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ORIGINAL ARTICLE

Histochemical changes of carbohydrate and protein contents in the digestive gland cells of the land snail *Monacha cartusiana* following starvation

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KEYWORDS

Starvation;
Land snails;
Monacha cartusiana

Abstract The present study was designed to investigate histochemically the detection of carbohydrate and protein in the normally feeding snails and after 15 and 30 days of starvation. Generally, abundant carbohydrate and protein materials were detected in the component cells of the digestive gland of normally feeding snails. The results of this investigation revealed a pronounced decline of carbohydrates in the digestive gland cells of *Monacha cartusiana* snails after starvation. Severe decline in carbohydrate content was observed especially after 30 days of starvation. Moreover, protein inclusions have exhibited a week stainability in the digestive gland cells of these snails as a consequence of starvation.

1. Introduction

The stylommatophoran species including land snails and slugs have gained an economical importance since they became among the pests attacking several crops in many parts (El-Okda, 1980; Baker, 1988; Baker and Vogelzang, 1988; Hegab, 2003; Ghamry et al., 1993; Ismail, 1997; El-Massry et al., 1998; Hegab et al., 2006; Amina et al., 2008).

Several species of the land snails are recorded on vegetable crops, ornamental plants, orchard trees and in field crops in different Governorates of Egypt (El-Okda, 1980, 1984; Hashem et al., 1992; Mersal, 1992; Azzam, 1995; Arafa, 1997; Shahawy, 1998). Most of these studies concluded that the population densities of the land snails depend mainly on the relative humidity and temperature. The control of these pests biologically requires an understanding of their biological and physiological activities with their ecological conditions – photoperiod, temperature and starvation – therefore, it warrants detailed investigations from different angles aiming to provide as much information as possible, that could be of some values in the control and eradication of these harmful organisms.

However, some studies were made in this respect, but in a limited and sporadic manner, as they were mainly focused on the taxonomical aspects of those snails. But eventhough,

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taxonomists mainly took into consideration the morphological and anatomical characteristics of these snails.

Histochemical investigations (Stickle, 1971; Veldhuijzen and Van Beek, 1976; Negabhushanam and Dhamne, 1977) postulated that many molluscs which store glycogen in large amounts were noticed to utilize it during starvation period as a primary source of energy. According to Beddiny (1979) carbohydrates were the first materials in the digestive gland in the two snails *Helisoma duryi* and *Physa acuta* to be used during starvation. Additionally, El-Emam and Ebeid (1989), Saad (1990), El-Saadany et al. (1994) and Heiba (1988) elucidated that starvation caused apparent decline of protein contents in the digestive gland cells of *Biomphalaria alexandrina*, *Eobania vermiculata* and *Theba pisana*, respectively.

Hence, the present work was planned to follow the localization of carbohydrate and protein in one of the important body organs, namely the digestive gland of the land snail *Monacha cartusiana* following starvation.

2. Materials and methods

Adult herbivorous terrestrial snails *M. cartusiana* were reared under laboratory conditions, being fed on green lettuce for 2 weeks to be acclimatized.

To examine the tolerance of the snails for complete starvation a group of control snails ($n = 30$) were allowed to normally feeding and another container ($n = 30$) without any food supply (treated group).

The digestive gland from both feeding and fasted snails (starved for 15 and 30 days) were taken out of their shells and dropped immediately into the appropriate fixative, then processed in the usual manner for the histochemical inspections under the light microscope.

General carbohydrates were illustrated in the digestive glands fixed in alcoholic Bouin and treated according to the periodic acid Schiff's technique (PAS) of Hotchkiss (1948). A positive reaction was indicated by the appearance of a pink (magenta) coloured material.

For revealing total proteins, fixation was satisfactorily achieved by the use of 10% calcium formol. The mercury bromophenol blue of Mazia et al. (1953) was used. The presence of total proteins was reflected by the appearance of bluish colouration.

However, it is well known that both pattern and intensity of developed colouration is exponential with the amounts and varieties of amino acids prevailing in such cases, as marked by Mazia et al. (1953).

3. Results

3.1. General carbohydrates

The digestive gland cells of normally feeding snail *M. cartusiana* are known to comprise two main types: digestive and secretory cells.

3.1.1. The digestive cells

These cells were characterized by the presence of intensively stained coarse carbohydrate granules located mainly in both the apical and basal regions of the cells (Plate I(A)).

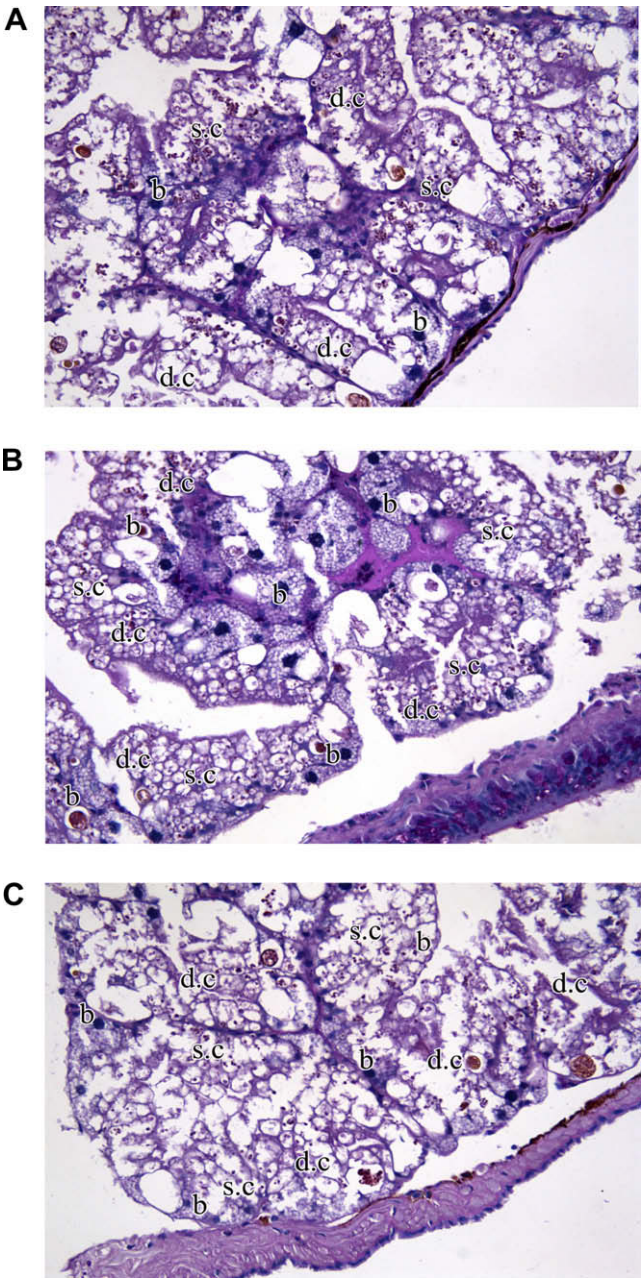

Plate I Periodic acid Schiff's (PAS) preparations of transverse sections of the digestive gland of normally feeding and starved snails *Monacha cartusiana* (40×). (A) Deeply stained coarse carbohydrate granules in the digestive cells (d.c) of normally feeding snails. In the secretory cells (s.c), these elements appear as strongly coloured fine particles. The secretory bodies (b) exhibit a strong reaction with PAS. (B) In starved snails, strongly PAS-positive carbohydrate contents have occurred as coarse granules in the apical parts of the digestive cells (d.c). The basal regions are crowded with moderately coloured fine particles. Moderately stained fine carbohydrate granules are scattered homogeneously in the secretory cells (s.c). (C) In the digestive cells (d.c) of snails starved for 30 days, carbohydrate are visualized as weakly stained fine particles lying in the apical portions, whereas they are moderately coarse in the basal regions. The secretory cells (s.c) are occupied with weakly PAS-reactive fine granules.

The apical portions of the digestive cells of snails starved for 15 days, have almost retained their inclusions of carbohydrate as strongly stained coarse granules, whereas such inclusions appeared as moderately stained fine particles in the basal regions of the cells (Plate I(B)).

On the other hand, after 30 days of starvation the digestive cells of *M. cartusiana* were faintly stained toward PAS technique and carbohydrate inclusions were displayed as moderately coarse granules in the apical portions of the cells and fine particles in the basal ones as illustrated in Plate I(C).

3.1.2. The secretory cells

In case of normally feeding snails, carbohydrate contents were illustrated in these cells taking the form of deeply stained fine particles, which have turned to be fine in structure and moderate in stainability after 15 days of starvation. Reduction in both size and reactivity of those inclusions proceed in a progressive manner to the extent that an almost negative result for PAS was displayed following 30 days of starvation. Nevertheless, there were still many excretory bodies (b) exhibiting deep stainability in the excretory cells as observed in Plate I(A).

3.2. Total protein

3.2.1. Digestive cells

Protein inclusions illustrated in the bromphenol preparations of the digestive cells of normally feeding *M. cartusiana* exhibiting a coarse granular structure with a strong reactivity. these elements were distributed allover the cytoplasm as revealed in Plate II(A).

In snails starved for 15 days, protein particles displayed moderately blue-colouration in the digestive cells (Plate II(B)), while they have become faintly stained after 30 days of fasting (Plate II(C)).

3.2.2. The secretory cells

The secretory cells in the digestive glands of normally feeding animals have exhibited their protein inclusions as deeply stained fine granules scattered homogeneously in the cytoplasm.

Such inclusions appeared moderately coloured in the corresponding cells in the snails starved for 15 days being more fainter or rather negatively stained after 30 days of starvation.

The small excretory bodies in those cells were not stained with mercury bromophenol blue, which indicates their lack of protein substance.

4. Discussion

The land snails are considered as the most injurious of the stylommatophoran species on Egypt. These snails have been steadily increasing in recent years as an abundant agricultural pests in several areas. For this reason, the land snails gained a considerable attention due to their economic importance. In the present study, the positive results obtained regarding the mode of occurrence of carbohydrate inclusions in the digestive gland cells of normally feeding snails are in agreement with the results presented by Abolins-Krogis (1980)

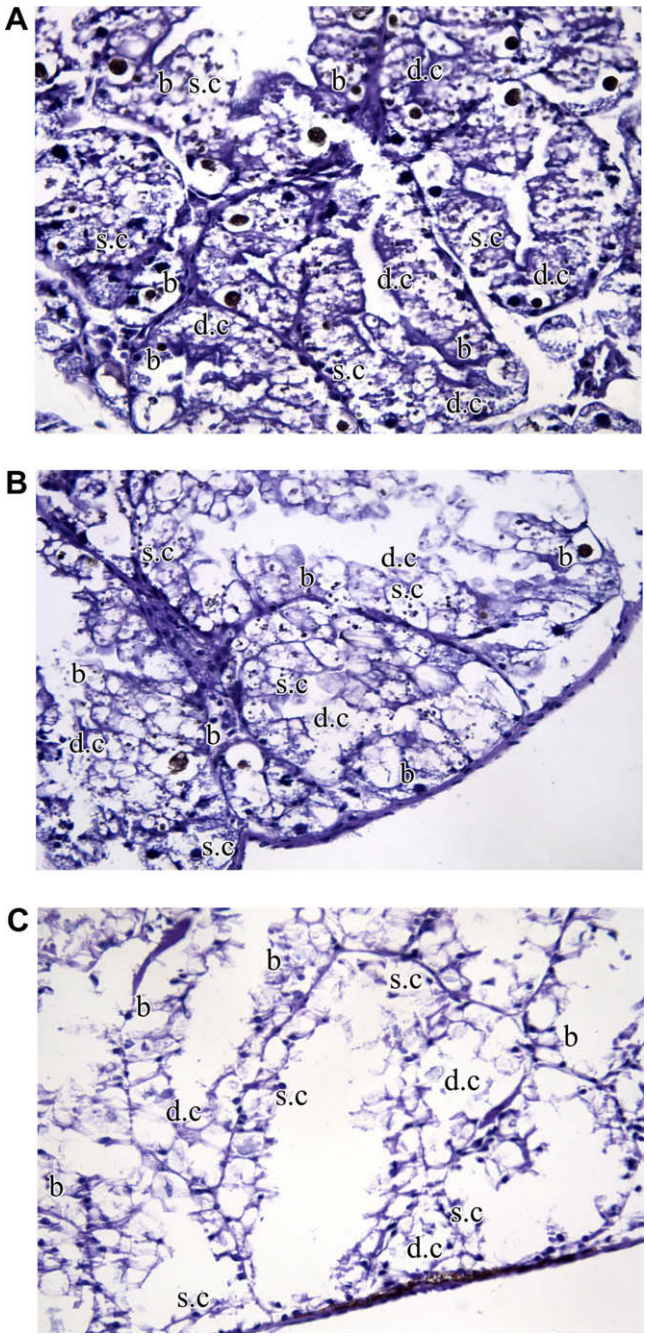

Plate II Bromophenol blue preparations of transverse sections of the digestive gland of normally feeding and fasted snails (40×). (A) Transverse section of the digestive gland revealing deeply stained moderately coarse protein granules in the digestive cells (d.c) of normally feeding snails. These inclusions appear in the secretory cells (s.c) as strongly coloured fine granules. In the secretory bodies (b), these components did not exhibit any reaction with mercuric bromophenol blue. (B) Moderately stained protein inclusions in both digestive (d.c) and secretory cells (s.c) of a snails starved for 15 days, but they have moderately coarse sizes in the digestive cells and fine granulation in the secretory ones. (C) After 30 days of starvation, protein particles exhibit a faint colouration in both the digestive (d.c) and secretory cells (s.c).

and El-Saadany et al. (1994) in their study on the digestive gland of *Helix pomatia* and *E. vermiculata*, respectively.

Also, the observed decline of carbohydrates in the digestive gland of the present snail after starvation conforms the findings of Veldhuijzen and Van Beek (1976) who reported that polysaccharides were markedly decreased in the digestive gland of *Lymnaea stagnalis* after 15 days of starvation. Also these results are in agreement with those obtained by Beddiny (1979) and Saad (1990) as they mentioned that glycogen was seemingly utilized during starvation in the digestive gland of *P. acuta* and *E. vermiculata*, respectively.

Concerning the protein inclusions in the digestive gland of *M. cartusiana*, the strong bromophenol blue positive results designated in case of normally feeding snails support those reported by Abolins-Krogis (1980), Saad (1990) and Walker (1970) in their studies on the digestive glands of *H. pomatia*, *E. vermiculata* and *Agriolimax reticulates*, respectively.

Protein inclusions have exhibited a weak stainability in the digestive gland cells of *M. cartusiana* as a consequence of starvation. The results are supported by the observations declared by El-Emam and Ebeid (1989) in their studies which implied that the reduction of protein contents have taken place in the digestive gland of *B. alexandrina* as a result of starvation. It could be concluded that starvation had an adverse effect on carbohydrate and protein contents of the digestive gland cells in the land snails *M. cartusiana*.

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التغيرات النسيجية الكيميائية لمحتوى الكربوهيدرات و البروتين في خلايا الغدة الهاضمة للقوقع الأرضي *Monacha Cartusiana* بعد التجويع .

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**العنوان الحالي: قسم علم الحيوان – كلية العلوم – جامعة الملك سعود. ص. ب. 2455 . الرياض. المملكة العربية السعودية.*

الملخص :

صممت الدراسة الحالية لفحص و اكتشاف المحتوى الكربوهيدراتي و البروتيني من الناحية النسيجية الكيميائية في القواقع عادية التغذية مقارنة بتلك المعرضة للتجويع لمدة 15 ، 30 يوما. بصفة عامة تم اكتشاف كميات وفيرة من الكربوهيدرات و البروتين في خلايا الغدة الهاضمة للقواقع عادية التغذية. أوضحت نتائج الدراسة الحالية نقصا واضحا في المحتوى الكربوهيدراتي في خلايا الغدة الهاضمة لقوقع *Monacha Cartusiana* بعد التجويع وكان النقص أكثر حدة بعد 30 يوما من التجويع .علاوة على ذلك أظهر المحتوى البروتيني لتلك الخلايا للقوقع تحت الدراسة قابلية ضعيفة للصبغ خاصة بعد التجويع لمدة 30 يوما مما يدل على انخفاض المحتوى البروتيني بوضوح لخلايا الغدة الهاضمة للقوقع.