

Observations on the Histochemistry of the Alimentary Canal of *Chalcides levitoni* (Reptilia, Scincidae)

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ABSTRACT. The distribution of carbohydrates, lipids and proteins including enzymes in the alimentary canal of *Chalcides levitoni* are described. Both neutral and acidic mucosubstances are identified in the epithelial cells. Neutral mucosubstances are most evident in the stomach and acid mucosubstances are more abundant in the intestine. Sulfomucins as well as sialomucins show different patterns of distribution. The present investigation shows that the principal digestive enzymes: acid phosphatase, alkaline phosphatase, non-specific esterases, aminopeptidases, endopeptidases, trypsin-like enzyme, beta-galactosidase and beta-glucuronidase are revealed histochemically. Lipases and alpha-glucosidase do not appear to be produced anywhere in the alimentary tract epithelium.

Chalcides levitoni is a squamata lizard sympatric with *C. ocellatus* in different districts in Saudi Arabia (Pasteur 1978). Ocellated skink has a wide distribution all throughout the Mediterranean area (Ibrahim 1977). A survey of literature revealed that little has been published on the histochemistry of the alimentary tract of lizards. Most studies have been limited to a specific area of gastrointestinal tract of a single species (Odebode *et al.* 1979), several unrelated species (Chazev 1966, Willems and Gerard 1969), or various closely related species (Read and Burnstock 1968). Few other investigators have described the distribution of mucosubstances throughout the entire gastrointestinal tract of a single species (Ganter and Marche 1970, Taib 1981) or closely related species (Suganuma *et al.* 1981).

The present study was undertaken to determine the histochemistry of the alimentary

tract of *Chalcides levitoni* with the intention of furthering knowledge about the digestive physiology of the squamata.

Material and Methods

Adult male and female *Chalcides levitoni* lizards were used in this study. The experimental animals were fed on boiled eggs and kept in cages where free movement was allowed. Twenty six specimens were sacrificed during this study. Each animal was anaesthetized with chloroform and the whole of the alimentary canal removed. The various parts of the gastrointestinal tract were identified as indicated in Table 1, then isolated, flushed of, and frozen with liquid nitrogen onto blocks. Unfixed cryostat sections (6-15 μm , at -25°C) were used routinely. Methods devised to reveal classes of compounds were employed as indicated in Table 1.

The following methods were used to investigate enzymes present: the α -naphthol acetate method (Gomori 1952) for nonspecific esterase; the Gomori's lead nitrate method (Pearse 1972) for acid phosphatase; the calcium cobalt method (Gomori 1952) for alkaline phosphatase with sodium β -glycerophosphate as substrate; the Gomori's (1952) tween method for lipase; the McCobe and Chayen (1965) method for exopeptidase with leucyl- β -naphthylamide as substrate; the Glenner and Cohen (1960) method for trypsin like enzyme using N-(.025- α -benzoyl-DL-arginine-naphthylamide) as substrate; the silver proteinate method (Yumada and Ofugi 1968) for endopeptidases; the azo-dye method (Pearse, 1972) for α - and β -glucosidases and galactosidases; the naphthol AS method (Hyashi *et al.* 1964) for β -glucuronidase. Control in each case consisted of parallel incubation of sections in media lacking a specific substrate.

In addition to these histochemical tests for enzymes, the distribution of glycogen and mucopolysaccharides was studied by means of Best's carmine (Best 1906) periodic acid-schiff (McManus 1948) with and without prior malt diastase digestion of tissue sections (McManus and Mowry 1964); and Alcian blue (Mowry 1956). Lipids were demonstrated by the Nile blue sulphate, Oil red O, and Sudan black B methods. Protein-bound amino groups were identified by Yasuma and Ichikawa's (1953) ninhydrin-schiff method.

Visual estimation of the intensity of the dye deposited in different regions using light microscopy examination was used as a measure of the relative activity of the enzymes and presence of other compounds.

Results and Discussion

The alimentary tract of *Chalcides levitoni* consists of oesophagus, stomach (fundus and pylorus), small intestine (duodenum and ileum), and large intestine (caecum, colon and rectum). The oesophagus of this skink consisted of an epithelial lining with both ciliated columnar and goblet cells. The lower part of the oesophagus was lined exclusively by mucous cells. The oesophagus was devoid of oesophageal glands. The

Table 1. Summary of the result of the histochemical tests in the lining epithelium and lumen of the alimentary canal of *Chalcides levitoni*.

	* 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Periodic acid-schiff	++	++	+	++	++	+	++	++	++	++	±	++	++	++	±
Diastase digestion	±	±	-	-	-	±	++	-	-	-	-	-	-	-	-
Best's carmine	±	±	-	±	±	-	++	-	±	±	-	±	±	±	-
Alcian blue pH 2.5	++	++	++	±	±	-	±	?	++	++	+	++	++	++	++
Alcian blue pH 1.0	++	++	+	-	-	-	-	-	++	++	+	++	++	++	±
Nile blue sulphate	±	±	-	±	±	-	±	-	±	±	-	++	++	+	-
Sudan black B	±	±	-	±	±	±	±	-	±	±	±	±	±	±	±
Oil red O	-	±	-	±	±	-	±	-	±	±	-	±	±	±	-
Ninhydrin-schiff	++	++	+	++	++	±	±	±	++	++	±	++	+	++	±
Acid phosphatase	-	-	-	++	++	-	-	-	-	-	-	-	-	-	-
Alkaline phosphatase	+	+	-	-	-	-	++	-	±	±	-	±	±	±	-
Nonspecific esterases	±	++	-	++	++	±	+	-	++	++	±	++	++	++	±
Lipases	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Aminopeptidases	-	-	-	-	-	-	-	++	++	±	-	-	-	-	-
Endopeptidases	-	±	-	±	+	±	+	-	++	++	-	+	±	-	-
Trypsin-like enzyme	-	-	-	+	+	+	+	-	±	±	-	±	±	±	-
Alpha-glucosidase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Beta-glucuronidase	±	+	-	++	++	-	-	-	±	±	-	±	±	±	-
Beta-galactosidase	-	-	-	-	-	-	?	-	+	+	-	±	-	-	-
Alpha-galactosidase	-	-	-	±	±	-	±	-	±	±	-	-	-	-	-

- * Key: 1) Anterior oesophagus lining 2) Posterior oesophagus lining 3) Oesophagus lumen
 4) Fundus lining 5) Pylorus lining 6) Stomach lumen
 7) Gastric glands lining 8) Gastric glands lumen 9) Duodenum
 10) Ileum 11) Small intestinal lumen 12) Caecum
 13) Colon 14) Rectum 15) Large intestinal lumen

** Code of reaction:

—, absent; ±, very weak; ±, weak; +, moderate; ++, strong; ±+, very strong; ?, doubtful

surface epithelium of the stomach consisted of a single layer of non-ciliated mucous columnar cells. The fundic and pyloric portions have different glandular morphological structures. The intestinal mucosa of this skink was lined by both striated columnar and goblet cells.

Carbohydrates

The distribution of carbohydrates in the alimentary tract is summarized in Table 1. The epithelial lining of the gut responded positively to the carbohydrate tests. Nonetheless, there were some notable variations between the different regions of the tract. In the oesophagus, the apical surface of some columnar cells demonstrated presence of sialomucins whereas others had sulfomucins. Although the acid mucosubstances in the goblet cells of the upper oesophagus consisted of sulfomucins and sialomucins, those cells lining the lower oesophagus displayed prominent sialomucins (Fig. 1 and 2). These findings are in conformity with those reported for the skink *Eumeces latistutatus* (Suganuma *et al.* 1981).

The surface epithelium of the stomach stained positively for neutral mucosubstances (Fig.3) similar to those of many mammalian species studied (Krause 1973, Sheahan and Jervis 1976, Suganuma *et al.* 1981). The gastric glands responded strongly to PAS reaction (Fig.3) and responded partially to the diastase digestion (Fig.4). However, no



Fig.1. Unfixed cryostat section through the posterior oesophagus. Goblet cells lining the oesophagus revealed sulfomucins (blue color). Alcian blue (2.5). $\times 200$.



Fig.2. Unfixed cryostat section through the posterior oesophagus. Goblet cells lining the oesophagus stained for sialomucins (greenish blue). Alcian blue (1.0). $\times 200$.

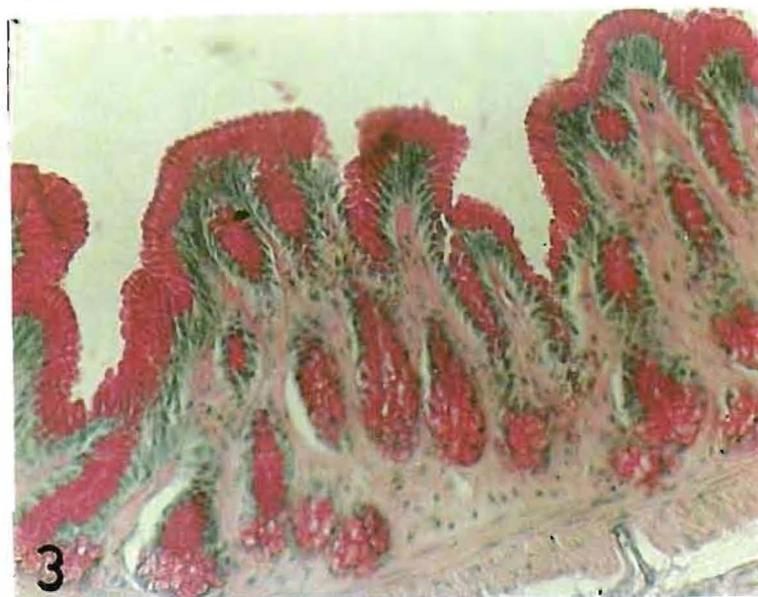


Fig.3. Unfixed cryostat section through the stomach. The epithelial lining as well as gastric glands revealed strong PAS-reaction. Periodic acid-schiff. $\times 200$.

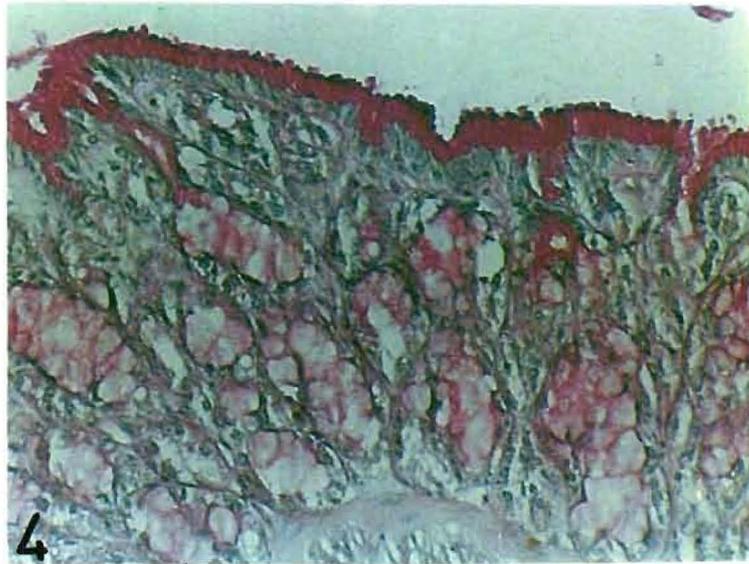


Fig.4. Unfixed cryostat section through the stomach. The epithelial lining showed no diastase activity. The gastric glands content digested partially by the diastase. Diastase treatment. $\times 200$.

reaction was encountered with Alcian blue at pH 1.0. These findings would indicate that glycogen and sulfomucins were among the contents of the gastric glands.

The striated border of the intestinal epithelium was stained for sialomucins but not sulfomucins. PAS-positive granules were seen in the cytoplasm of the intestinal columnar epithelial cells. In the goblet cells of the small intestine, sulfomucins were present in greater quantities than sialomucins (Fig. 5 and 6). In the large intestine, sulfomucins predominated sialomucins in the goblet cells.

Neutral mucosubstances were most evident in the stomach, but acid mucosubstances were more heavily distributed in the small and large intestine. This pattern of carbohydrate distribution is closely similar to many reptiles species such as the skink *Eumeces latistutatus*, the turtle *Clemmys japonica*, and the snake *Elaphe climacophora* (Suganuma *et al.* 1981). Also, the pattern of mucosubstances distribution was slightly different, but in general agreement with some other vertebrate species such as mouse, hamster, cat, bison, mule deer, and baboon (Krause 1973, Sheahan and Jervis 1976). These together may allow to conclude that the pattern of mucosubstance distribution throughout the entire alimentary canal is not correlated with the different orders of vertebrates species and appears to be unrelated to diet.

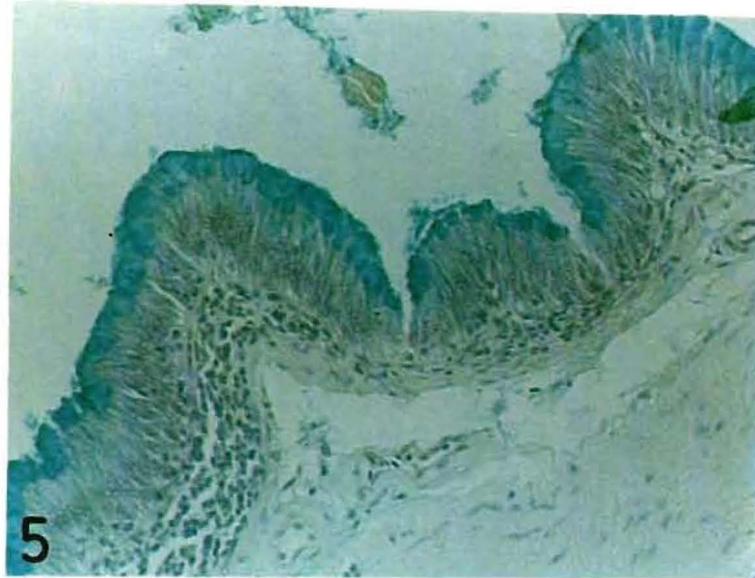


Fig.5. Unfixed cryostat section through the caecum. Goblet cells lining the caecum stained for sulfomucins (blue color). Alcian blue (2.5). $\times 200$.

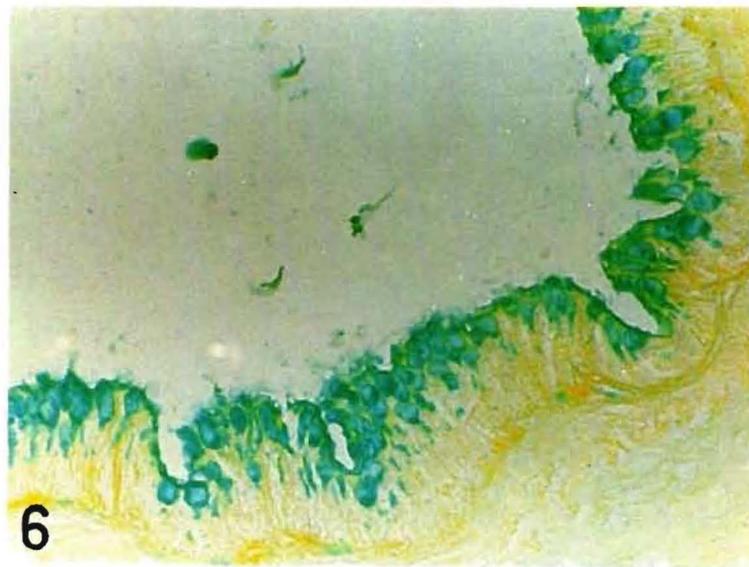


Fig.6. Unfixed cryostat section through the caecum. Goblet cells lining the caecum revealed sialomucins (greenish blue). Alcian blue (1.0). $\times 200$.

Lipids

The distribution of lipids in the alimentary canal, as revealed by Sudan black B, Oil Red O, and Nile blue sulphate, is summarized in Table 1. Most of the lipid was found in the cells lining the stomach and large intestine with lesser quantities in the oesophagus and small intestine. In the oesophagus, the apical surface of some columnar ciliated cells was stained for lipids. The upper half of the cells composing the lining of the stomach was stained for lipids. Some of the cells lining the gastric glands demonstrated a weak positive reaction for presence of lipid. Of the two types of cells lining the intestinal mucosa, only the striated cells responded positively to lipid test. Goblet cells throughout the entire alimentary canal displayed a negative reaction for lipids.

Proteins

The relative amounts of proteins in the epithelial lining and its glands which was revealed by ninhydrin-schiff method are summarized in Table 1. The wall of all regions of the alimentary canal gave positive reactions for protein. In the oesophagus, the apical cytoplasm of the ciliated columnar cells demonstrated the most pronounced staining. The epithelial lining of the stomach showed a notable staining reaction which was mostly confined to the cytoplasm of gastric cells beneath the free border. Gastric glands were stained positively with ninhydrin-schiff. This indicated the presence of a considerable amount of stored or enzymatic proteins. The epithelial lining of the intestinal mucosa also showed a strong positive reaction.

Proteins were detected in the lumen of all regions of the alimentary canal. Presence of protein could not be demonstrated in goblet cells of the alimentary canal. This is in agreement with previously reported studies that among some lizards goblet cells are purely mucous as in *Anguidae*, *Anniellidae* and *Scincromopha* (Gabe and Girons 1972). It was reported previously that some goblet cells contain both proteins and carbohydrates (Bolognani-Fantin and Bolognani 1964, Leppi 1968, Porcelli and Mansoni 1969, Askawa 1970). It should be noted that, among some lizards, some of the goblet cells may contain a small quantity of histochemically discernible proteins. This is true in *Helodermatidae*, *Varanidae*, *Gekkota* and *Iguania* (Gabe and Girons 1972).

Enzymes

Acid phosphatase

As shown in Table 1, this enzyme occurred in the epithelial lining of the stomach (Fig.7). It is noteworthy, however, that the reaction was confined to the intestinal columnar cells and was most pronounced just outside the free border of the epithelial lining of the stomach. This may be associated with the structure of the stomach free border. The remainder of the alimentary canal showed no acid phosphatase activity.

Alkaline phosphatase

The most pronounced reaction of this enzyme occurred in the epithelial lining of the gastric glands (Fig.8) and oesophagus. Slighter reactions were observed in the



Fig.7. Unfixed cryostat section through the gastric pyloric portion. The epithelial lining stained for acid phosphatase (black deposits). The gastric glands revealed no reaction for acid phosphatase. Lead nitrate method for acid phosphatase. $\times 90$.



Fig.8. Unfixed cryostat section through the stomach. The gastric glands revealed strong reaction for alkaline phosphatase (brownish black). The epithelial lining revealed no alkaline phosphatase. Calcium-cobalt method for alkaline phosphatase. $\times 200$.

epithelial lining of the intestine. This enzyme was found to be completely absent in the stomach epithelial lining. The pronounced reaction in the gastric glands may be related to the involvement of alkaline phosphatase in the supply of energy during active transport or in the process of intracellular digestion and the degradation of nucleoproteins (Taib 1976).

The occurrence of phosphatases in the epithelial lining of the gastric mucosa may indicate that these enzymes are involved in the uptake of food products (Crane 1960, Moog 1962).

Nonspecific esterases

As shown in Table 1, nonspecific esterases were widely distributed in the gut. Most of the activity was observed in the epithelial lining of the stomach and large intestine (Fig. 9 and 10). A significant amount of activity also was observed in the epithelial lining of the oesophagus, especially at its posterior region. Feeble activity appeared in the lumen of the stomach but it was absent in the gastric glands lumen.

Lipases

Gomori's Tween method failed to reveal any lipolytic activity anywhere in the gut. Possibly the strong activity of nonspecific esterases could be responsible for the absence of lipase activity. As has been suggested by Barrington (1957), fat droplets



Fig.9. Unfixed cryostat section through the gastric fundic portion. The epithelium and gastric glands revealed a strong reaction for non-specific esterases (brownish black). The α -naphthol acetate method. $\times 200$.

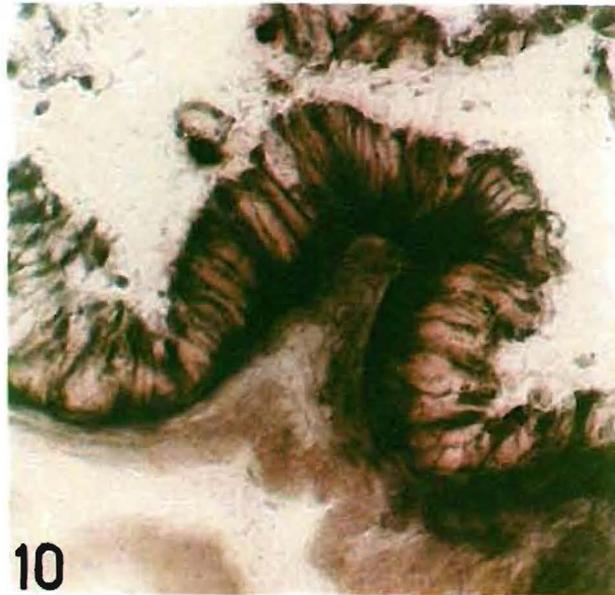


Fig.10. Unfixed cryostat section through the colon. The epithelial lining revealed nonspecific esterase (Brownish black). The α -naphthol acetate method. $\times 200$.

could pass through the mucosa; therefore, lipid digestion could be intracellular, not requiring a soluble lipase. These results are similar to those reported on *Uromastix aegyptia* (Taib 1981).

Aminopeptidases

Distribution of aminopeptidases appeared only in the epithelial lining of the small intestine most notably in the bottom part of the crypts. The absence of the enzyme from the stomach digestive glands and large intestine is notable (Table 1). This enzyme probably is concerned with absorption of material from the gut lumen.

Endopeptidases

The most pronounced reaction of this enzyme appeared in the epithelial lining of the small intestine (Fig.11). A significant amount occurred in the stomach and large intestine. Lesser quantities were observed in the epithelial lining of the gastric glands. Although a feeble activity of the enzyme was observed in the stomach lumen, no activity was detected in the gastric glands lumen.

Trypsin-like enzyme

This enzyme is similar in distribution to endopeptidase. However, although the



Fig.11. Unfixed cryostat section through the ileum. The epithelial lining revealed strong reaction for aminopeptidase (purple color). The McCabe and Chyen method with leucyle - β - naphthylamide as substrate. $\times 90$.

latter is more pronounced in the epithelial lining of the small intestine, the former was more pronounced in the stomach (Table 1).

Alpha-glucosidase

No activity was observed for this enzyme anywhere in the alimentary canal.

Beta-glucuronidase

The most pronounced reaction was observed in the epithelial lining of the stomach, localized in the distal half of the epithelial cells. Clear, but moderate, reaction also was observed in the apical surface of the ciliated columnar cells of the posterior portion of the oesophagus. The intestinal epithelial lining showed weak reaction. This enzyme was completely lacking in the gastric glands.

Beta-galactosidase

Significant activity was observed only in the striated border of the small intestine. The remainder of the alimentary tract showed no activity. This enzyme was reported to be localized in the small intestine brush border in the human, rat, and monkey (Lojda 1970, Lojda *et al.* 1974). It is believed that this enzyme is concerned with catabolism

of mucosubstances and glycolipids (Furth and Robinson 1965).

Alpha-galactosidase

The localization of this enzyme was noticed in the epithelial lining of the stomach, gastric glands and small intestine. The remaining parts of the alimentary tract showed no activity (Table 1).

Conclusion

The absence of oesophageal glands in *Chalcides levitoni* alimentary canal may possibly be compensated for by the bulk secretion of mucosubstances from the epithelial lining of the oesophagus.

Although the knowledge of the enzymes involved in the alimentation of vertebrates is relatively extensive, nothing hitherto has been known of the enzymes in *C. levitoni*. In the present study, the authors report the varied nature of these enzymes in *C. levitoni*. The bulk of enzymes include beta-glucuronidase, beta-galactosidase, alpha-galactosidase, aminopeptidase, endopeptidase, acid and alkaline phosphatases and nonspecific esterases. This is to be expected, since the diet consists of animal microscopic forms which may support the notion that this animal is mainly an insectivore (Ibrahim 1977).

The pronounced reactions of the phosphatases may be related to the active process of secretion occurring in the epithelial lining cells and they could possibly be involved in the supply of energy for the process. Phosphatases could also be involved in the process of intracellular digestion and in the degradation of ingested nucleoproteins by converting nucleic acids into nucleosides and phosphate.

The absence of lipases is noteworthy. Lipases appear to hydrolyse the glycerol ester of fatty acids which consist of long carbon chains, *i.e.* neutral fats. The deficiency of lipases may be compensated for by the strong secretion of non-specific esterases which could be involved in the hydrolysis of simple esters (carbon chain length below 12).

The histochemical investigation of glycosidases and proteolytic enzymes indicated an obvious increase in the concentration of some of these enzymes, due to the presence of food, whereas less secretion was noticed in the starved animals.

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ملاحظات حول كيمياء أنسجة القناة الهضمية في السحلية كالسيدس ليفيتوني

نوري طاهر الطيب، بشير محمود جرّار
قسم علم الحيوان - كلية العلوم - جامعة الملك سعود

يدرس البحث توزيع انتشار المواد الكربوهيدراتية والدهنية والبروتينية بالإضافة إلى الإنزيمات الهاضمة في القناة الهضمية للسحلية كالسيدس ليفيتوني .

وجد أن الخلايا الطلائية تحتوي على كلا النوعين من المواد المخاطية الحمضية والمتعادلة؛ كما وجد أن المواد المخاطية الحمضية أكثر وجوداً في الأمعاء، بينما المتعادلة منها أكثر وجوداً في المعدة.

معظم الإنزيمات الهاضمة الرئيسية وجدت في طلائية القناة الهضمية بشكل متفاوت وتوزيع مختلف .

بعض الإنزيمات، مثل الليباز وأنزيم المالتاز، لم تظهر في أي جزء من أجزاء القناة الهضمية .