

HISTOCHEMICAL EFFECT OF 20-HYDROXY ECDYSONE ON THE SECRETORY LOBE CELLS OF THE FOVEAL GLAND IN THE TICK (*Hyalomma (Hyalomma) dromedarii* Koch) (Acari: Ixodoidea : Ixodidae)

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ABSTRACT

The histochemical effect of topical application of 20-Hydroxy Ecdysone (20-HE) to nymphal *Hyalomma (H.) dromedarii* stimulated a marked increase in the content of proteins, lipids and carbohydrates in the secretory cells of the secretory lobe of the foveal gland of female *Hyalomma (H.) dromedarii* in unfed, semi-fed females and fully fed-females. The histochemical changes of content of proteins, lipids and carbohydrates are studied in females treated with 20-Hydroxy Ecdysone and compared to untreated females at different physiological conditions.

INTRODUCTION

The camel tick, *Hyalomma dromedarii* Koch, is widely distributed in desert semidesert, steppe and savannas biotopes of the Polarctic and Ethiopian Regions and in the western zone of the oriental region. In Egypt, *Hyalomma (Hyalomma) dromedarii* Koch is an important pest of lives tock parasitizing chiefly camels. This species transmits the agents of several diseases including the arboviruses of Crimean-Congo haemorrhagic fever, Dera Ghazi Khan (Buny aviridae), Dhor, Tettang (Ungrouped), Kadam (Togaviridae) and Quaranfil (Unclassified), the rickettsiae of *Richjettisia conori* (boutonneuse, fever) and *Coxiella burneti* (Q fever) and the protozoa *Theileria annulata*, *I. dispar*, *Theileria* of sheep and *Ehrelchia bovis* (Barnett, 1977; Hoogstraal, 1956, 1967, 1973, 1975; Smith and Ristic, 1977; Uilenberg, 1976; Wood *et al.*, 1982).

The foveal glands serve as the female sex pheromone glands in *Dermacentor variabilis* (Say) and *Dermacentor andersoni* Stiles (Sonenshine *et al.*, 1977). Foveal glands also occur in the camel tick, *Hyalomma (Hyalomma) dromedarii* Koch (Marzouk, 1992).

No available literatures have been found in the recent years concerning this subject.

Exogenous ecdysteroids may have a variety of effects on arthropods depending on the concentrations used and, on the route of application (Ruscoe, 1974; Mkhize and Gupta, 1980; Philips *et al.*, 1996). In ticks, ecdysteroids were reported to break larval diapause (Wright, 1969; Sannasi and subramoniam, 1972), cause high mortality (Mansingh and Rawlins, 1977), influence moulting (Mango, 1979) and supermoulting (Kitaoka, 1972). Exogenous ecdysteroids were also found to influences oogenesis (Solomon *et al.*, 1982; Connat *et al.*, 1983; Khalil *et al.*, 1984) Salivary glands development (Harris and Kaufman, 1981; 1985; Kaufman, 1984; Lindsay and Kaufman, 1988; Shelby *et al.*, 1989), cuticle formation (Germond *et al.*, 1982;

Diehl *et al.*, 1983), neurosecretory cells activity (Khalil, *et al.*, 1988; Marzouk, 1988) and sex pheromone activity (Dees *et al.*, 1984a,b).

This study was undertaken to determine the histochemical effect of 20-HE on the secretory cells of the secretory lobe of the foveal gland of female *H. dromedarii* at different physiological conditions.

MATERIALS AND METHODS

H. dromedarii colony originated from engorged females collected from camels in the Imbaba camel market, Giza Governorate, Egypt. The Ticks were colonized at $28 \pm 1^\circ\text{C}$ and 75% relative humidity. The ticks were fed on the rabbits *Oryctolagus cuniculus* using the method of Berger *et al.* (1971). Unfed females 0, 3, 5 and 7 days after moulting referred to herein after as odm female, 3dm female, and 7dm female respectively and semifed females (5 days post-attachment) and fully-fed females on the day of engorgement and detachment from the host were examined. For histological examination, unfed females were dissected in 0.7% saline solution under a stereoscopic binocular microscope (Wild M8 type). The ticks were fixed in a Bouin fixative (Humason, 1962) for 24 hours, dehydrated in an ascending series of ethyl alcohol, transferred to methyl benzoate for 24 hours, placed in a solution of 2% celloidin in methyl benzoate, cleared in benzoyl for 2 min. and embedded in paraplast. Serial sections, 5-7 μm thick, stained with Gomori's (1941) chrome haematoxylin phloxine stain (CHP) were examined. For histochemical examination, proteins, freshly dissected ticks were fixed in carony fixative for 5-10 minutes (Gatenby and Beams, 1950) and stained using Mercury Bromphenol Blue (Hg-BPB), method (Chapman, 1975). To detect lipids and glycogen, freshly dissected ticks were fixed in modified, formalin fixative for 24 hours (Barker, 1958). For glycogen demonstration, sections were stained using Periodic Acid-Schiff, (PAS) stain (Lillie, 1951). For detecting lipids, sections were stained with Sudan Black (SB) (Chiffelle and Putt, 1951) stain. In treated ticks, 20-Hydroxy Ecdysone (20-HE) (Sigma Chemical Co., St., Louis, Missouri, U.S.A.) was dissolved in absolute ethanol and applied topically in aliquots of 1 μl /tick to nymphs on dropping day. The dose applied was 20 $\mu\text{g}/1 \text{ ul}/\text{tick}$ (Dees *et al.*, 1985). Five to 10 specimens of each stage were examined treated and control.

RESULTS

The foveal glands of female *H. dromedarii* are located beneath the dorsum, posterior to the scutum. The foveal glands secretory lobe zone appear as rosette like clusters of 15-21 bulbous lobes of varying sizes formed of large secretory cells intermingled with small support cells (Fig. 1).

The large basally located secretory cells possess highly vacuolated cytoplasm containing finely granular distributed material and their nuclei contain irregularly distributed, coarse chromatin granules with one or two nucleoli (Fig. 1). The support cells are spindle shaped with their lower ends

embedded among the secretory cells (Fig. 1). The secretory lobe zone is connected via ducts to ampullae. The ampullae link pore tubes from the external fovea and ducts from the secretory lobes (Fig. 1).

In the present study, histochemical sections passing through the secretory lobe zone of the foveal glands of unfed, semi-fed and fully-fed females *H.dromedarii* were examined after treatment with 20-HE and compared to untreated females at the same physiological conditions to study proteins, lipids and carbohydrates content (Fig. 2-8).

Proteins:

Positive reaction to general proteins stain in treated females increased from unfed female to semi-fed and fully-fed females.

However, when compared with untreated females, the proteins in the cytoplasm of secretory cells of the secretory lobe of treated unfed, semi-fed and fully-fed females showed an intense staining reactivity. This is indicated by a darker blue colour using Hg-BPB stain (Figs. 2,3 and 4).

Lipids

Lipid content detected by positive reaction to SB in the cytoplasm of the secretory cells in treated females increased from unfed females, to semi-fed and fully-fed females. However, when compared with the untreated females, the reactivity for lipid droplets detectable in the cytoplasm of the secretory cells of foveal glands in treated female exhibited a stronger reaction in unfed, semi-fed and fully-fed females as indicated by a deeper staining affinity (Figs. 5,6 and 7).

Carbohydrates:

The carbohydrates detected in the cytoplasm of the secretory lobe in treated unfed females using PAS staining exhibited a denser staining affinity in the secretory cells of foveal glands. A similar dense reactivity was also demonstrated in treated semi-fed and fully-fed females. However, when compared with untreated females, the PAS reactivity in the cytoplasm of cells in treated females showed a stronger reaction, as indicated by a deeper purplish red stain (Fig. 8).

**Histochemical Effect of 20-Hydroxy Ecdysone on Ticks
Plate I**

- Fig. (1):** Transverse section in the foveal gland (FG) of female *H.dromedarri*. showing the secretory cells (S) connected Via ducts (U) to pore tubes (P) from the external fovea. A (ampullae), D (dorsum), FD (fovea dorsales) and O (support cells). (CHP, 250X).
- Figs. (2,3):** Transverse sections passing through the foveal glands of 7dm female *H.dromedarii* in untreated and treated ticks respectively showing secretory cells (S) stained with Hg-BPB. D (dorsum). (400 x).
- Fig. (4) :** Transverse section passing through the foveal glands of semi-fed female *H. dromedarii* showing secretory cells (S)

stained with Hg-BPB. D (dorsum) and FD (fovea dorsales)
(100x).

**Histochemical Effect of 20-Hydroxy Ecdysone on Ticks
Plate II**

Figs. 5-7: Transverse sections passing through the foveal glands of untreated and treated *H. dromedarii* respectively showing the secretory cells (S) stained with SB. D (dorsum), FD (Fovea dorsales) and P (Pore tubes). (Figs. 5 and 6) odm female. (Fig. 7) 7 dm female. (250 x).

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Fig. (8) : Transverse section passing through the foveal glands of treated odm female *H. dromedarii* showing secretory cells (S) stained with PAS. (400 x)

DISCUSSION

The natural occurrence of steroid hormones ecdysone and 20-HE was found in several species of ixodid and argasid ticks including *Amblyomma hebraeum*, *Ornithodoros moubata*, *Rhipicephalus appendiculatus*, *Dermacentor variabilis* and *H.dromedarii* (Delbecque *et al.*, 1978; Germond *et al.*, 1982; Solomon *et al.*, 1982; Dees *et al.*, 1984b).

The exogenous dose of 20-HE applied to *H.dromedarii* nymphs and studied in unfed, semi-fed and fully-fed females was not found to alter the normal developmental pattern of the foveal gland's secretory lobe responsible for the secretion of the sex pheromone (Marzouk, 1992)

In the present study, generally proteins, lipids and carbohydrates content increased in the secretory cells of the secretory lobe of the foveal gland in treated unfed, semi-fed and fully-fed females. However, when compared with untreated ticks, 20-HE was found to stimulate a marked increase in the content of proteins, lipids and carbohydrates in secretory cells of the secretory lobe of the foveal glands of the treated females. It is therefore, possible to suggest the application of 20-HE may stimulate increased synthesis of protein. The activation may further induce increased production of secretory proteins, lipids and carbohydrates, necessary for the secretion of sex pheromone 20-HE may control on some cellular activities in the developing oogenesis. Ecdysteroids probably act directly in the form of an ecdysteroid receptor complex on the genetic material. In most cases, the hormone increases the rate of biosynthesis of protein (Gadallah *et al.*, 1990). Lipids provide a source of energy, essential components of the cell membrane and have predominant, roles in controlling oogenesis, embryogenesis, growth and morphogenesis (Gilbert *et al.*, 1977).

Dees *et al.* (1985) reported increases in ecdysteroids concentration to occur normally in *H.dromedarii* during the first few days following female enclosion indicating a regulatory role for stimulation of sex pheromone production. It is therefore possible to suggest that in the present study the application of an external dose of 20-HE stimulated increased cellular activity in the unfed female which may further induce an increase in the production of proteins, lipids and carbohydrates in the secretory material of the secretory lobe of the foveal gland.

Also, since Dee *et al.* (1984 a,b, 1985) found that the greatest increase in all ecdysteroids in *H.dromedarii* occurred following mating and during repletion. Therefore, it is possible to suggest that in the present study the application of an external dose of 20-HE mimics the natural increase in ecdysteroids in feeding and fully-fed females leading to increased production of proteins, lipids and carbohydrates in the cells of the foveal gland.

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**الأثر الهيستوكيميائي لاستعمال هرمون ٢٠- هيدروكسي ايكدايسون على الخلايا المفترزة في الغدة النقرية في القراد من نوع هيالوما (هيالوما) دروميدرياي. زكية عيسى عشموي درويش
قسم علم الحيوان- كلية العلوم- جامعة الأزهر**

تمت معالجة الحوريات في القراد من نوع هيالوما دروميدرياي بجرعة من هرمون ٢٠- هيدروكسي ايكدايسون وتتبع الأثر الهيستوكيميائي لهذه الجرعة على الخلايا المفترزة في الغدة النقرية في الأنثى المعالجة ومقارنتها مع الأنثى غير المعالجة.

وقد وجد أن هذه الجرعة قد أدت إلى زيادة ملحوظة في كمية كلا من البروتين والدهون والكربوهيدرات مما يدل على ازدياد النشاط الإفرازي في الخلايا المفترزة في الأنثى غير المغتذية والنصف مغتذية وكذلك في تلك المغتذية اغتذاءً كاملاً. وقد لوحظ كذلك زيادة ملحوظة في كمية كلا من البروتين والدهون والكربوهيدرات في الخلايا المفترزة للأنثى المعالجة بعد مقارنتها بتلك الغير المعالجة في كلا من الأنثى غير المغتذية والأنثى النصف مغتذية والمغتذية اغتذاءً كاملاً.