being only longitudinal in the oesophagus, thin longitudinal and circular in stomach and more thick in the large intestine.

INTRODUCTION

The reptilian fauna of Qatar was briefly reported few years ago [1]. Reptiles of Kuwait were also recored [2]. However no previous studies have been made on the Histology and Histo-chemistry of reptiles recorded in Qatar.

Diplometopon zarudnyi is a worm-like burrowing reptile classified at one time with the lizards but now generally given a subordinal rank within the Squamata in parallel with lizards and snakes since it has some characters typical of snakes and lizards [3]. It is limbless, long and cylindrical with blunt head, short pointed tail and very small eyes. It feeds on small i nvertebrates, a nts and termites, and live in burrows in s andy areas. Its dorsal side has a spotted brown colour while the ventral side is whitish with smooth scales, It is locally known as "Nadus". It is known in El-Karaana area, south-west of Doha and Qatari western desert near Umm-Bab. Few studies are known regarding histology of the digestive tract of certain reptilian species [4,5]. Other investigations concerned with histochemical and ultrastructural observations on the digestive tract of reptiles are also reported [6,7,8,9,10].

The main objective of this work was to investigate the histological structure of the digestive tract of *D. zarudnyi* with special reference to the polysaccharides in the tissues of the digestive system.

MATERIALS AND METHODS

Animals were collected in November 1995, identified and some individuals of them from both sexes were injected with formalin and preserved in jars containing 10% formalin. Eight other animals, were dissected under chloroform anaesthesia, the digestive tract was distinguished and then injected with 10% formalin with 1% CaCl₂ in phosphate buffer saline (PBS).

Transverse strips (5 mm wide) were cut at different parts of the digestive tract: The oesophagus, the stomach, the small intestine and the large intestine. Samples were fixed by extra immersion in the buffered 10% formalin with 1% CaCl2 overnight then washed and routinely processed for paraffin embedding and 5µm sections were prepared. These were investigated by H & E technique for general histological studies and by PAS for polysaccharide histochemistry.

RESULTS

Different organs of the digestive tract of *D. zarudnyi* exhibited histological and histochemical variations which can be summarized as follows:

OESOPHAGUS

The mucosa of the oesophagus showed many longitudinal folds. The muscosal epithelial lining is formed of stratified columnar epithelium with middle well-defined polyhedral cells (Fig.1). Wide layer of longitudinal muscle fibres occurs underneath the distinct lamina propria. After PAS reaction, the oesphageal mucosa exhibited a moderate regular distribution of polysaccharides on the lining epithelial cells (Fig. 2). However, the polysaccharide content was more intense in the cytoplasm of basal cells while surface polyhedral cells showed only PAS positive cell membranes lamina propria, submucosa and muscle layer were very poor in polysaccharide content.

STOMACH

It shows prominent muscosal folds with a short simple gastric villi supported by a submucosal core. There was a thin layer of inner longitudinal and outer circular muscle fibres (Fig.3) The gastric mucosa is built of mucous columnar cells with basal nuclei. The mucous columnar cells of the gastric mucosa revealed a uniformly polysaccharide distribution with PAS reaction (Fig.4).

SMALL INTESTINE

It is characterised by long and narrow folds, with very poor musculature and submucosal lymph cell aggregations (Fig.5) and well defined lamina propria with no special intestinal glands, The lining epithelium is composed of columnar brush-border absorptive cells with distinct microvilli and prominent large goblet cells (Fig.6). Intestinal lining epithelium is provided by goblet cells which are larger and more common than the absorptive cells. The former cells have abundant amounts of mucin granules (Fig.7) and PAS positive lamina propria.

LARGE INTESTINE

The mucosa of the large intestine shows a characteristic pattern with short, branched but fewer folds than those of the small intestine. Submucosal cores and lymph aggregations are prominent. The large intestine is provided by thicker patches of inner longitudinal and outer circular muscle fibres than those of the small intestine, vascularized submucosa is also observed (Fig.8). The lining epithelium was formed of a single layer of mucous columnar cells incorporated with goblet cells (Fig.9). Large intestinal mucosal cells are covered by a layer of mucin with large number of goblet cells which are completely filled with larger amounts of mucins coming out of the goblet cells all over the mucosal surface (Fig.10). Some columnar cells are binucleated.

Figure (1) Section of the oesophagus showing mucosa lined by stratified columnar polyhedral epithelial cells with longitudinal muscle fibres undermeath the lamina propria. H & E, x 100

Figure (2) Moderate regular distribution of polysaccharides in the oesophagus lining. Basal cells exhibit more intense reaction than the surface cells. PAS, x 100

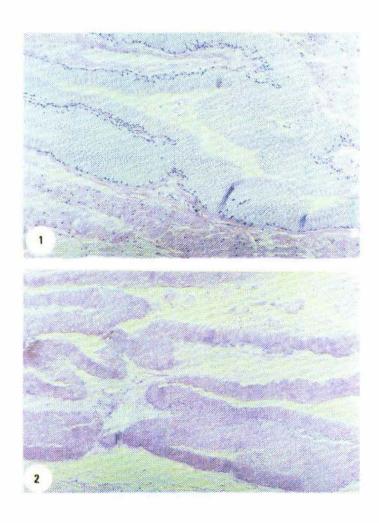


Figure (3) Gastric mucosa of mucous columnar cells with basal nuclei and thin layer of outer circular and inner longitudinal muscle fibres. H & E, x 100

Figure (4) PAS positive materials distributed along the gastric mucosal surface. PAS, x 100



Figure (5) Long and narrow folds of the small intestinal mucosa with scattered lymph aggregations (arrow). H & E, x 100

Figure (6) Mucosa of small intestine showing the columnar absorptive cells with brush – borders (arrow) and large goblet cells (asterisks). H & E, x 200

Figure (7) Large goblet cells of the small intestinal mucosa exhibited ideal PAS positive reaction with a distinct lamina propria (arrow) PAS, x 400

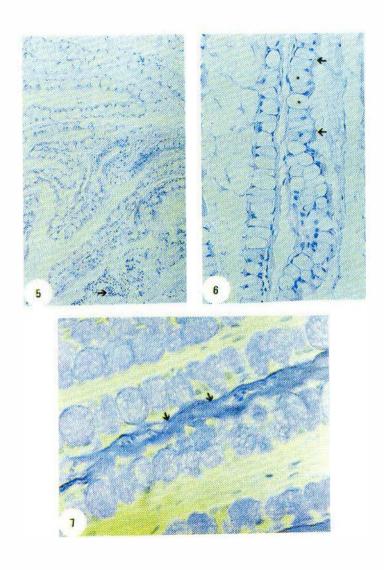
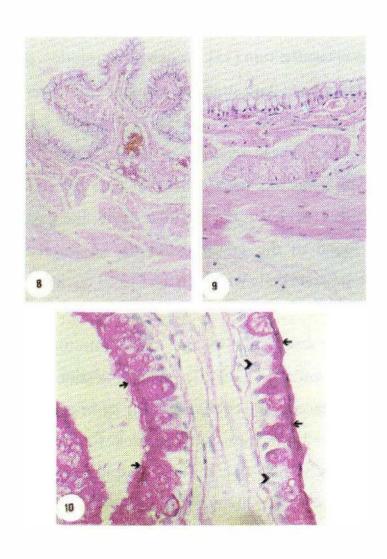


Figure (8) Section in the large intestine showing short and branched villi with distinct inner longitudinal and outer circular muscles. H & E, x 100

Figure (9) Goblet cells and mucous columnar cells of the large intestine mucosal lining. H & E, x 100

Figure (10) Mucosa of the large intestine with goblet cells completely filled by mucins forming a uniformal layer on the mucosal surface (arrows) and the binucleated columnar cells (arrowheads). PAS, x 400

p.



DISCUSSION

Previous studies showed that the epithelial lining of the oesophagus in reptiles consists of ciliated columnar type [11,12,13,14]. The oesophagus of a fresh water turtle was described as being lined by keratinized stratified squamous epithelium [15]. Unlike other reptiles, the oesophagus of *D.zarudnyi* has is lined with stratified column cpithelium.

The reptilian stomach was reported as having a gastric mucosa of 2 different cell type: mucous neck cells with their pyramidal shape and oxyntiopeptic cells [16,17,10]. On the other hand, the gastric mucosa of *D.zarudnyi* is found in the present work to be formed only of tall columnar oxynticopeptic cells with a distinct basal nuclei.

Goblet and absorptive cells of the small intestinal mucosa were described in many reptiles [4,5,10]. The small intestine of *D. zarudnyi* is lined by prominent large goblet cells and also by brush-border columnar absorptive cells which increase surface area for absorption. It was estimated that the such brush-border clumnar epithelium would in arease the obsorptive surface area by about 600 folds [18].

In agreement with other findings in tortoises [10], the lining epithelium of large intestine of **D**. zarudnyi was composed of exclusively large goblet cells and mucous columnar cells. This confirms that in Testudo

graeca [9] where no absorptive cells were reported. The musculature of the digestive tract of *D. zarudnyi*, exhibits different forms, being only longitudinal in the oesophagus where ther is no need for transverse contractions of the circular muscles since the oesophagus is relatively short. Both longitudinal and circular muscle fibres form a thin layer which may help in the gastric contractions on both axes generating the peristaltic movements of the gastric wall during digestion. The poor muscular layer of the small intestine is probably enough for the passage of absorbed digested food after to the large intestine. The latter has highly developed thick longitudinal and circular muscle layers which can produce two dimensional contractions to facilitate the process of defaecation. Lymph cell aggregations in the submucosa of the large and small intestines of *D.zarudnyi* are apprently elements of the immune system in these animals.

Mucins exhibited moderate uniform distribution in the oesophageal and gastric lining epithelium especially the basal cells. This may protect the mucosa against mechanical injuries during engulfing and against chemical and bacterial attack during digestion. PAS positive mucins in the small intestinal mucosa may also be protectine to the mucosa and allowing wastes passase of into the large intestine. The entire mucin coverage of the large intestinal mucosa confirms that intestinal mucins

partly play an important role in the protection of the mucosa against the physical and chemical damage [19] and also against bacterial invasion [20]. Such mucin lining facilitates well the excretion of foeces including hard wastes such as exoskeletons of invertebrates, ants and termites.

In conclusion, the histological features and PAS positive results in the digestive tract of *D. zarudnyi* common in Qatari fauna proue different from other reptiles in having oesophageal stratified columnar epithelium, gastric oxynticopeptic cells, small intestinal mucosa of brush-order columnar epithelium with goblet cells and a classical reptilian large intestinal mucosa.

REFERENCES

- [1] Mohammed, M. B. H., 1988. Survey of the reptiles of Qatar. Proc. Zool. Soc. A. R. Egypt, 16: 17-26.
- [2] Eissa S. M. And Y. S. El-Assy, 1975. Record of certain reptilian species found in Kuwait. J. Univ. Kuwait (Sci.), 2, 123-146.
- [3] Webb J. E., J. A. Wallwork and J. H. Elgood, 1978. Guide to living reptiles. The Macmillan Press Ltd., Lon-don.
- [4] Ferri, S., L. C. V. Junqueira, L. F. Medeiros, and L. O. Medeiros, 1976. Gross, microscopic and ultrastructural study of the intestinal tube of *Xenodon merremii*, Wagler, 1824 (Ophidia). J. Anat., 121: 291-301.
- [5] Martin-Lacave, I., C. Montero, J. M. Lopez-Munoz, J. L. Lopez-Campos, and H. Galera, 1982. Ultrastructural study of the epithelial mucous cells in lizards (Lacertilia). Cell Tissue Res., 221: 679-686.
- [6] Ferri, S., D. S. Sottovia, and L. O. Medeiros, 1975. Polysaccharides, proteins, nucleic acids, enzymes and sulphated uptake of the gastric epithelial cells of *Xenodon merremii* Wagler 1824 (Ophidia). An histochemical and autoradiographic study. Acta Histochem., 54: 175-182.
- [7] Ferri, S., F. D. Sottovia, L. F. Medeiros and L. O. Medeiros, 1977 a. Histochemical study of polysaccharids, proteins, nucleics acids and enzyme and sulphate uptake in the oesophagus epithelial cells of *Xenodon merremii* Wagler 1824 (Ophidia). Cell Molec. Biol., 22: 235-241.

- [8] Ferri, S., L. O. Medeiros and A. C. M. Stipp, 1977 b. Histochemical (polysaccharides, nucleic acids and enzymes) and autoradiographic (incorporation of 35S labelled sodium sulfate) study of the epithelial intestinal cells of *Xenodon merremii* Wagler 1824 (Ophidia). Acta Histochem., 58: 290-298.
- [9] Madrid, J. F., Ballesta J., Castells M. T., Marin J. A. and L. M. Pastor, 1989. Characterisation of Glycoconjugates in the intestinal mucosa of vertebrates by means of Lectin histochemistry. Acta Histochem. Cytochem., 22: 1-14.
- [10] Perez-Tomas R., J. Ballesta, J. F. Madrid, L. M. Pastor and F. Hernandez F., 1990. Histochemical and Ultrastructural study of the Digestive Tract of the Tortoise *Testudo graeca* (Testudines). Jour. Morph., 204: 235-245.
- [11] Ballmer, G. W. 1949. The comparative histology of the enteron of some American turtles. Papers Mich. Acad. Sci, Arts left. 1949: 91-100.
- [12] Wright, R. D., H. W. Florey and A. G. Sanders 1957. Observations on the gastric mucosa of Reptila. Quart. J. Exp. Physiol., 42: 1-14.
- [13] Patt, D. L., and G. R. Patt 1969. Comparative Vertebrate histology. New York: Harper and Row.
- [14] Gabe, M., and H. Saint Girons, 1972. Contribution a lihistologie de liestomao de liepidosauriens (Reptiles). Zool. Jb. Anat., 89: 579-599.

- [15] Andrew, W., and C. P. Hickman, 1974. Histology of the vertebrates. A comparative text. Mosby St. Louis: pp. 243-296.
- [16] Ferri, S., W. Gremski, A. C. M. Stipp, and L. O. Mcdeiros, 1974. Ultrastructure of the gastric epithelial cells of *Xenodon merremii*, Wagler 1824 (Ophidia). Gegenbaurs Morph. Jahrb., 120: 905-916.
- [17] Giraud, A. S., N. D. Ycomans and D. J. B. St. John, 1979. Ultrastructure and cytochemistry of the gastric mucosa of a reptile, *Tiliqua scincoides*. Cell tissue Res., 197: 281-294.
- [18] Hildebrand Milton, 1988. Analysis of vertebrate structure. 3rd cd. John Wiley & Sons, Inc., New York, Chichester, Brisbane, Toronto, and Singapore pp. 231.
- [19] Allen, A., D. A. Hutton, A. J. Leonard, J. P. Pearson and L. A. Sellers, 1986. The role of mucus in the protection of the gastroduodenal mucosa. Scand. J. Gastroenterol., 21 (Suppl. 125): 71-77.
- [20] Chadee, K., W. A. Petri Jr., D. J. Innes, and J. I. Ravdin, 1987. Rat and human colonic mucins bind to and inhibit adherence lectin of *Entamoeba histolytica*. J. Clin. Invest., 80: 1245-1254.

Part II

Anatomy of the worm-like reptile Diplometopon zarudnyi (Squamata) and study of the Histology and PAS-reaction in its liver and Kidney

ANATOMY OF THE WORM-LIKE REPTILE DIPLOMETOPON ZARUDNYI (SQUMATA) AND STUDY OF THE HISTOLOGY AND PAS-REACTION IN ITS LIVER AND KIDNEY

ABSTRACT

Anatomy of D. zarudnyi, which is common in Oatar environment, was described with special reference to the arrangement and shape of the internal organs. The left lung was found larger in size with a bout four folds compared to the right lung. Gut was found generally straight or slightly folded. Transition from oesophagus, stomach, small and large intestine and cloaca was in a form of just swelling or narrowing. Liver and kidney of **D**. zarudnyi were studied through H&E techniques. Unlike mammals, no lobules were observed in liver, it was found to be composed of hepatocytes surrounding blood sinusoids. The walls among sinusoids are of two cells thick and the adjacent hepatocytes are held together by the tiny bile channels. Liver capsule, ductules and cell surfaces lining sinusoids exhibited a moderate polysaccharide content. The metanephric kidney was typically formed of renal capsules and nephric tubules which are divided into proximal, intermediate and distal tubules without distinct cortex and medulla like mammals. Proximal tubules were of brush-border cuboidal or low columnar cells while the intermediate segment was of thin ciliated cells and again cells lining the distal tubules were cuboidal with few or without microvilli. The parietal walls of renal capsules were of simple Squamous epithelium. Proximal tubules showed a light polysaccharide content while the visceral walls of the renal corpuscles exhibited more content. Anatomy of D. zarudnyi exhibits a classical mode of almost a straight reptilian gut. Structure of liver and kidney of D. zarudnyi confirms the metabolic and water conservation mechanisms of reptiles.

INTRODUCTION

Reptiles of Qatar peninsula were recorded (Mohammed, 1988) without an extra anatomical study. AL-Thani, and EL-Sherif 1996 studied some histological and histochemical characteristics on the digestive tract of the worm-like reptile *D.zarudnyi* which is known as "Nadus" as common name in Qatar .D. zarudnyi is a worm-like reptile classified at one time with 'the lizards but now generally given subordinal rank within the Squamata in parallel with lizards and snakes having some typical characters of both snakes and lizards (Webb et al.,1978). It is of limbless, long and c ylindrical trunk with blunt head, very small eyes and short pointed tail. It feeds on the small invertebrates, ants and termites, so, it lives underground sandy areas with spotted brown colour transversally above and whitish below with smooth scales. (Fig. 1).

It was classified according to Mohammed (1988) as:

Phylum: Chordata Subphylum: Vertbrata Class: Reptilia

Subclass : Lepidosauria Order : Squamata

Suborder : Sauria(Lacertilia) Inferaorder : Amphisbaenia Family : Amphisbaeniadae

Distribution in Qatar: El-Karaana area, south-west of Doha and Qatari western desert near Umm-Bab.

Liver has many diverse functions like storage of carbohydrates, release of nitrogenous wastes which is transported to kidneys for elimination, removing of the old red blood cells and toxicants from the blood and releasing bile which emulsifies fats and preparing it to digestion via the pancreatic enzymes (Hildebrand, 1988).

Reptilian liver exhibits some characteristic morphological features since it occupies the majority of the anterior half of the coelom and divided unequally into two halves by the gall bladder (Juanita and Thomas, 1980). Hildebrand, 1988 described the kidney of reptiles as being composed of proximal tubules have cuboidal or columnar cells with microvilli. The intermediate segment is thin and ciliated. Cells of the distal tubules are cuboidal with few or without microvilli. Glomerulus is a tuft of capillary loops and a nastomoses that hangs into renal capsule which is cup-shaped with an outer parietal wall of Squamous or cuboidal epithelia and a visceral wall. The proximal tubules returns sugars, amina acids, vitamins and various salts to the blood streams and may secrete certain foreign material into the filtrate. The distal tubules acidifies the filtrate and removes sodium and chloride ions. Both tubules acting for returning water to the blood. Zausti et al., 1987 discussed the ultrastructure of tubular nephron of Testudo gracea as a comparison between hibernating and non-hibernating animal. The meta-nephric kidney of reptiles was studied by Beuchat and Braun, 1988 and compared to that of mammals. Liver was described in reptiles by Hildebrand, 1988 and its role in the metabolic pathways was investigated.

In the present work the gross anatomy and both kidney and liver of *D. zarudnyi* will be investigated histolgically and their poly- saccharide content will also be checked.

MATERIALS & METHODS

Animals collected in November 1995, of about 35 cm length identified and dissected under chloroform anaesthesia. Abdominal wall was opened directly above the cloacol opening and across the ventral mid-line till the ventral surface of the head. Pieces (5mm wide) were cut from liver and kidney, samples were fixed by immersion in buffered 10% formalin with 1% CaCl2 overnight then washed and routinely processed for paraffin embedding and 5 μ m sections were prepared. These were investigated by H&E technique for general histological studies and by PAS for polysaccharide histochemistry.

RESULTS

Heart was located anterior to the lungs at the extreme anterior end of the gut. The lung lobes exhibited two different sizes since the right lung was found as about one quarter size of the left lung. The gastrointestinal tract was found to be almost equal to the whole body length (Fig.2).

Oesophagus is a straight muscular tube dorsal to the liver. Small intestine is nearly straight while the large intestine is straight. Transition from oesophagus to the stomach, at the gross level was nothing more than a gradual widening of the gut in the region of the gall bladder to a uniformly spindle-shaped swelling. The division between the foregut and the hindgut is marked by a muscular pyloric sphincter.

Anterior to the small intestine, duodenum arises near to the pancreas, as a diverticulum of the small intestine which is thin walled and almost straight leading to the large intestine and colon and then to the cloacal opening without district gross anatomical charact-eristics.

Liver covers most other organs of the foregut and ventrally attaches to the gut in the form of two right and left lobes and in between lies the gall bladder which divides the liver unequally. Kidneys are located beside each other at the extreme posterior end of the coclomic cavity.

Regarding the histology of liver, it was a simple mass of hepatic cell cords and contained relatively small amounts of connective tissues, the whole liver lined with a capsule of simple squamous cells (Fig.3), with no internal lobulation. In (Fig.4), the mass of liver cells was found to be fenestrated by blood sinusoids and capillaries. The cellular walls among sinusoids were of two cells thick, the adjacent hepatocytes are held

together through tiny bile ductules. PAS reaction exhibited almost uniformal distribution of polysaccharides in the cytoplasm of liver cell mass (Fig.5). However, liver capsule, sinusoidal walls (Fig.6) and also capillary and ductules surfaces (Fig.7) as well exhibited more PAS +ve reaction compared to that inside hepatocytes.

Kidney of *D. zarudnyi* (Fig 8) showed no distinct cortex and medulla. Renal tubules were presented in three different segments. The proximal tubuls were typically brush-border cuboidal or low columnar cells, the intermediate segments were of thin ciliated cells (Fig. 9) and cells of the distal tubules were almost cuboidal with few or lacking microvilli. The renal corpuscle was formed of an outer parietal wall of simple squamous epithelium and an inner visceral wall with folds of glomerular capillaries with relatively poor vascular system (Fig.9&10). Polysaccharides of renal tissues revealed PAS +ve reaction in the cells of proximal tubules slightly more than that of the intermediate and distal tubules while the renal capsule was also of high PAS reaction (Fig11). Renal capsules however, revealed the maximum amount of poly-saccharides among the whole renal tissues (Fig. 12).



Figure (1) The worm like reptile (*D. zarudnyi*) with spotted dark and light brown transverse striations.

Figure (2) General viscera of D. zarudnyi

HT: heart.

RL: right lung.

LL: left lung.

OE: oesophagus.

LV: liver.

GB: gall bladder.

ST: stomach.

PA: pancreas.

DU: duodenum.

S1: small intestine.

PS: pyloric sphinter.

L1: large intestine.

KD: kidney,

CL: colon.



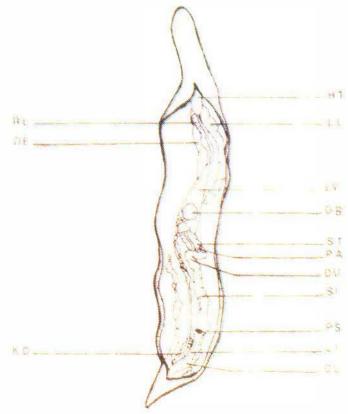
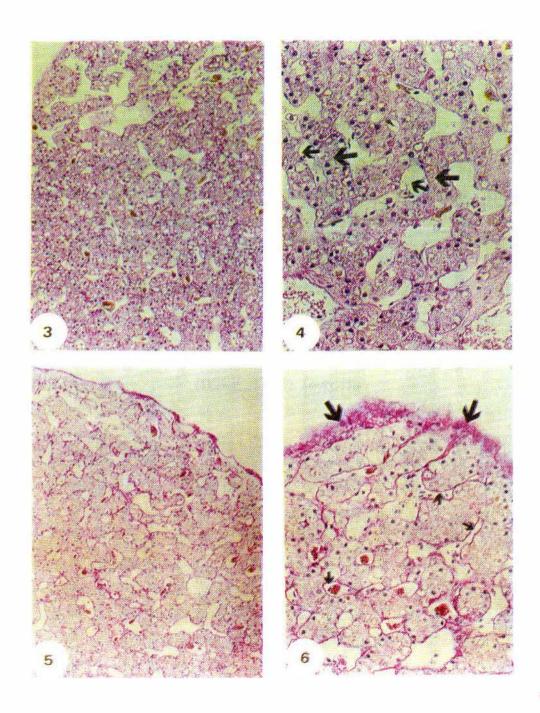


Figure (3) The mass of liver with cell cords surrounding the hepatic sinusoids with small amounts of connective tissues and lacking lobulation (H & E- X100).

Figure (4) Hepatic cell corde of two cells thick (large arrows) around the bile ductules (small arrows). (H&E, X200).

Figure (5) The normal distribution of polysaccharides in the hepatocytes . (PAS,X100).

Figure (6) Hepatic capsule (large arrows) and sinusoidal walls (small arrows) with higher (PAS , X200).



- Figure (7) Capillary (small arrows) and bile ductules (large arrows) walls also exhibited higher PAS reaction. (PAS, X400).
- Figure (8) Kidney of *D.zarudnyi* lacking distinction between cortex and medulla with three types of renal tubules (proximal intermediate and distal. Large, small arrows and arrowheads respectively). (H&E, X100).
- Figure (9) Proximal tubules (large arrows) of brush-border cuboidal to low columnar cells. Intermediate segment with thin cells (asterices). Distal tubules compsed of cuboidal without or with few microvilli (small arrows). (H&E.X200).
- Figure (10) The parietal wall (of simple squamaus epithelium, large arrows) and the visceral wall (of folded capillaries, small arrows) of the renal corpuscle. (H&E .X 400).

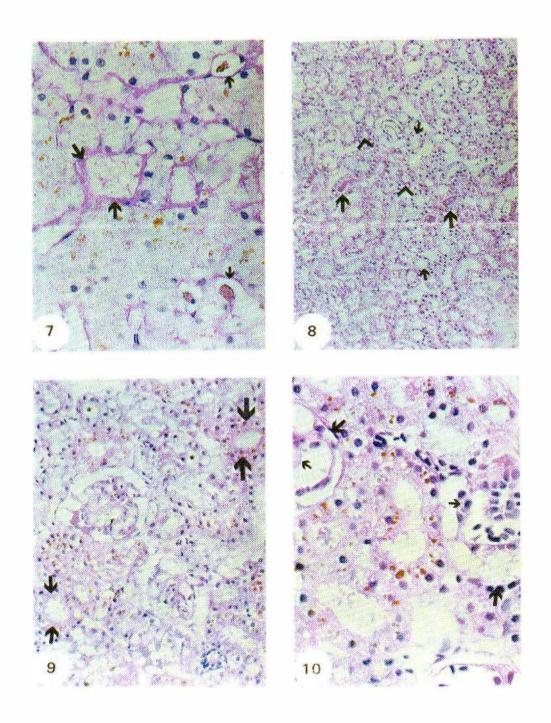
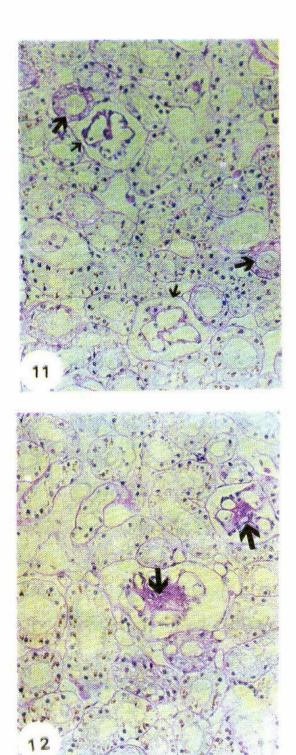


Figure (11) Both proximal tubules (large arrows) and renal capsules (small arrows) exhibited more PAS +ve reaction. (PAS, X100).

Figure (12) The visceral wall of the gromerulus (arrows) revealed the maximum amount of polysaccharides. (PAS. X200).



DISCUSSION

Reptilian tissues are not common enough in the majority of the histological investigations since histologists used to design their current studies using mammalian tissues as models. Other studies applied on tissues except that of mammals e.g. reptiles and birds are limited and used for some specific aims

Histology of reptiles, as in all lower animals, is highly related to the relatively primitive anatomical and functional characteristics of these groups of animals. External features of *D.zarudnyi*, however, confirm well its middle systematic position between the higher lizards and primitive snakes inside order Squamata. This species exhibits many typical characters of both lizards and snakes as described by Webb et al. (1978).

•n the other hand, reptiles belong to the inferaorder amphisbaenia, including *D. zarudnyi*, have many special characteristics like the massive skull, the enlarged medial premaxillary tooth and a uniquely specialized middle ear as discussed by Juanita and Thomas (1980). Regarding the internal gross anatomy, general viscera exhibited a model confirms the general morphological features of *D. zarudnyi* as elongated and cylindrical amphisbaenian reptile.

The anatomical location of the heart of D. zarudnyi as the start point of the blood vessels specially those of lungs and the very short trachea reflects a pure amphisbaeian anatomical feature which agrees with Smalian (1884).

The almost straight pattern of the digestive tract of D. zarudnvi was found extremely similar in almost all other amphisbaenians studied by Junita and Thomas (1980) which is considered anatomically close to the snake-like pattern rather than of true lizards. This may suits, however, the simple food stuffs since it feeds on the sandy minute invertebrates. ants and termites which is in on need for the complicated long time chemical and mechanical digestion performed and aided by the highly folded gastrointestinal tract. The rudimentary right lung and the large sized left one and also the extreme location of the kidneys at the posterior end of the coelom beside each other in D. zarudnyi confirms pure unique characters of amphisbaenions rather than lizards and snakes as Butler stated, (1889b and 1895). On the other hand, the anatomical relation between duodenum and pancreas of D. zarudnvi is a pure snake-like character while the location of the gall bladder in a notch between the liver lobes is a characteristic feature for lizards in an agreement with Miller and Lagios, 1970 (for the pancreas) and de Carlo, 1957 (for the gall bladder).

The histological features of liver of D. zarudnyi reflect a considerable variations than that of the higher vertebrates e.g. mammals and birds. Liver of this amphisbaenian worm-like reptile lacks any lobule arrangement as in the lower vertebrats in an agreement with Tanuma, 1980 and Barni etal, 1985. It is well known that walls between sinusoids are one cell thick in mammals and same birds but the sinusoidal walls in the liver of D. zarudnyi were found two cells thick which a grees with Hidbrand, 1988. The thick walled sinusoids with the tiny bile ductules helding the hepatocytes together may provide this lower vertebrate an opportunity to integrate the function of the whole mass liver as a unit since it lacks the systematic distribution of the central veins and the portal spaces among lobules containing branches of the hepatic arteries, portal veins and bile ductules which is the common mode of the mammalian liver. However, the increase of the PAS +ve material in the hepatic capsule, sinusoidal walls, surfaces of the capillaries and ductules may be attributed to relatively long periods between available feeding processes which causes a loss of the hepatocyte glycogen to provide the animal energy till the next feeding chance.

Regarding the structure of kidney, the microvillous cuboidal or low columnar cells of the proximal tubules confirm its main function which is the return of sugars, amino acids, vitamins and various salts to the blood stream and secreting the foreign materials into the filtrate. On the other

hand the distal tubule cells with few or without microvilli acidify the filtrate and remove sodium and chloride ions (Hildebran . 1988) . This may support well the significant role of the reptilian kidney in the regulation of water and ion balance through a water conservation mechanism as confirmed by Beuchat and Braun .1988. Reptiles have only a small high-pressure flow to the glomeruli so, the filtration will be limited, this may attributed to the histological structure of the poorly vascularized renal corpuscle and the glomerular capillaries to minimize the water loss in contrast to the mammalian kidney. However, these structural and functional characteristics of the kidney of D. zarudnyi is fit well to the excretion of the insoluble and semisolid uric acid instead of soluble urea in mammals. The higher polysaccharides content in the corpuscles and proximal tubules confirms and decreases water filtration which increases water conservation. Distal tubules contain lower amounts of polysaccharides since they regulate removal of sodium and chloride ions. We may attribute as well, the thin-walled intermediate segment to also the water conservation mechanism through a water reabsorption role.

In conclusion, the general anatomical features of the internal viscera of *D. zarudnyi* confirm the reptilian mode in between lizards and snakes. However, the histological structure and poly-saccharide contents of both liver and kidney are integrated to minimize the water excretion which is functionally critical in reptiles.

REFERENCES

- 1- Al-Thani A. S. and EL-Sherif G., (1996): Histological and histochemical study of the digestive tract of the worm-like reptile D. zarudnyi (Squmata), Qatar Univ. sci. J., 16 (1): 113-117.
- 2- Barni S., Bernocchi G., and Gerceli G., (1985): Morphomitrical changes in hepatocytes during the life cycle of European eel. Tissue cell. 17: 97-109.
- 3- Beuchat C. A., and Braun E. J., (1988): Allometry of the kidney: implications for the ontogeny of osmoregulation. Am. J. Phsiol., 255 (5pt 2): 760-767.
- 4-Butler, G. W., (1889b): On the relations of the fat bodies of the sauropsida. Proc. Zool. Soc. (Lond) 1889, 602-613.
- 5- Butler, G. W., (1895): On the complete or partial suppression of the right lung in the Amphisbaenidae and of the left lung in the snakes and snake like lizards. Rivadevia and amphisbains, Pro. Zool. Soc. (Lond.) 1895, 691-712.
- 6- de Carlo, J. M., (1957): Consideraciones anatomo-histological sober el higado y vias biliares de "*Amphisbaena derwini*" D. Y. B. Rev. Mus. Arogentino Cien. Nat. Bernar-dino. Rivadavia, Cien. Zool. <u>3</u> (3): 155-186.
- 7- Hildbrand M., (1988): Analysis of vertebrate structure 3rd ed.. John Wiley Sons, Inc., New York, Chichester, Brisbane, Toronto and Singapore. PP. 231.
- 8- Juanita and Thomas, (1988). Visceral anatomy of the Amphi-sbaenia.
- J. Morphol., <u>136</u>: 99-133.

- 9- Miller, M. R. and Lagios, M. D., (1970): The pancreas. In: Biology of the Reptilia. C. Gans and T. S. Parsons, eds. Academic Press, London, 3: 319-346.
- 10- Mohammed M. B. H. (1988). Survey of the reptiles of Qatar. Proc. Zool. Soc. A. R. Egypt. 16: 17-26.
- 11- Smalian, C. (1884): Beitrage zur Anatomie der Amphisboaeniden. Zeitschr. Wiss. Zool. 42: 126-202.
- 12- Tanuma Y., (1980): Eletron microscope observation on the intrahepatocytic bile canalicules and sequent bile ductules in the crucian. *Carassius carassius*. Arch. Histol.Jap., <u>43</u>: 1-21.
- 13- Webb J. H. Wallwork J. A. and Elgood J. H., (1978). Guide to living reptiles. The Macmillan Press Ltd., London.
- 14- Zausti, A., Ferrer, C., Ballesta, J. and Pastor L.M.(1986): Ultrastracture of the tubular rephron of Testudo graeca (Chelonia). A comparison between hibernating and non-hibernating animals. Histol. Histopathol. <u>2</u>(4) 391-400.

Part III

Histological studies on the Gastro-intestinal tract of the Snake, Cerastes vipera, a new local record in Qatar

Histological Studies On The Gastrointestinal Tract Of The Snake, Cerestes vipera – A New Local Record In Qatar

ABSTRACT

The histological structure of the gastrointestinal tract of the non-horned snake, *Cerestes vipera* and the pattern of the PAS-positive materials were described. Both the histological findings and also the mucin content in the GIT of *C. vipera* was found to be related to the nature of the food stuffs and the feeding mechanism of the snake. The digestive action of a high enzyme content of the snake's saliva reflects the differences between snakes and other animals. Mucins play a critical role in digestion and excretion in *Cerestes vipera*.

INTRODUCTION

Qatar peninsula is almost pale desert in the northwest Arabian Gulf and approaching the sea level in most directions. Reptiles in Qatar were recorded by Mohammed, (1988). Among the species recorded by the auther was the snake *Cerestes cerestes*. Eissa and El-Assy (1975) studies the distribution of reptilian species in an other state, Kuwait, in the north Arabian Gulf. They recorded the horned snake, *Cerestes cerestes* and the non-horned snake *Cerestes vipera* as well.

Earlier, other authors described the distribution of reptiles in the neighbouring regions of Q atar, n amely A rabia, (Anderson, 1889; H ass, 1957; Hass, 1961; Corkill & Cochrane, 1966 and Hass & Weerner, 1969), in Eastern Arabia (Mandaville, 1965), in Northeastern Arabia (Mandaville, 1967), in Abu-Dhabi (Leviton & Anderson 1967) and later in Bahrain (Summer, 1954 and Gallagher, 1971).

Reptiles have been attracted the attention of many investigators mostly as survey studies. On the other hand, no parallel studies on the histological structure of reptile organs have been established. Histology of the gastrointestinal tract (GIT) of some ophidian species, rather than Cerestes vipera attracted attention of some investigators (Reis & Lyons, 1943: Allen & Lhotka, 1961 and Ferri, et.al., 1974). It is well known that GIT plays a vital role in the feeding mechanism in snakes due to the characteristic anatomical and histological features of these predators.

This may help in more explanation for digestion and absorption in the GIT of the snakes. Feeding habitat of reptiles is a very critical factor in any ecosystem especially in the desert ecology, which is common in Qatar.

CLASSIFICATION OF Cerestes vipera, (Mohammed, 1988):

Class : Reptilia

Subclass : Lepidosauria Order : Squamata

Suborder : Ophidia (serpents).

Infraorder : Caenophidia. Family : Viperidae. Viperinae.

Cerestes vipera, (non-homed cerestes).

In this work the first record for *Cerestes vipera* in Qatar was established and the histological structure of its GIT was described in relation to the animal locality, taxonomy and habitat.

MATERIALS AND METHODES

Adult specimens of *Cerestes vipera* were collected from different localities at the middle area of Qatar peninsula. Animals were identified and recorded according to Eissa & El-Assy (1975) as a well-known representative of genus *Cerestes*. Snakes of both sexes were chloroformed and then quickly dissected at different parts of the gastrointestinal tract (oesophagus, stomach, small intestine, large intestine and cloaca). These tubular organs were internally injected by cold saline solution to clear the organs from all traces of previous meals. This was followed by injection of cold buffered 10% formol-calcium for quick fixation of the lining mucosa. Specimens were then extra fixed by immersion through the same buffered fixative. Paraffin sections of 5-6

µm thick were subjected to the Haematoxylin and Eosin technique for histological investigations. Other paraffin sections were treated through the PAS reaction (Mc Manus & Mowry, 1960) for the mucosubstances. Sections then were examined and photographed to check the general histological structure and PΛS-positive materials.

RESULTS

Oesophagus:

The internal mucosal villi of oesophagus (Fig. 1) are lined by ciliated columnar epithelium with a lot of goblet cells. Oesophageal mucosalining cells (Fig. 2) are based on well-developed lamina propria of supporting tissues. In Fig. 3 submucosa is rich with blood vessels and there is a mass of elastic cartilage extends directly underneath the lining mucosa to the border of the circular muscle layer. Muscularis layer is typically formed of an inner thick circular smooth muscles and an outer longitudinal muscle fibers with an extra outer highly vascularised scrosa (Fig.4).

Regarding the PAS reaction, goblet cells exhibit an intensive heavy PAS-positive mucos-substances (Fig. 5) extend superficially and covering the cilia. Goblet cells are stained with PAS (Fig. 6) with clear different intense reactions.

Stomach:

Gastric mucosa (Fig. 7) is almost flat and composed of columnar mucous cells while submucosa is completely engaged by the gastric gland. There is a very thin internal circular smooth muscle layer compared with the thick outer longitudinal muscle layer which is lined externally by serosa of simple squamous epithelium.

The gastric mucous-secreting cells are characterized by basal nuclei while the gastric gland cells contain centrally located nuclei (Fig. 8). Columnar mucous-secreting cells and mucous glands of the stomach exhibit an over-loaded amount of the PAS-positive materials (Fig. 9) which occupy almost the cytoplasm of the mucous cells (Fig. 10).

Small intestine:

Composed of relatively thin wall (Fig. 11), mucosa of which consists of high columnar mucous cells with distinct extreme basal nuclei and a thin submucosa. There is a narrow band of circular smooth muscles which represents the muscularis mucosa of the small intestine. The muscularis layer is formed of another thicker layer of circular muscles only. The small intestine is covered externally by a thin serosa composed of simple squamous epithelium.

The small intestinal mucosal cells exhibited a regular pattern of the PAS-positive materials (Fig. 12) which is lining also the whole inner surface of the mucosa.

Large intestine:

It is still thin walled (Fig. 13) with a columnar cells and some goblet cells. Muscularis is remarkably reduced to patches of circular and/or oblique smooth muscles. Submucosa is highly vascularised with many blood vessels.

An ideal form of goblet cells filled with PAS-positive reaction product is exhibited but the reaction is extremely reduced in the cytoplasm of the lining columnar cells (Fig. 14).

Cloaca:

Cloacal mucosa is characterized by more goblet cells among the columnar lining cells (Fig. 15). Submucosa is reduced and the smooth circular muscles of the muscularis layer is still thin.

Goblet cells of the cloacal lining mucosa exhibited PAS-negative reaction (Fig. 16) while the positive reaction is clearly observable on the outer most surfaces of the mucsal lining.

×

FIG.(1): Oesophagus with ciliated columnar mucosa and goblet cells.

11&E, x400.

FIG.(2): The well-developed lamina propria of the oesophageal lining. H&E, x1000.

FIG.(3): Submucosa of the oesophagus showing a lot of blood vessels and cartilage. H&E, X100.

FIG.(4): Inner thick circular and outer longitudinal muscles of oesophagus. H&E, x100.

FIG.(5): PAS+ve materials lining the ciliated lining of the oesophagus.

PAS, x400.

FIG.(6) Clear different intense goblet cells in the •esophageal mucosa. PAS, x1000.

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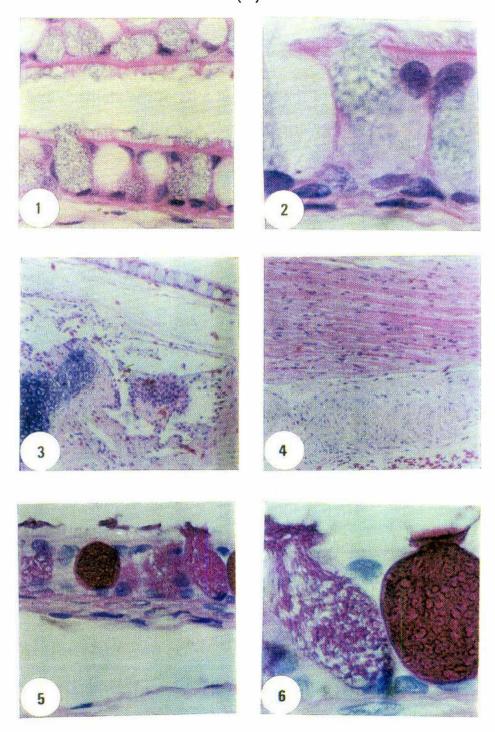


FIG.(7) : Gastric columnar mucous cells. H&E, x200.

FIG.(8): Basal nuclei of the mucous cells of the stomach and central nuclei of the gastric glands. PAS, x400.

FIG.(9) : High PAS+ve reaction in the both gastric mucosa and gastric glands. PAS, x200.

FIG.(10): C olumnar cells with PAS+ve materials in the cytoplasm. PAS, x400.

FIG.(11): Small intestinal columnar cells mucosal with extreme basal nuclei. H&E, x400.

FIG.(12): Regular pattern of the PAS+ve materials in the small intestine. PAS, x400.

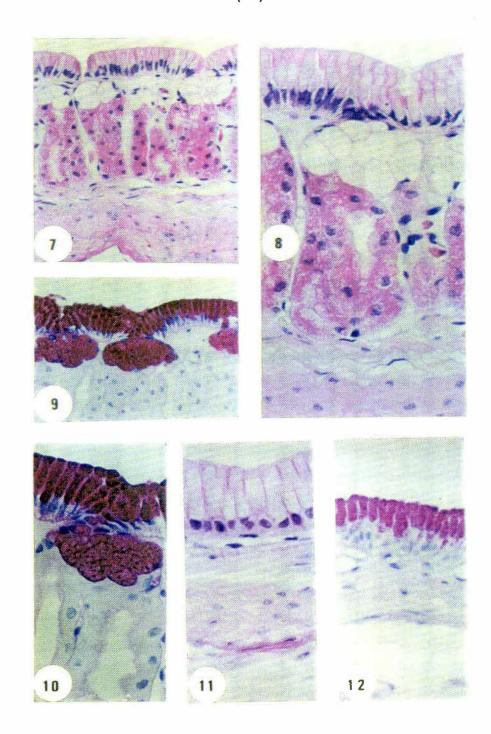
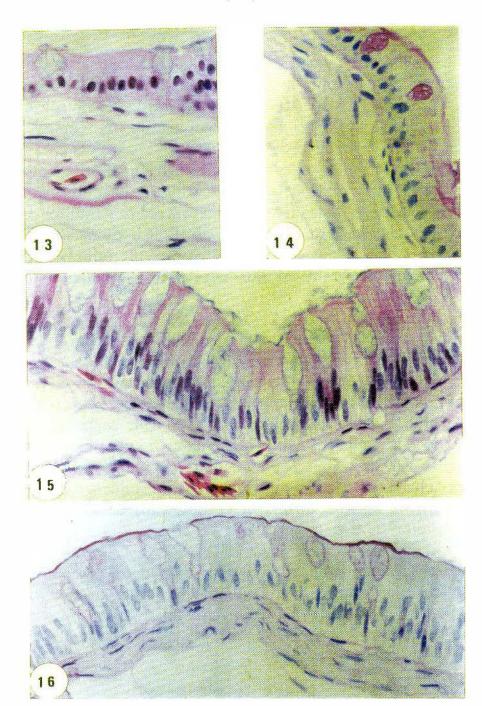


FIG.(13): Goblet cells distributed in the large intestinal mucosa. H&E, x400.

FIG.(14) : Goblet cells rich with PAS+ve materials in the large intestine. PAS, x400.

FIG.(15) : Cloacal mucosa with more goblet cells. H&E, x400.

FIG.(16): PAS-ve reaction in the goblet cells of the cloaca. PAS, x400.



DISCUSSION

Vipers usually feed on small vertebrates like rats, mice, lizards and less often on frogs and birds swallowed as whole. Since viper snakes cannot tear their prey apart, they have highly effective chemical digestion mechanism depending on the high proteolytic enzyme content in their venom secreted by the modified salivary glands. Snakes are also provided by an effective mechanical digestion mechanism which begins outside the body. Biting the prey and injecting saliva inside not only paralyzes or leads the prey to die, but also it may be considered as a trial for preparing the prey to be digested. Strong contractions of the body muscles integrate the process and help in pushing the prey toward the following digestive organs which are generally of less developed musculature (Grizmek 1979).

Both highly advanced chemical and mechanical aspects of the digestion mechanism of snakes are not enough to overcome the absence of the tearing, crushing or chewing organs. So, the wholly swallowed preys with their extra rough bodied and appendages should be lubricated well to facilitate the glinding of the prey into the GIT. This begins in the mouth cavity by the highly mucous saliva of the unmodified salivary glands and aided partly by the body muscle contractions (Coborn, 1994). Building on these snake characteristics, we can make a trial to confirm the histological structure and the distribution of PAS-positive materials in the GIT of Cerestes vipera.

The oesophageal longitudinal folds and submucosal smooth muscles may help in a maximum degree of distension during swallowing of the prey as stated by Coborn (1994). We also attribute the presence of the

longitudinal supporting elastic cartilages, the inner circular and the outer longitudinal smooth muscles most probably to support the distended wall of the oesophagus against tearing. Swallowing of the prey is effectively aided by the contractions of the neck and the body (Coborn, 1994). The high vascularity of the submucosa and serosa provides an adequate amount of blood carrying oxygen and nutrients for energy production to help in pushing the prey to the stomach. The ciliated lining columnar cells may help partly in completion of this process, the distinct lamina propria provides the energy requirements for these ciliated cells and also for the goblet cells to secrete muco-substances which are also very important to lubricate the outer surface of the prey's body.

The high PAS-positive neutral mucopolysaccharides in the goblet cells in the wall of the oesophagus agree with Suganuma et.al., (1981). It may protect the internal oesophageal wall against the effect of the protedolytic enzymes secreted with saliva. Other goblet cells stained lesser may be due to acid mucosubstances which assure the rapid swallowing of the prey as lubricants during feeding (Gabe, 1971).

The stomach is histologically fitted to be main complementary digestive organ. The gastric wall which is provided by poor circular muscles and relatively developed longitudinal pattern may help in pushing the prey downward since the prey was already churned by the oesophagus and body muscles (Coborn, 1994). The lining cells and the basal gastric gland may secrete digestive enzymes and HCl to digest bones of the prey and the soft parts in an agreement with Coborn. (1994).

The neutral mucins secreted by both the PAS-positive lining columnar cells and the gastric mucous glands mainly protect the gastric wall against the higher concentration of the proteolytic enzymes and HCl in the gastric juice. The PAS-negative basal gastric glands may secret enzymes and acid mucines as lubricant.

However, the small intestine is provided only by thin circular smooth muscle, this may be attributed to the squeezing contraction mechanism of the small intestinal wall to facilitate the beginning of absorption which confirm the basic snake pattern and not only in viperidae (Reis and Lyons, 1943). The columnar cells lining the small intestinal mucosa are also attributed to the same review and they are mainly absorptive cells.

The high content of the PAS-positive material is partly attributed to protect the internal lumen of the small intestine from the lytic effect of the gastric juice containing HCl. On the other hand, it may also insure the role of such organin absorption as stated by Bishai, (1960) and AL-Nasser, (1976).

Meanwhile, large intestine muscle layer is thin as the squeezing movement becomes less. But, the goblet cells arise among the columnar brush-border cells which is confirmed with Bishai, (1960) as the main water absorptive organ of reptiles and to our opinion especially in snakes.

Lacking of PAS-positive material in the large intestinal mucosa may be explained as the digestive juice is altered to be diluted and so the thin layer of mucins lining the brush-borders and secreted by the scattered goblet cells provides enough protection in this stage. This may also attributed to Bishai, (1960) since these mucosubstances initiate the water absorption in the large intestine.

In the cloaca, at which urinary and genital system also ends, the main function of the cloaca is to compress the fecal matter into solid pellets and store it until defecation (Coborn, 1994). This may agree with the present findings since the circular smooth muscles are close to the mucosa and there is no submucosa to increase the ability of compression of fecal pellets.

The thin PAS- positive material extends to the cloaca lining from the large intestine is to protect and insure water reabsorption. But is more clear that goblet cells in the cloacal mucosa are more prominent and interesting that they—are PAS-negative, so this may attributed to Thiruvathukal and Kuriakosa. (1965) as these mucins act as good lubricant to assure the solid pellets of faeces.

In conclusion, both the histological structure and the distribution of the PAS-positive materials appear functionally fitting and the sufficient for the viper snakes pattern of feeding with special reference to *Cerestes vipera*.

REFERENCES

- Allen, R.F. & Lhotka, J. F. JR. (1961): Studies on *Crotalus atrox*. Histology of fundic glands of western diamond black rattlesnake, Anat. Rac. 2:202.
- AL-Nasser. N.A. (1976): Anatomical studies, osteology and gut Histology of the Amphisbaenian *Diplometopon zarudnyi* inhibiting Kuwait, M. Sc. Thesis. Kuwait University.
- Anderson, J. (1889): Zoology of Egypt. Vol. (1), London. Bernard Oatrich.
- Bishai, H. (1960): The anatomy and Histology of alimentary tract of *Chamaeleon Vulgaris*, Bull. Fac. Sci. Cairo Univ. 15(29): 44-61.
- Coborn, J. (1994): The mini-atlas of snakes of the world. T. F. H. Puplications, Inc. NJ, USA.
- Corkill, N.L. & Cochrane, J. A. (1966): The snakes of the Arabian Peninsula and Socotra. J. Bombay Nat. Hist. Soc. 62(3): 475-506.
- Eissa, S. M. & EL-Assy, Y. S. (1975): Records of certain reptilian species found in Kuwait, J. Univ. Kuwait (Sci.). 2: 124-146.
- Ferri, S.: Medeiros, L. •. & Stipp, A. C. (1974): Gastric wall histological analysis and cellular type distribution in *Xendon merremii*, Wagler, 1824 (Ophidia), Morphol. J. 120: 627-637.
- Gabe, M. (1971): PolySaccharides in lower vertebrates, in "Handb Uch Der Histochemie". 2/3 gustav Fisher, Stuttgart, West Germany.

Gallagher, M. D. (1971): Amphibians and reptiles of Bahrain. Published by the author. 37 pp.

Grizmek, B. (1979): Animal life encyclopedia, Vol. (6), reptiles. Van Nostraud Reinhold Company, NY, USA.

Hass, G, (1957): Some amphibians and reptiles from Arabia. Proc. Calif. Acad. Sci. Ser. 29: 47-89.

Hass, G, (1961): On a collection of Arabian reptiles. Ann. Carnegie Museum. 36:19-28.

Hass, G, & Weerner, Y. L. (1969): Lizards and snakes from southwestern Asia.

Collected by Henry Field. Bull. Mus. Comp Zool. 138: 327-406.

Leviton, A. E. & Anderson, S. C. (1967): Servey of the reptiles of the Sheikhdom of Abu-Dhabi. Arabian Peninsula. Part II. Systematic account of the collection of reptiles made in Sheikhdom of Abu-Dhabi by Jon Gasperetti. Proc. Calif. Acad. Sci. Ser. 35: 157-194.

Mandaville, J. P. Jr. (1965): Plants Eaten By *Cromastyx microlepis* Blanford and other notes on this lizard in Eastern Arabia. J. Bombay Nat. Hist. Soc. 62(1): 161163.

Mandaville, J. P. Jr. (1967): The hooded Malpolon. *M moilensis* Reuss and notes on other snakes of northeastern Arabia J. Bombay Nat. Hist. Soc. 64 (1): 115-117.

Mc manus, J. F. A. & Mowry, R. W. (1960): Staining methods, histolgic and histochemical (Hoeber, New York 1960).

Mohammed, M. B. H. (1988): Survey of the reptiles of Qatar. Proc. Zool. Soc. A. R. Egypt. 15 July (1988) 16: (17-26).

Reis, R. & Lyons, W. (1943): Histology of the small and large intestinal of the common green snake *Thamphois sirtalis*. I, Duodenum, Wassman coll. 5: 82-90.

Suganuma, T.; Tsukahara, m.; Tatemastsu, M.; Sakakura, y. & Murata, F. (1981): Comparative histochemical study of the alimentary tracts with special reference to the mucous neck cells of the stomach. Amer. J. Anat. 161: 219-238.

Summer, M. (1954): A comedy of lizards. Bahrain Islander. Bapco. Bahrain.

Thiruvathukal, K. V. & Kuriakusa, M. V. (1965): The histology of digestive tract of the fresh water *Chrysemys picta*. J. A nimal Morph. Physiol. 12: 220-230.

Part IV

Histological and PAS-reaction studies in the liver and kidney of the snake Cerastes vipera (Ophidia, Viperidae)

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HISTOLOGICAL AND PAS- REACTION STUDIES IN THE LIVER AND KIDNEY OF THE SNAKE CERESTES VIPERA (OPHIDIA, VIPERIDAE)

ABSTRACT

The histological structure of both liver and kidney in the snake *Cerestes vipera* has been studied in this work. Liver appeared as a mass of hepatocytes without lobulation. Liver cells appeared polygonal in shape and arranged in either two cells thick or irregularly distributed around sinusoids and capillaries. PAS+ ve materials were reported on the boundary liver cells and moderately around the hepatic veins. Kidney showed neither cortex nor medulla, the glomerulus and the renal corpuscle were small and structurally poor. The proximal and distal tubules are ideally reptilian but the intermediate tubules were found to be modified. The relation between the structure of liver and kidney and their role in the metabolic activities of *C. vi pera* was also discussed.

INTRODUCTION

Although the basic and the functional mechanisms of both liver, in digestion, and kidney in excretion are now well understood in higher vertebrates with special interest to mammals, these investigations are often rare in lower vertebrates specially in reptiles. Snakes represent a group of reptiles at which its bioactivities are almost still not so clear except on the level of venom-related studies.

The non-horned snake, *Cerestes vipera* was firstly recorded in Qatar by Al-Thani (1998) and the functional histology and the distribution of the PAS-positive materials in the digestive tract of the snake were discussed. The relation between the feeding mechanisms in one side and both the histological's tructure and PAS-positive materials on the other side was established.

Liver plays a central role in digestion, partly as secretory mechanisms for fat digestion and also as a store for carbohydrates. Liver also converts proteins into fats or carbohydrates. It releases nitrogenous wastes which are transported into kidneys for elimination. Liver is also involved in removal of RBCs and elaborating of the yolk necessary for the growing eggs (Hildebrand, 1988).

However, kidney was reported as being the main pathway for removal of the nitrogenous wastes of protein digestion and also for exerction of other harmful materials resulted after the metabolic activities. It was also stated that it eliminates a controlled amount of water and salts (Hildebrand, 1988) for maintaining an adequate water balance for all the body functions.

Snakes have a characteristic mode of feeding (Coborn, 1994) since they are predators of preys often large in size and usually ingested as whole with its skin and rough appendages. The digestive tract of snakes is also structurally unique and hence, the co-role of liver as a digestive gland in digestion is different than that in higher vertebrates. On the other hand, removal of metablolic wastes is too critical since kidneys should also minimize the excreted amounts of water due to the habitual and the environmental requirements of snakes.

In the present work, the relation between the histological structure and the PAS-positive materials of both liver and kidney and their specific metabolic role in *C. vipera* snake was investigated.

MATERIALS AND METHODS

Snakes were collected from the middle area of Qatar peninsula. Specimens were identified and recorded by Al-Thani (1998) after Eissa and El-Assy (1975) and Mohammed (1988) as a well-known representative of genus *Cerestes*. Adult snakes of both sexes were chloroformed and then quickly dissected. Liver and kidneys were collected and washed for few minutes in cold saline solution and then immersed in cold buffered 10% formol-calcium fixative. Specimens were roughly cut into 2-3 mm blocks and then were extra fixed in the same fixative, washed and prepared for paraffin embedding. Paraffin sections of 5-6 µm thick were treated through the haematoxylin and eosin technique for histological investigations while other sections were subjected to PAS- reaction for the mucosubstances (Mc Manus and Mowry, 1960).

RESULTS

Liver:

The general histological characteristics of the liver of *C. vipera* exhibited a mass of hepatocytes without clear defined lobulation (Fig. 1), like higher vertebrates e.g., mammals. Liver cells were arranged as walls of either two-cell thick around the hepatic sinusoids or as irregular mass of hepatocytes around the blood capillaries. Hepatocytes showed a polygonal shape (Fig. 2), and relatively large nuclei with one or two nucleoli and few elements of connective tissue. The whole liver mass appeared fenestrated by blood sinusoids and bile canaliculi. Cytoplasm of hepatocytes (Fig. 3), appeared vacuolated with clear cellular borders among cells of the liver parenchyma.

Liver cells just underneath the liver capsule exhibited a clear high PAS+ ve reaction around the cytoplasmic vacuoles (Fig. 4). On the other hand, the internal parenchymal cells showed drastic decrease in the PAS+ ve materials in a uniformal distribution pattern (Fig.5). However, cells around the hepatic vein exhibited moderate amounts of the PAS+ ve materials (Fig. 6).

Kidney:

Unlike mammals, the histological appearance of the kidney in *C. vipera* showed no distinct cortex and medulla (Fig. 7). The renal capsule is formed of simple squamous epithelium and the renal tubules exhibited different staining affinities and shape patterns. The proximal tubules

appeared as relatively thick walls composed of cuboidal to low columnar cells with brush-borders (Fig. 8) and clear eosinophilic cytoplasm. The intermediate tubles exhibited different affinity to cosin. They are covered by a distinct outer simple squamous epithelium with extreme basal nuclei and inner ciliated lining. Renal capillaries are distributed among the loose renal tubules. The distal tubules composed of cuboidal cells (Fig.9) with few or without cilia. Glomeruli appeared with an outer simple squamous parietal wall and relatively poor folded vascular capillaries.

The ciliated internal surfaces of the intermediate tubules appeared rich with PAS+ ve materials (Fig. 10), as compared to the brush-border of the proximal tubules. In the distal tubules (Fig. 11), both the few ciliated internal surfaces and also the cytoplasm of some cuboidal cells exhibited high PAS+ ve reaction. Both the parietal and the visceral walls of the golmeruli (Fig. 12) showed a marked PAS- ve reaction.

DISCUSSION

The general structure of the liver in *C. vipera* is similar to that found in many reptiles specially in lacking lobulation (Tanuma, 1980; Henninger, 1982; Barni et al., 1985). The arrangement of the hepatocytes as two cells thick or the irregular distribution around the sinusoids and capillaries was also reported in other reptiles (Henninger, 1982; Ferrer et al., 1987; El-Sherif and Al-Thani, 1997). The absence of the portal triads and the decreased amounts of connective tissue may provide reptiles with an intermediate postion between mammals and birds, which are more advanced, and fishes and amphibia, which are less, in this respect (Tsuneki and Ichihara, 1981). This may lead to the belief that the liver in *C. vipera* can regulate its function not so finely like higher vertebrates but still better than the lower vertebrates.

The high PAS+ ve reaction on the superficial hepatocytes rather than the internal parenchymal cells was also recorded in other worm-like reptile (El-Sherif and Al-Thani, 1997). This may be explained as the main function of the reptilian liver is the storage of carbohydrates (Hildebrand, 1988), and due to the long periods between meals in snakes, carbohydrates may be depleted firstly from the central parenchymal cells through the hepatic capillaries and remain in the liver boundary cells. However, the moderate amounts of PAS+ ve materials in the hepatocytes around the hepatic veins may be resulting from the ability of reptilian liver to convert proteins into carbohydrates or fats (Hildebrand, 1988) which are circulated via the blood. This may provide the snake with carbohydrates for energy until the next feeding process.

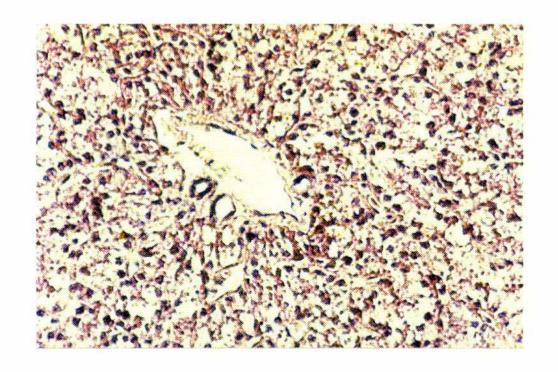
The histological structure of the kidney of C. vipera confirms the general pattern of the reptilian kidney with no clear cortical or medullary areas (Hildebrand, 1988). Kidney is simply covered by fine capsule of epithelial monolayer since it is deeply inserted and protected in the postabdominal cavity. The presence of cuboidal or the low columnar cells of the proximal tubules was Confirmed by (Hildebrand, 1988) and also by (El-Sherif and Al-Thani, 1997) as well since the microvillous brushborders return carbohydrates, amino acids, vitamins and salts from the renal filtrate to the blood stream in an agreement with (Guo et al., 1996). The intermediate tubules with their cells of specific affinity to eosin and their large basal nuclei may reflect some secretory mechanisms which, together with the membrane covering these tubules, are still in need for more investigations to clear up their metabolic functions. The cuboidal cells of the distal tubules were stated to acidify the filtrate, which is mainly uric acid, and also to remove sodium and chlorine ions (Hildebrand, 1988). The mucous-like blebs in some cells of the distal tubules also reported by (Guo et al., 1996). This, and the internal PAS+ ve as well, may provide the filtrate with viscous characters after the reabsorption processes in the proximal and distal tubules. The simple

glomerulus and the small size of the renal corpuscle may place a limitation on the glomerular filtration rate (Peek and Mc Millan, 1979). The poor vascularized capillaries may also minimize the water loss in the filtrate (Zausti et al., 1986). These structural findings in the kidney of *C. vipera* may support its critical role in regulating the loss of water and the balance of ions (Beuchat and Braun, 1988) in reptiles. (Coborn, 1994), stated the relation between liver and kidney in the excretory mechanisms of snakes. Liver converts the toxic ammonium compounds resulted from the breakdown of proteins into uric acid. The relatively water insoluble uric acid is transported from liver to kidneys via the blood stream. Therefore, much water is recycled into the body by the kidneys.

In conclusion, the histological structure of both liver and kidney of the snake *C. vipera* confirms their central role in the metabolic activities, the body osmoregulation and the water conservation mechanisms of this species.

Fig. (I): A photomicrograph of a section in the liver of *C. vipera* showing a mass of hepatocytes. (H. & E.; X 200)

Fig. (2): A photomicrograph of a section in the liver of C. vipera showing the polygonal hepatocytes with large nuclei. (H. & E.; X 400)



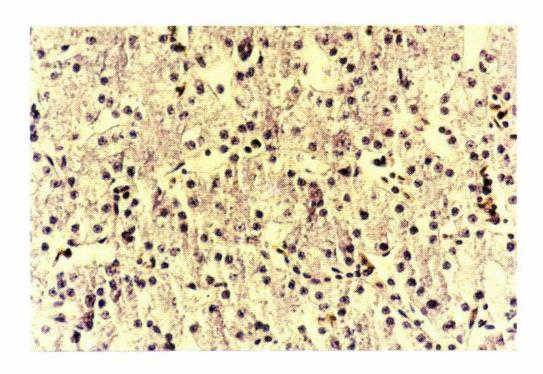
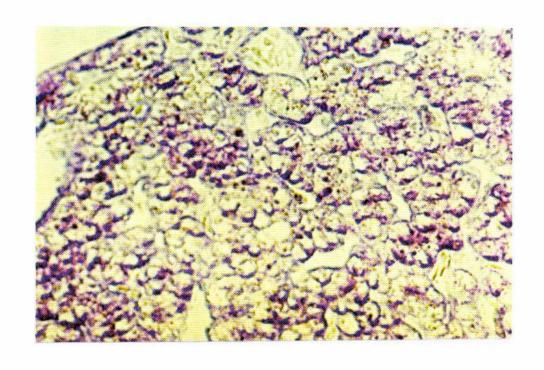


Fig. (3): A photomicrograph of a section in the liver of *C. vipera* showing the vacuolated cytoplasm of the parenchymal liver cells. (H. & E.; X 400)

Fig. (4): A photomicrograph of a section in the liver of *C. vipera* showing high PAS+ ve reaction on the surface liver cells. (PAS; X 400)



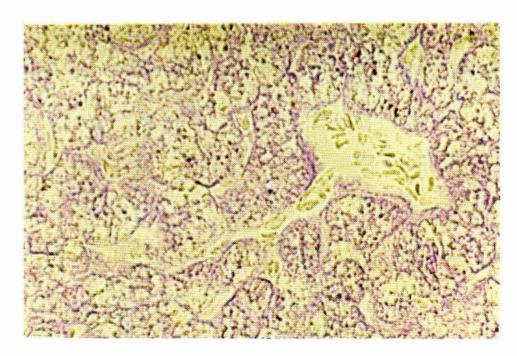
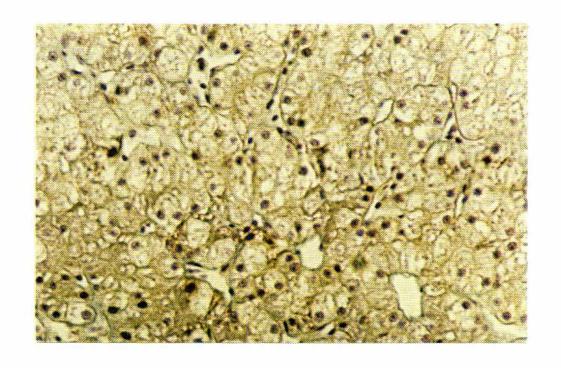


Fig. (5): A photomicrograph of a section in the liver of C, vipera showing the internal parenchymal cells exhibited less PAS+ ve materials. (PAS: X 400)

Fig. (6): A photomicrograph of a section in the liver of C, viperal showing the hepatic vein with moderate amounts of PAS+ ve materials. (PAS: X 400)



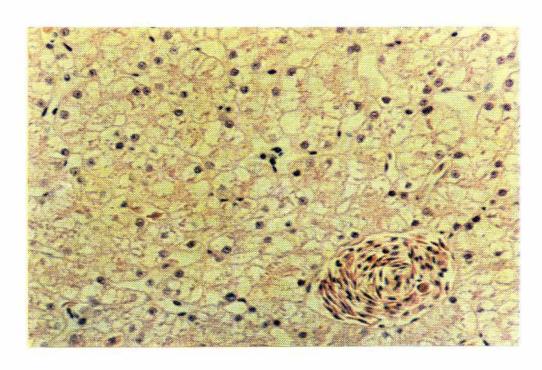
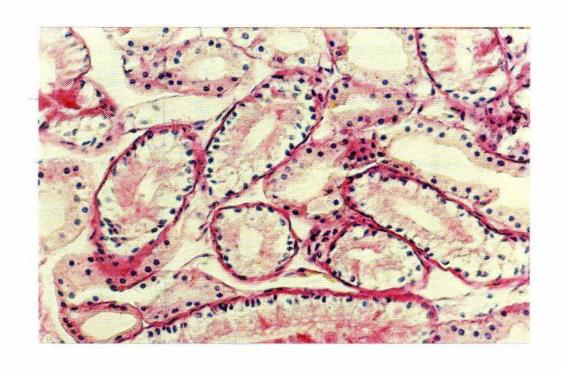


Fig. (7): A photomicrograph of a section in the kidney of C, vipera. Notice that the cortex and medulla are not distinct. (H. & E.; X100)

Fig. (8): A photomicrograph of a section in the kidney of C, vipera showing the proximal tubules (small arrows) and the intermediate tubules (large arrows). (H. & E.; X 400)



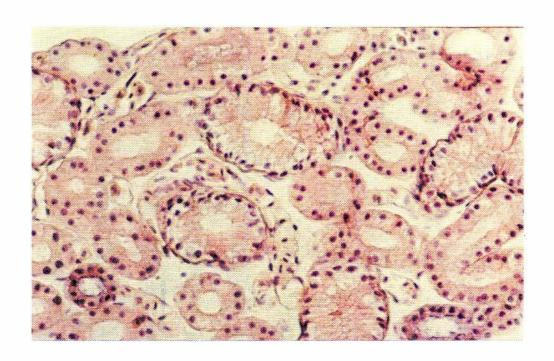
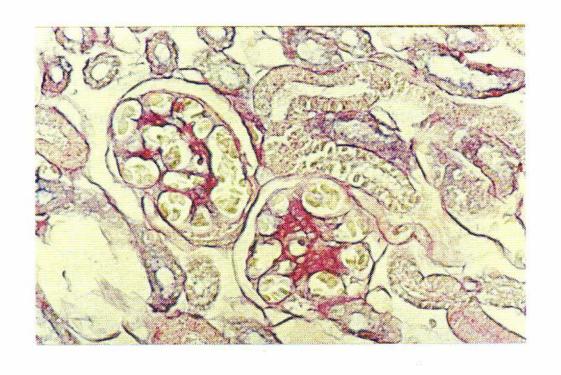


Fig. (9): A photomicrograph of a section in the kidney of *C. vipera* showing the glomeruli (small arrows) and the distal tubules (large arrows), (H. & E.; X 400)

Fig. (10): A photomicrograph of a section in the kidney of C, viperal showing the internal lining of the intermediate tubules with PAS+ vereaction. (PAS; X 400)



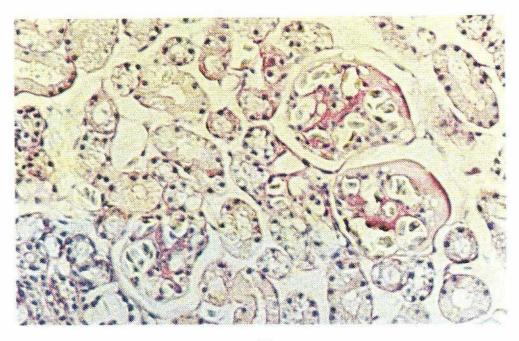
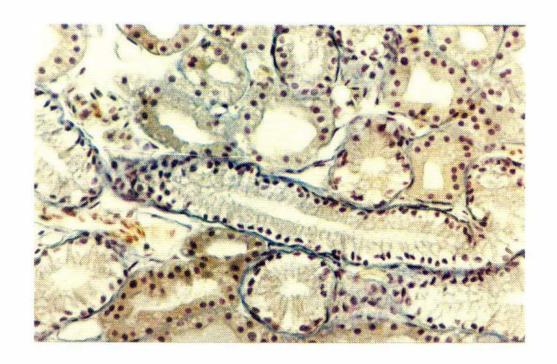
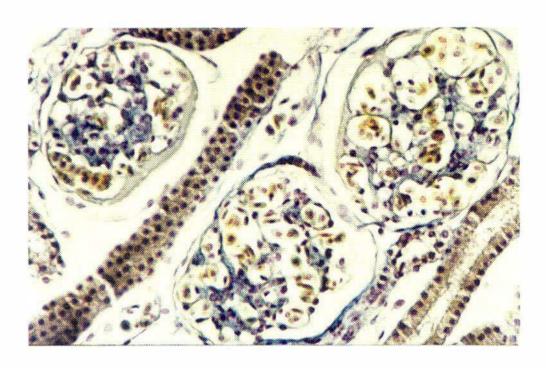


Fig. (11): A photomicrograph of a section in the kidney of *C. vipera* showing the internal lining and cytoplasmic blebs exhibiting PAS+ ve reaction.(PAS; X 400)

Fig. (12): A photomicrograph of a section in the kidney of *C. vipera* showing the glomeruli exhibiting PAS- ve reaction.(PAS; X 400)





REFERENCES

- 1. Al-Thani, A. S. (1998): Histological studies on the gastrointestinal tract of the snake, *Cerestes vipera* A new local record in Qatar. Presented to the 22nd conference. Egyptian Society of Histology and Cytology. 14th Dec. 1989. Cairo, Egypt.
- 2. Barni, S.; Bernocchl, G. and Gercell, G. (1985); Morphometrical changes in hepatocytes during the life cycle of European eel. Tissue cell, 17: 97-109.
- 3. Beuchat, C. A. and Braun, E. J. (1988): Allometry of the kidney: implications for the Ontogeny of osmoregulation. Am. J. Physiol., 225: 760-767.
- 4. Coborn, J. (1994): The mini-atlas of snakes of the world, T. F. H. Publications, Inc., NJ, USA.
- 5. Eissa, S. M. and El-Assy, Y. S. (1975); Records of certain reptilian species found in Kuwait, J. Univ. Kuwait, (Sci), 2: 124-146.
- 6. El-Sherif, G. and Al-Thani, A. S. (1997): Anatomical studies of the worm-like reptile, *Diplometopon zarudnyi* (Squamata) and study of the histology and PAS-reaction in its liver and kidney. Egypt. J. Histol., 20 (1): 175-185.
- 7. Ferrer, C.: Zausti, A.; Ballesta, J.; Hernandez, F. and Pastor, L. M. (1987): The liver of *Testudo gracea* (Chelonia): A comparative study of hibernating and non-hibernating animals. J. Submicrosc. Cytol., 19 (2): 275-282.
- 8. Guo, M.; Ditrich, H. and Splechtna, H. (1996): Renal vasculature and Urini ferous tubules in the common Iguana. J. Morphol., 229: 97-104.

- 9. Henninger, J. M. (1982): Histology of the liver in the box turtle. Anat. Rec., 202 (79A).
- 10. Hildebrand, M. (1988): Analysis of vertebrate structure and function. 3rd ed., John Willey Sons Inc., New York, 231.
- 11. McManus, J. F. A. and Mowry, R. W. (1960): Staining methods, histologic and histochemical, Hoeber, New York.
- 12. Mohammed, M. B. H. (1988): Survey of the reptiles of Qatar. Proc. Zool. Soc. A. R. Egypt, 16: 17-26.
- 13. Peek, W. and Mc Millan, D. (1979): Ultrastructure of the renal corpuscle of the garter snake *Thamnophis sirtalis*. Am. J. Anat., 155: 83-101.
- 14. Tanuma, Y. (1980): Electron microscope observations on the intrahepatocytic bile canaliculus and sequent bile ductules in the crucian, *Carrassius carrassius*. Arch. Histol. Jap., 43: 1-21.
- 15. Tsuneki, K. and Ichihara, K. (1981): Electron microscope study of vertebrate liver Innervation. Arch. Histol. Jap., 44 (1): 1-13.
- 16. Zausti. A.; Ferrer, C.: Ballesta, J. and Pastor, L. M. (1986): Ultrastructure of the tubular nephron of *Testudo gracea* (Chelonia): A comparison between hibernating and non-hibernating animals. Histol. Histopathol., 2 (4): 391-400.

ARABIC SUMMARY

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الجزء التالث يشمل دراسات هستولوجية عنى الفناة الهضمية الحية القرعاء سيراستس فيبرا التي تم رصدها حديثا في دولة قطر وقد تعت دراسة التركيب النحيجي بالإضافة إلى نظام توزيع المواد عديدات التسكر المخاطية في الفناة الهضمية والتي سجلت لأول مرة في دولة قطر خلال هذه الدراسة. وجدت علاقة وتيقة بين كل من التركيب النسيجي والمحتوى المخاطي المفاطي المهضمية وبين طبيعة الغهدذاء وألية التغذية ، كما وجد أن الحية القرعاء (كما هو معروف في كل التعابين) تتميز بمحتوى عهدالي مسن إنزيمات الهضم في اللعاب عن الانواع الحيوانية الأخرى ، كما أن المواد المخاطية تقوم بدور أساسي فسي الهضم والإخراج في هذا النوع من الحيات .

أما الجزء الرابع فقد عني بدراسة التركيب النسيجي ومحتوى الكربوهيدرات في الكبد والكلية الحية القرعاء سيراستس فيبيرا وقد أسفرت الدراسة عن ظهور خلايا الكبد على شكل كتلة واحدة دون فصوص واضحة وكانت الخلايا الكبدية عديدة الأضلاع ومرتبة في صفين من الخلايا أوتنتشر حول الجيوب والشعيرات الدموية دون نظام معين. كما أظهرت خلايا الحواف في الكبد تفاعل إيجابي للكربو هيدرات وبدرجة أقل بالنسبة للخلايا الكبدية حول الأوردة الكبدية. لم يظهر بالكلية فاصل تركيبي واضح بين القشرة والنخاع وظهرت الكبات والكريات الكلوية صغيرة الحجم وذات تركيب نسيجي بسيط. كانت الأنابيب القاصية والدانية مماثلة للتركيب المعروف في حين كانت الأنابيب المتوسطة مختلفة عن الزواحف. تم كذلك مناقشة العلاقة بين التركيب والوظيفة وكل من الكبد والكلية ودورهما في النشاط الأيضي لهذا النوع من الزواحف.

ARABIC SUMMARY

يعنى هذا الجزء من المشروع بالتركيب النسيجي والهستوكيميائي ليعض أنواع من الزواحف الشهيرة والشائعة في البيئة القطرية. فقد تمت دراسة الاعضاء المختلفة مثل الفناة الهضمية والكبد والكلية لكل من السطلية شبيهة الديدان والحية القرعاء

لراوحت الطبقة العضلية بين الألياف الطولية فقط في المريء والطولية والعرضية الرقيقة في والجزء الأول يغتص بنراسة بالمجهر الضوئي للتركيب النسيجي وكذلك تفاعل حمض البيريوديك وكاشف شيف. وقد لوحظ أن مخاطية المريء تتكون من طاكية عمودية مصففة بينما مخاطية. كما وجدت الخلايا الإمتصاصية ذات الحواف الفرجونية وانعد الكاسية في مخاطية الأمعاء الدقيقة وفي الأمعاء الغليظة نوحظ وجود مخاطية هرمية الشكل وعدد كبير من الغدد الكاسية مع طبقة محددة بوضوح من المواد المخاطية. كما بينما تميزت أيغدد الكاسية بكميات كبيرة من عديدات التسكر المخاطية شديدة التفاعل. وقد أظهرت خلايا المرىء والمعدة تفاعل إيجابي بصورة موحدة مع تفاعل البيريوديك وكالنف شيف تعتبر السحلية شبيهة الديدان من الزواحف المعروفة في البيئة القطرية باسم الناوس ، المعدة بينما تميزت الأمعاء الغليظة بوجود طبقتين سميكتين من الآلياف الطولية والعرضية.

فقد وجدت تتكون من كريات وأنيبيات كلوية لكن يدون فاصل وأضح بين القشرة والنخاع يعكس الثدييات ، تتكون القطع القريبة من قطع يعيدة وقريبة ومتوسطة فتتكون القطع القريبة من خلايا من خلايا القطعة المتوسطة رقيقة ومهدبة خلايا مكعبة ام عمودية قصيرة ذات حواف فرجونية بينما خلايا القطعة المتوسطة رقيقة ومهدبة والخاريا العريات الكلوية من النوع الطّلالي العرشفي البسيط اما المحتوى المخاطي فقد نوحظ أن خلايا القطعة القريبة بها كميات قلبلة بينما تعيزت الأوعية داخل الكريات بكميات اكبر من المخاطيات. إلى المعدة فالأمعاء الدقيقة والغليظة والمجمع على هيئة ضيق أو اتساع ، وجد أيضا أن الرينة اليسري اربعة أضعاف الرئة اليمني حجما. لوحظ أن الكبد عديم الفصوص ويتكون من الخلايا الكبدية التي تحيط بالجيوب الدموية. الفواصل بين الجيوب بسمك خليتين والخلايا المتجاورة المخاطبات في الغشاء المحيط بالكبد والقنبات وأسطح الخلايا المبطنة للجيوب الكبدية. اما الكلية ترقبيط ببعضها عن طريق القنبات الصغراوية الدقيقة. لوحظ أيضا وجود كميات متوسطة من تترتب بحيث تبدو القناة الهضمية مستقيمة تقريبا أو بها ثنيات قليلة كما ان الأنتقال من المريع النسيجي ومحتوى المخاطبات لكل من الكبد والكلية ، وقد نوحظ أن الأحشاء العامة للحيوان الجزء الثاني يعنى بالتركيب التشريحي للسحلية شبيهة الديدان مع دراسة التركيب رقم الايداع ددار الكتب القطرية: ٢٣ / ٢٠٠٤ الرقم الدولي (ردمك): ٢ - ٥٣ - ٢٥ - ٩٩٩٢١

رصد وتسجيل طائفة الزواحف في البيئة القطرية ودراسة التركيب النسيجي والمحتوى الإنزيمي لأعضائها وعلاقته بالتغيرات البيئية

مشروع رقم (HE/45/95)

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