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A Comparative Study of Enzyme Histochemistry of the Alimentary Tracts of *Chalcides ocellatus*, *Uromastyx microlepis* and *Mauremys caspica*

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ABSTRACT. A comparative histochemical study on the distribution and localization of nine digestive enzymes in the alimentary tracts of the skink (*Chalcides ocellatus*), spiny-tailed lizard (*Uromastyx microlepis*) and the stripe-necked terrapin (*Mauremys caspica*) was undertaken. Variations in the activities and localization of these enzymes in the three reptiles are discussed in relation to their feeding habits.

A survey of the literature revealed that little has been published on the digestive physiology of reptiles. What knowledge is available deals with the distribution of mucosubstances (Anwar and Mahmoud 1975, Chavez 1966, Williams and Gerard 1969, Suganuma *et al.* 1981) and few studies were conducted on the distribution and localization of digestive enzymes (Chou 1977, Taib 1981, Taib and Jarrar 1983b).

The present study deals with the localization and distribution of nine digestive enzymes in the alimentary tracts of *Chalcides ocellatus* (Forskal), *Uromastyx microlepis* (Bladford) and *Mauremys caspica* (Gmelin). The selected species have a wide distribution throughout Saudi Arabia and represent different types of feeding habits.

The present work is an attempt to throw some light on the lacuna that exists in our knowledge of the digestive physiology of reptiles. It is hoped that it will help to stimulate further work on this little-known aspect of reptiles for further comparative studies of vertebrate digestive physiology.

Material and Methods

Twenty skinks (*Chalcides ocellatus*), twenty spiny-tailed lizards (*Uromastyx microlepis*) and seventeen stripe-necked terrapins (*Mauremys caspica*) were collected from various districts of Saudi Arabia, killed with chloroform and three pieces were removed from each part of their alimentary canals as indicated in Table 1. Paraffin as well as unfixed fresh cryostat sections (5-12 μm at −25°C) were cut from these portions and studied by the following methods: The α-naphthol acetate method (Gomori 1952) for nonspecific esterases, the Gomori's lead nitrate method (Pearse 1972) for acid phosphatase, the calcium cobalt method (Gomori 1952) for alkaline phosphatase, the McCobe and Chayen's (1965) method for exopeptidase, the silver proteinate method (Yamada and Ofugi 1968) for endopeptidase, the azo-dye method (Pearse 1972) for β-galactosidase, the naphthol-AS method (Hayashi *et al.* 1964) for β-glucuronidase, and Häusler (1958) method for carbonic anhydrase. The control in each case consisted of parallel sections in media lacking a specific substrate or by using heat incubation of sections.

Results

The enzyme activities of the various parts of the epithelium of the three species shown in Table 1.

Acid phosphatase

A diffuse cytoplasmic reaction was obtained in most of the chief cells lining the gastric glands of the three species (Fig. 1, 2) but a nuclear reaction was only sometimes clearly observed. A significant reaction was noticed in the free border of cells lining the lower part of the oesophagus of *M. caspica* and *U. microlepis*, but such activity was completely lacking from the whole oesophagus of *C. ocellatus* and from the intestine of both *C. ocellatus* and *U. microlepis* except for a light reaction in the distal half of the columnar cells lining the anterior portion of the duodenum.

Alkaline phosphatase

A moderate activity of this enzyme was detected in the epithelial lining of gastric glands and duodenum (Fig. 3), together with a slight reaction in the apical portion of columnar cells lining the lower oesophagus (Fig. 4) and the anterior portion of the large intestine. No differences were observed among the three species under study.

Lipase

Apart from a weak reaction in the anterior portion of the duodenum of *U. microlepis*, the activity of this enzyme was not detected anywhere in the alimentary tract of the three reptiles.

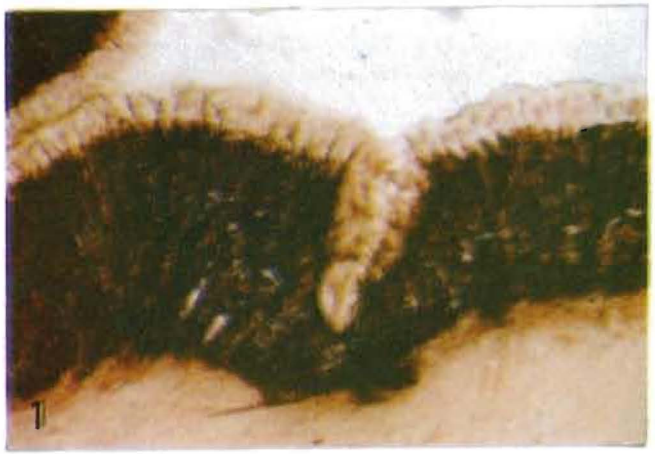

Fig. 1. Transverse section through the stomach of *M. caspica*. Lead nitrate method for acid phosphatase. X200.

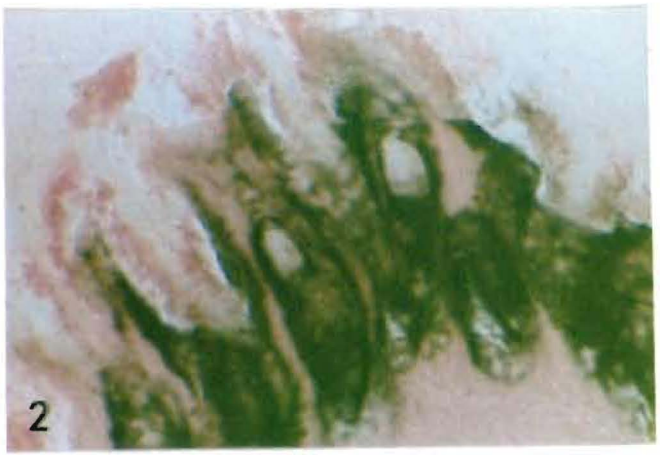

Fig. 2. Transverse section through the stomach of *U. microlepis*. Lead nitrate method for acid phosphatase. X180.

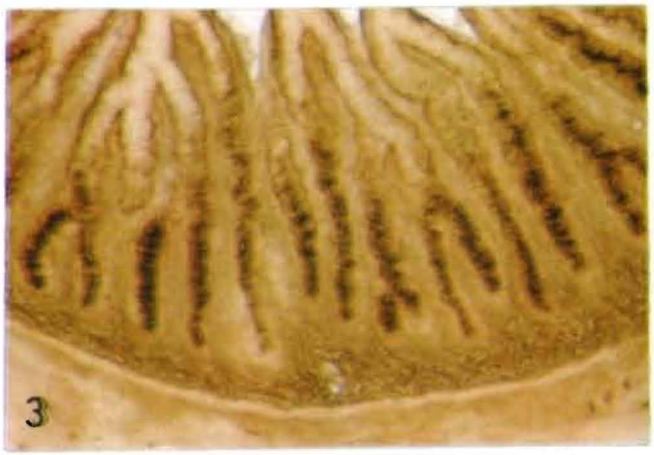

Fig. 3. Transverse section through the duodenum of *M. caspica*. Calcium-cobalt method for alkaline phosphatase. X90.

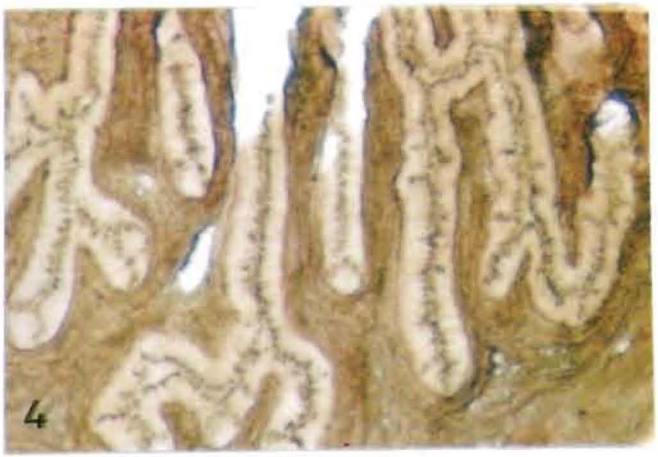

Fig. 4. Transverse section through the posterior portion of the oesophagus of *C. ocellatus*. Calcium-cobalt method for alkaline phosphatase. X135.

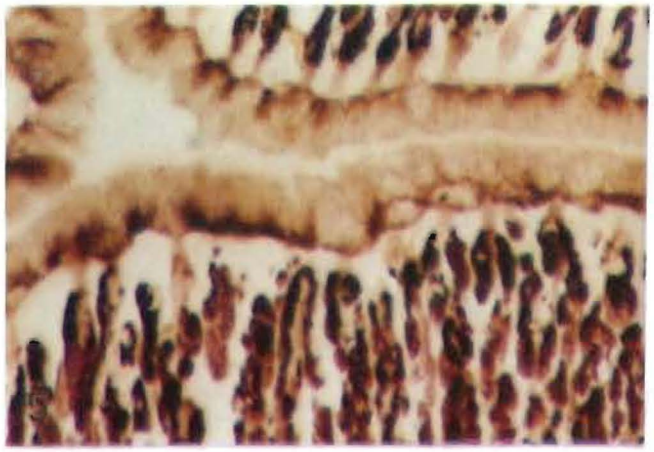

Fig. 5. Transverse section through the stomach of *C. ocellatus*. The α-naphthol acetate method for nonspecific esterases. X180.

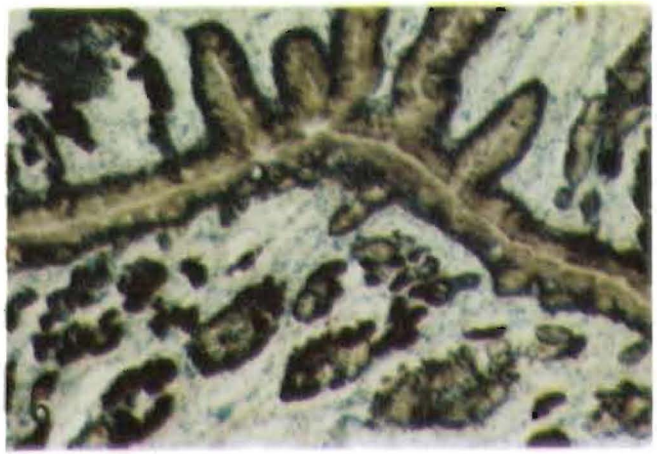

Fig. 6. Transverse section through the stomach of *M. caspica*. The α-naphthol acetate method for nonspecific esterases. X180.

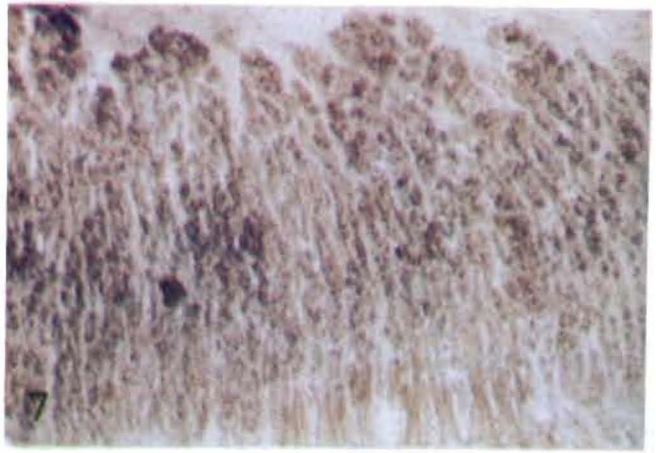

Fig. 7. Transverse section through the stomach of *U. microlepis*. The α-naphthol acetate method for nonspecific esterases. X135.

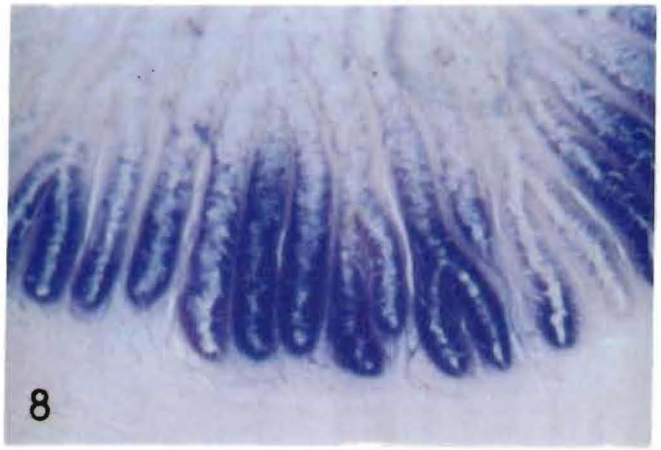

Fig. 8. Transverse section through the duodenum of *C. ocellatus*. McCobe and Chayen method for exopeptidase. X135.

Table 1. Enzyme activity in the alimentary tracts of *C. ocellatus*, *M. caspica* and *U. microlepis*.

		* 1	2	3	4	5	6	7	8	9
		**								
Acid phosphatase	*C. ocellatus*	−	−	++	−	++	−	−	−	−
	M. caspica	−	±	±	+++	+	±	±	−	−
	U. microlepis	−	+	++	+	++	−	−	−	−
Alkaline phosphatase	*C. ocellatus*	±	+	−	+	−	+	±	±	−
	M. caspica	±	±	−	+	−	+	±	±	±
	U. microlepis	±	+	−	+	−	±	±	±	−
Lipase	*C. ocellatus*	−	−	−	−	−	−	−	−	−
	M. caspica	−	−	−	−	−	−	−	−	−
	U. microlepis	−	−	−	−	−	±	−	−	−
Nonspecific esterase	*C. ocellatus*	±	+	++	+	++	++	++	+	+
	M. caspica	±	+	−	+++	−	++	++	+	+
	U. microlepis	−	±	−	+++	−	++	++	+	+
Exopeptidase	*C. ocellatus*	−	−	−	+	−	+++	±	−	−
	M. caspica	−	±	±	+	±	++	+	±	−
	U. microlepis	−	−	−	+	−	++	+±	±	−
Endopeptidase	*C. ocellatus*	−	−	±	+	±	++	+	±	−
	M. caspica	−	±	±	+	±	++	+	−	−
	U. microlepis	−	−	±	±	±	++	±	−	−
β-Glucuronidase	*C. ocellatus*	±	±	+	−	+	±	±	±	±
	M. caspica	±	±	−	+	−	++	±+	±	−
	U. microlepis	−	−	+	++	+	+++	++	±	−
β-galactosidase	*C. ocellatus*	−	−	−	?	−	+	±	−	−
	M. caspica	−	−	−	?	−	±+	±	−	−
	U. microlepis	−	−	−	±	−	++	+	−	−
Carbonic anhydrase	*C. ocellatus*	−	±	−	++	−	+	−	−	−
	M. caspica	±	±	?	++	?	+	±	−	−
	U. microlepis	−	±	−	++	−	+	−	−	−

* Key:
1. Anterior oesophagus lining
2. Posterior oesophagus lining
3. Fundus lining
4. Gastric glands lining
5. Pylorous lining
6. Duodenum
7. Ileum
8. Colon
9. Rectum

** Code of reaction:
−, nonreactive; ±, very weak; ±, weak; +, moderate; ++, strong; +++, very strong; ?, doubtful.

Nonspecific esterases

Both parietal and chief cells were stained for nonspecific esterases, the former more intensely in all three species (Fig. 5, 6 & 7). Significant activity was also observed in the epithelial lining of the lower oesophagus and the intestines with gradual diminution of activity posteriorly. Considerable activity was observed in the epithelium of the stomach of *C. ocellatus* but was absent from the other two species.

Exopeptidase

A vivid and strong reaction was observed in the duodenal lining of the three reptiles (Fig. 8), together with considerable activity in the gastric glands and the lining of the anterior portion of the ileum and very weak activity in the oesophagus and the large intestine.

Endopeptidase

The gastric glands of *C. ocellatus* and *M. caspica* showed a moderate reaction, while those of *U. microlepis* demonstrated a less intense reaction (Fig. 9). The lining of the duodenum of the three species, on the other hand, revealed a strong reaction.

β-glucuronidase

The activity of this enzyme was intense in the distal parts of the cells lining the small intestine of *U. microlepis* followed by *M. caspica* and *C. ocellatus*. A considerable amount of activity was also detected in the gastric glands of both *U. microlepis* and *M. caspica* and was negligible in those of *C. ocellatus* (Fig. 10, 11). Moreover, some activity was also noticed in the cells lining the stomach of both *C. ocellatus* and *U. microlepis* but not in *M. caspica*.

β-Galactosidase

Considerable activity was observed in the apical portion of columnar cells lining the small intestine of *U. microlepis* (Fig. 12) while lesser activity was observed in those of both *M. caspica* and *C. ocellatus*. The surface epithelium of the oesophagus and the large intestine were nonreactive while the reaction in the gastric glands was uncertain.

Carbonic anhydrase

A pronounced reaction was observed in the lining of the gastric glands of the three species (Fig. 13, 14). Less activity was detected in the lining of the duodenum while the reaction was almost absent in the lining of the large intestine of the three species.

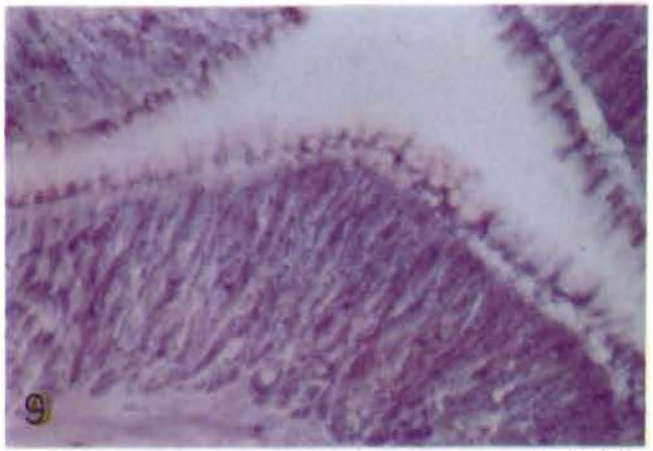

Fig. 9. Transverse section through the stomach of *M. caspica*. Silver proteinate method for endopeptidase. X180.

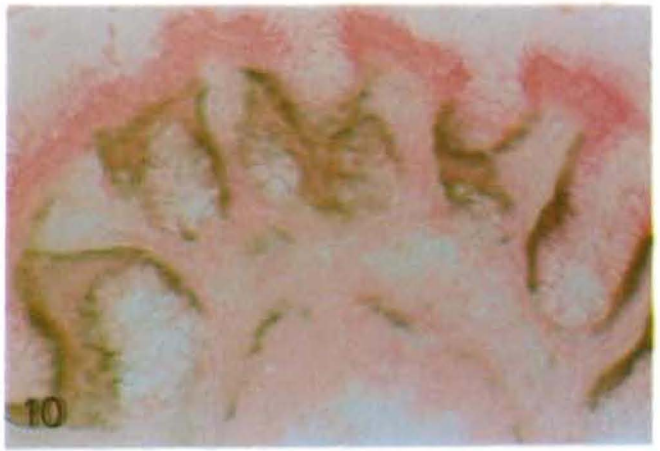

Fig. 10. Transverse section through the stomach of *M. caspica*. Naphthol AS-BI Glucuronide method for β-glucuronidase. X200.

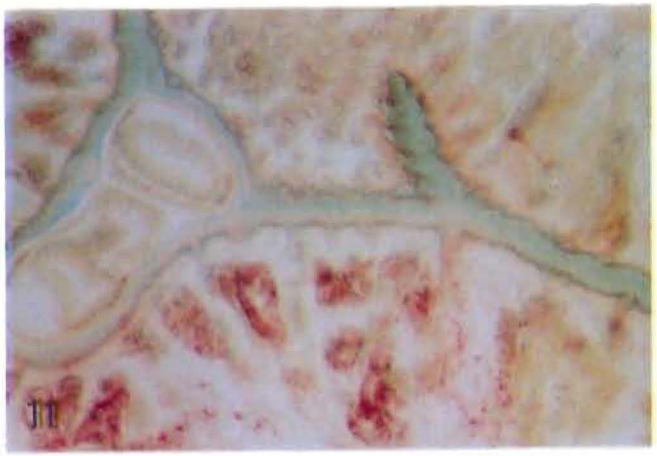

Fig. 11. Transverse section through the stomach of *U. microlepis*. Naphthol AS-BI Glucuronide method for β-glucuronidase. X180.

Fig. 12. Transverse section through the duodenum of *U. microlepis*. The azo-dye method for β-galactosidase. X200.

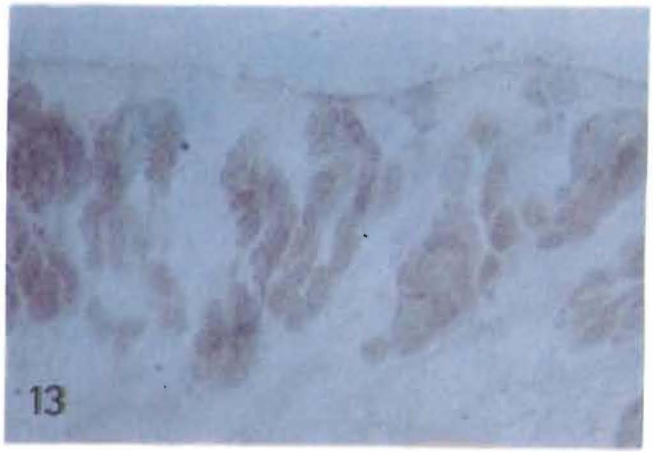

Fig. 13. Transverse section through the stomach of *M. caspica*. Haüsler method for carbonic anhydrase. X180.

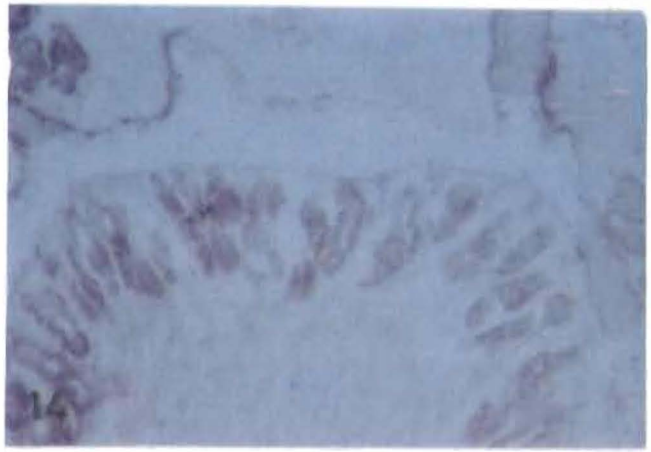

Fig. 14. Transverse section through the stomach of *C. ocellatus*. Haüsler method for carbonic anhydrase. X180.

Discussion

The detailed histological structure of the alimentary canal of the three species has already been investigated (El-Toubi and Bishai 1955, Taib *et al.* 1982, Taib and Jarrar 1983a), but the present study indicates that the alimentary canals of the three species possess an armoury of various digestive enzymes to ensure the maximum utilization of food ingested.

The pronounced reaction of phosphatases in the alimentary tract of the three species may be related to the involvement of these enzymes in the supply of energy during active transport and in the process of intracellular digestion, but a digestive role for these lysosomal enzymes can not be excluded. The results show that phosphatases are found in high concentration in the cells which play a role in secretion and absorption of material from the lumen of the gut.

The absence of lipase activity from the alimentary canal of the three species may be due to the fact that fat droplets can pass through the mucosa and hence results in its intracellular digestion (Barrington 1957). However, this deficiency seems to be compensated for in these reptiles by the intense activity of nonspecific esterases which might have resulted from the combined activities of several closely related enzymes.

The pronounced activity of the proteolytic enzymes observed in the anterior portion of the small intestine could be due to the fact that these enzymes are activated by partial digestion and hence concerned with the later stages of digestion and absorption from the gut lumen (Smith 1960).

Carbonic anhydrase occurs in active conditions and is associated with the production of hydrochloric acid in the stomach (Jennings 1962). This may explain the localization of this enzyme in the gastric glands of the three reptiles.

The variations observed in the activities of digestive enzymes might reflect the differences in the feeding habits of the three reptiles. The results show that *Chalcides ocellatus* has the highest activity of nonspecific esterases and endopeptidase but less of β-glucuronidase and β-galactosidase. This is in agreement with the feeding habit of this animal as an insectivore (Ibrahim 1977).

Uromastyx microlepis is a pure herbivore (El-Toubi and Bishai 1955). This may explain the weak lipolytic and proteolytic enzyme activities and the strong activity of carbohydrases. The latter group of enzymes is concerned with oligosaccharide hydrolysis and with catabolism of mucosubstances and glycolipids (Furth and Robinson 1965).

According to Mahmoud and Klicka (1979), *Mauremys caspica* has a varied diet of plants, insects, small invertebrates and amphipods. This may explain the considerable activity of nonspecific esterases, endopeptidase, β-glucuronidase and β-galactosidase to ensure the maximum utilization of the varied diet of this animal.

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مقارنة لنشاط بعض الإنزيمات الهاضمة في أنسجة القناة الهضمية لكل من *كالسيدس أسوليتس و يورماستكس مايكرولبس و مورميس كاسبكا*

نورى طاهر الطيب و بشير محمود جرار

قسم علم الحيوان - كلية العلوم - جامعة الملك سعود - الرياض - المملكة العربية السعودية

تضمنت هذه الدراسة مقارنة وجود وتوزيع تسعة إنزيمات هاضمة في أنسجة القناة الهضمية لثلاثة أنواع من الزواحف وهي السحلية (كاليدس أسوليتس) والسحلية شوكية الذيل (يورماستكس مايكرولبس) والسلحفاة مخططة العنق (مورميس كاسبكا). كما تتناول هذه الدراسة شرحا لأوجه التشابه والاختلاف لنشاط الإنزيمات الهاضمة ومدى ارتباط ذلك بنوعية غذاء الأنواع الثلاثة من الزواحف تحت الدراسة.