# HISTOLOGY AND DEVELOPMENT OF ARGAS (ARGAS) HERMANNI (IXODOIDEA: ARGASIDAE) SALIVARY GLANDS

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# التركيب النسيجي وتطور الغدد اللعابية في أرجاس (أرجاس) هرماناي (القراديات: القراد الطري)

جلیلة خلیل و علیة مرزوق محمود مهلل و حصة آل ثانی

تمت دراسة التركيب التشريحي والنسيجي للغدد اللعابية في أرجاس (أرجاس) هرماناي أثناء نمو طوري الحورية والحيوان البالغ. وتتكون الغدد اللعابية من نوعين من الحجيرات ، تتكون الأولى منها من عدة خلايا في منطقة حافية ذات خطوط مميزة تحيط بخلية مركزية . ولا تظهر في هذا النوع من الحجيرات تغيرات نسيجية أثناء نمو القراد أو بعد أن يغتذي ، ولكن ينقص حجمها بعد الاغتذاء ثم يزيد أثناء فترة النمو . وعلى الأرجح فإن نشاط هذا النوع من الحجيرات ذو علاقة بالتنظيم الأسموزي ونقل السوائل. ويتكون النوع الثاني من الحجيرات من سبعة أنواع وتحت أنواع من الخلايا التي تحتوي على كريات إفرازية ذات أحجام وأشكال وصفات صبغية مختلفة . وينقص حجم هذه الحجيرات نقصاً كبيراً بعد الاغتذاء مباشرة ثم يزيد أثناء فترة النمو التالية . وقد لوحظ أن الخلايا من النوع أ, و أ, و ب لا تتغير كثيراً بعد الاغتذاء بينما تلك من النوع ج و د و هـ, و هـ ب تفقد معظم أو كل محتوياتها من الكريات الإفرازية . ومن المحتمل أن الخلايا من نوع أى تختزن مواداً إفرازية أولية بحيث تحل هذه الخلايا محل نوع (أو أكثر) من الضلايا الأخرى التي فقدت محتوياتها أثناء الاغتذاء. أما الفجوات التي تظهر في الضلايا من نوعى أن و ب بعد الاغتذاء فربما تمثل مركبات ذائبة ناتجة من تغير في تركيب بعض محتويات هذه الخلايا أو تمثل مناطق خارج الخلايا يحيطها غشاء رقيق بعد أن أفرزت الخلايا بعض محتوياتها . وعلى الأرجح فإن التركيب الكيميائي للكريات الافرازية يتغير قبل الاغتذاء بوقت طويل في الضلايا من النوع د بينما يتغير أثناء الاغتذاء في الخلايا من النوعين هر وهر وأثناء فترة النمو بعد الاغتذاء يتم إحلال خلايا جديدة بدلاً من تلك التي فقدت محتويات تماماً أثناء الاغتذاء (ج و د و هـ ، ، هـ ) أو يتم تخليق كريات إفرازية جديدة في نفس الخلايا التي فقدت محتوياتها .

Key Words: Argasidae, Argas hermanni, development, histology, Ixodoidea, salivary glands

#### **ABSTRACT**

The anatomy and histology of *Argas* (*Argas*) *hermanni* salivary glands were studied during nymphal and adult development. The paired salivary glands consist of 2 alveolar types. Type I alveoli consist of several cells forming a striated peripheral zone around a clear central cell. These alveoli do not exhibit significant histological changes during development or after feeding but their size decreases after feeding and increases during the postfeeding period. The activity of this type is probably related to osmoregulation and fluid transport by the glands. Type II alveoli consist of 7 cell types and subtypes containing globules of various staining reactions,

sizes, and/or shape. The alveolar size decreases after feeding and increases during the postfeeding period. After feeding, cell types a<sub>1</sub>, a<sub>2</sub> and b do not exhibit great changes, while types c, d, e<sub>1</sub> and e<sub>2</sub> lose most or all of their secretary globules. Type a<sub>1</sub> cells probably store a precursor material to replace a certain cell type(s) depleted during feeding. Vacuoles in type a<sub>2</sub> and b cells may represent soluble metabolites or extracellular spaces after apocrine secretion of some globules. The globule chemical structure probably changes before feeding in type d and during feeding in type e<sub>1</sub> and e<sub>2</sub> cells. Cells depleted during feeding are replaced by new cells and/or new globules are synthesized in depleted cells during the postfeeding period.

#### INTRODUCTION

Tick salivary glands are considered to be one of the most suitable sites for multiplication of micro-organisms transmitted during tick feeding and causing diseases of man and animals. In this study, we investigate the anatomy and histology of Argas (Argas) hermanni Audouin salivary glands during nymphal and adult development.

#### MATERIALS AND METHODS

An A. hermanni colony, originating from ticks collected from a domestic pigeon (Columbia livia) house in Suez, Egypt, was maintained in an incubator at 28+1°C and 75% RH and domestic pigeon were used as hosts.

The salivary glands of unfed first-  $(N_1)$  and second- instar  $(N_2)$  nymphs, 2 weeks (2WN) and 6 weeks (6WN) postmoulting, of adult males and females, 1 month (1M) and 2 months (2M) postmoulting, and of fed  $N_1$   $(FN_1)$ ,  $N_2$   $(FN_2)$ , males and females within 2 hr postfeeding were investigated. The dissected tick was flooded with 0.7% saline solution to which a drop of methylene blue was added to obviate the anatomy of the glands and their relationship with the other organs.

For histological examination, the dorsal cuticle was removed and the tick was fixed in Zenker-formol fixative. After dehydration in an ascending series of ethyl alcohol and double embedding in celloidin-paraplast, serial sections, 5-7 um thick, were prepared and stained with Harries, haematoxylin and eosin.

Surface areas of nuclei, cells and alveoli (referred to hereafter as size) were calculated as described by Marzouk *et al.* (1987). The means and standard errors were calculated for the size of each cell and alveolar type in all examined stages, and the data were compared using Student's *t*-test. When no significant difference was observed (P>0.05) between the nuclear dimension of a certain cell type within a certain stage, the mean and standard error was calculated for all the nuclei in that stage.

#### **RESULTS**

#### Anatomy

The paired salivary glands are anatomically similar in males, females and nymphs. They lie in the ventrolateral aspect of the body cavity and extend from near the capitular foramen anteriorly to the level of the third leg coxa posteriorly. Each gland consists of a translucent, grapelike cluster of alveoli. Methylene blue obviates 2 types of alveoli; type I stain deeply and form a narrow mass extending along the medial side of the anterior two thirds of the gland (Fig. 1) and type II stain light blue and constitute the rest of the gland.

The 2 main ducts traverse the entire length of the gland, emerge from the anterior end of each gland, pass through the capitular foramen on both sides of the pharynx and open posterolaterally into the salivarium. Type I alveoli open into short alveolar ducts which connect directly with the main duct (Fig. 2). Type II alveoli open into short alveolar ducts, 2 or

more of which usually unite into one common lobular duct that connects with the main duct (Fig. 3).

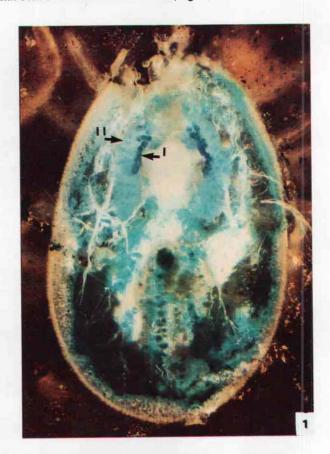


Fig. 1: Dissected A. hermanni showing the salivary glands with type I (deep blue) and II (light blue) alveoli (X 32).

## Histology

The salivary duct and type I and II alveolar structure is similar in all the examined unfed stages.

Salivary ducts: The main duct wall consists of a 1-cell-thick epithelial layer lined with a cuticle possessing spiral thickenings (Fig. 2). The boundaries between the cells are indiscernible and their vesicular nuclei are rounded or oval. The lobular and alveolar ducts are narrower but structurally similar to the main duct (Fig. 3). The ducts in 6WN<sub>1</sub>, 2WN<sub>2</sub>, 6WN<sub>2</sub>, 1M and 2M adults may contain fine grayish purple granules while after feeding purple granules may be observed.

Type I alveoli: Each alveolus consists of several cells with indiscernible boundaries, resting on a delicate basement membrane (Fig. 4). These cells form a peripheral zone with striations almost perpendicular to the alveolar boundary where numerous very fine granules occur. In this zone, there are 6-9 small, vesicular, rounded to oval nuclei, with chromatin lumps adhering to the nuclear membrane. Only one large oval, vesicular, nucleus with coarse chromatin granules adhering to the nuclear membrane occurs in he central clear zone.

Type II alveoli: These alveoli have a circular or oval outline and consist of 5-12 cells surrounding a central lumen and lying on a fine basement membrane. These cells appear mostly triangular in outline with the triangle base at the alveolar periphery. Between the basal parts of these cells lie small interstitial cells with numerous fine processes extending to the alveolar lumen (Fig. 5). The cuticular lining of the alveolar ducts extends into the alveolus to form a valve-like structure at the alveolar base (Fig. 6). Except for the interstitial cells, the cells in these alveoli contain secretary globules. According to the staining reaction, size and/or shape of these globules, 5 main cell types are distinguished.

**Type a cells:** These cells are close to the alveolar duct and contain rounded, bright red, globules (Figs. 7, 8). Two subtypes may be distinguished, type  $a_1$  with small globules, 0.4-1.5 um in diameter, and type  $a_2$  with large, more or less rounded globules, 1.8-3.8 um in diameter. The nuclei are vesicular, rounded, and usually lie near the basal part of the cells.

**Type b cells:** These cells are usually near the alveolar duct. They are packed with cup-shaped purple globules (Figs. 7,8) 1.0-4.6 um in diameter. The nuclei are rounded, compact and eccentric.

**Type c cells:** These cells usually lie away from the alveolar duct. They are triangular or oval, and contain rounded globules, 1.5-3.0 um in diameter. The globules posses a translucent peripheral zone, which may be colourless, purple or bluish purple, and a deep purplish blue central core, 0.4-1.5 um in diameter (Fig. 9). The globules are sometimes so closely packed together that their boundaries become indiscernible. The nuclei are rounded, compact and basally located.

Type d cells: When more than one of these cells are present in one alveolus, one may occur near the alveolar duct and the others among the other cell types. They contain more or less rounded globules, 2.3-6.1 um in diameter, which do not stain with haematoxylin or eosin (Figs. 8,9). The globule contents appear homogeneous and the interglobular spaces contain a finely granular, weakly basophilic cytoplasm. The nuclei are rounded, vesicular, and basally located.

**Type e cells:** These cells lie usually away from the alveolar duct. They contain blue or bluish-purple globules. Two subtypes may be distinguished according to the globule size (Figs. 7,9). In type e<sub>1</sub>, the globules are small, more or less rounded, measuring 1.0-3.8 um in diameter. In type e<sub>2</sub>, globules are large, usually with an irregular outline, measuring 2.0-3.0 x 7.6-8.0 um in their largest dimensions. The nuclei in both subtypes are rounded, vesicular, and may be centrally or basically located.

### Developmental changes in the salivary glands:

The salivary gland length is ca 0.694 mm in unfed  $N_1$ , 0.930 mm in unfed  $N_2$  and 1.256 mm in unfed adults, the increase in gland length during growth from one stage to the following one being about X1.3.

**Type I alveoli:** These alveoli do not show significant histological changes but exhibit a distinct pattern of change in size during nymphal and adult development (Table 1). During the starvation period, no change (P>0.05) occurs in their dimensions, but after feeding their size decreases (P<0.01). However, the alveoli are larger (P<0.001) in unfed  $N_2$  than in unfed  $N_1$ , and larger (P<0.01) in unfed adults than in unfed

N<sub>2</sub>. The alveolar size is similar (P>0.05) in males and females.

The striated zone thickness remains unchanged (P>0.05) during nymphal development, but is greater (P<0.01) in adults than in nymphs (Table 2). Both the large and small nuclei do not exhibit a significant change (P>0.05) in size throughout nymphal and adult development (Table 2).

**Type II alveoli:** These alveoli exhibit a similar pattern of change in size during  $N_1$ ,  $N_2$  and female development (Table 1); their size does not change significantly (P>0.05) during the starvation period but decreases (P<0.01) after feeding. However, the alveoli are similar (P>0.05) in size in 1 M males and fed  $N_2$ , enlarge (P<0.01) in 2 M males and decrease greatly (P<0.01) in size after the males feed. The alveoli are larger (P<0.01) in unfed  $N_2$  than in unfed  $N_1$ , larger (P<0.01) in unfed females than in unfed  $N_2$ , and larger (P<0.01) in females than in the corresponding male stages.

All cell types described above are observed in all unfed and fed stages. In all unfed stages, an average number of 1 or 2 type  $a_1$ ,  $a_2$  and b cells and 2-5 type  $e_1$  and  $e_2$  cells occur in each alveolus. However, some alveoli may contain either subtype  $e_1$  or  $e_2$  while others may contain both subtypes. Some alveoli may contain 1-3 type c and d cells while others may lack either or both types. In fed ticks, certain cell types exhibit a change in size, structure and/or number (Tables 3-6). No change (P>0.05) in the nuclear size or appearance in any of the cell types was observed throughout the tick development.

Table 1
Changes in salivary alveoli size during
Argas hermanni development

	Mean dimensions of alveoli (um) $\pm$ S.E. (range)					
stage	Туре І	Type II				
First-instar nymphs:						
2-week-unfed	20.8±3.73x26.8±3.79a* (14.0-26.0x18,0-34.0)	38.4±5.87x45.1±5.21 g (28.0-50.0x38.0-58.0)				
6-week-unfed	21.6±2.48x28.7±3.51 a (18.0-26.0x24.0-36.0)	37.7±5.23x44.2±3.53 g (22.0-38.0x34.0-48.0)				
Fed '	21.2±3.92x25.6±2.80 b (16.0-26.0x22.0-32.0)	29.9±3.87x37.8±4.81 h (24.0-38.0x30.0-50.0)				
Second-instar ny	mphs:					
2-week-unfed	25.5±3.07x34.3±4.89 c (20.0-30.0x26.0-42.0)	43.2±5.86x49.7±6.82 i (34.0-58.0x36.0-62.0)				
6-week-unfed	26.2±4.71x35.6±4.74 c (18.0-38.0x28.0-46.0)	41.3±4.43x47.6±6.42 i (32.0-48.0x40.0-62.0)				
Fed	23.4±5,18x31.3±3.35 d (16.0-36.0x26.0-38.0)	35.3±4.92x44.6±5.09 j (26.0-46.0x36.0-56.0)				
Males:	•					
1-month-unfed	28.7±5.86x39.8±6.18 e (18.0-40.0x30,0-54.0)	36.2±5.08x44.9±4.33 j (30.0-48.0x34.0-52.0)				
2-month-unfed	28.1±3.81x40.8±3.84 e (22.0-34.0x36,0-48.0)	42.8±6.62x51.6±6.08 k (32.0-62,0x42.0-64,0)				
Fed	26.7±4.13x36.7±4.50 f (24.0-40.0x34.0-54.0)	35.1±5.30x41.8±3.99 m (26.0-44,0x34.0-48.0)				
Females:	(2 (210/12 //2 0 //0)	(200 4100010 10.0)				
1-month-unfed	31.7±3.67x43.1±4.05 e (26.0-38,0x38,052.0)	46.3±5.29x60.8±7.93 n (34.0-56.0x42.0-72.0)				
2-month-unfed	30.0±4.00x42.7±4.51 e (24.0-40.0x36.0-50.0)	46.0±4.35x60.9±5.35 n (38.0-56.0x48.0-70.0)				
Fed	28.1±4.94x39.1±6.70 f (20.0-40.0x26.0-52.0)	39.5±6.09x46.2±6.55 p (30.0-52.0x38.0-60.0)				

<sup>\*</sup> Figures followed by similar letters are not significantly different (P>0.05); those followed by different letters are stastically different (P<0.05 - P<0.001).

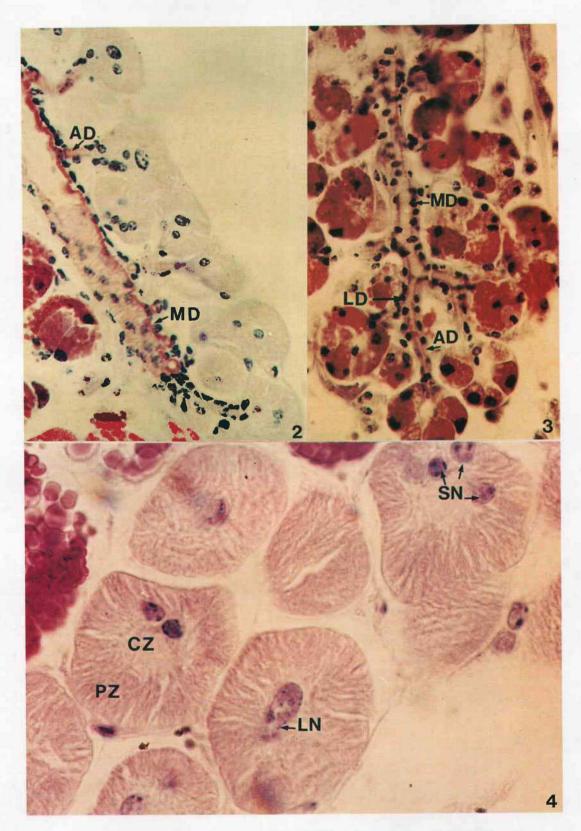


Fig. 2: L. S. in A. hermanni salivary gland showing type I alveolus opening into an alveolar duct (AD) connecting directly with the main salivary duct (MD) which is lined with cuticle possessing spiral thickenings (X 551).

Fig. 3: Section in A. hermanni salivary gland showing main (MD), lobular (LD) and alveolar (AD) ducts (X 551).

Fig. 4: Section in A. hermanni type I alveoli showing the large (LN) and small (SN) nuclei and the central (CZ) and peripheral (PZ) zones (X 1377).

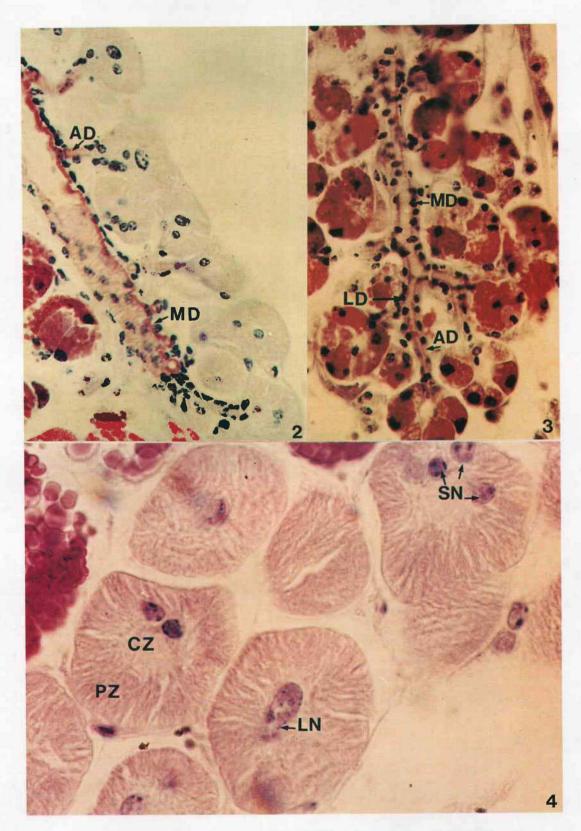
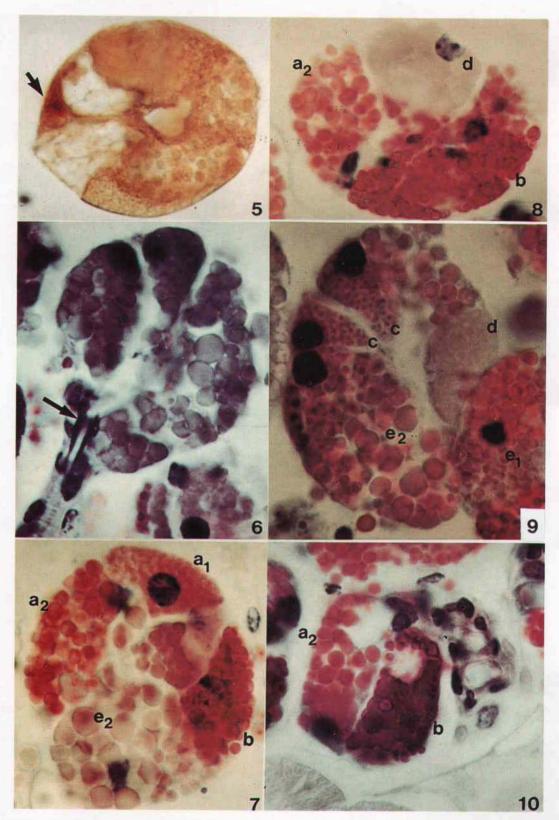


Fig. 2: L. S. in A. hermanni salivary gland showing type I alveolus opening into an alveolar duct (AD) connecting directly with the main salivary duct (MD) which is lined with cuticle possessing spiral thickenings (X 551).

Fig. 3: Section in A. hermanni salivary gland showing main (MD), lobular (LD) and alveolar (AD) ducts (X 551).

Fig. 4: Section in A. hermanni type I alveoli showing the large (LN) and small (SN) nuclei and the central (CZ) and peripheral (PZ) zones (X 1377).



Figs. 5-9: Sections in type II alveoli in unfed *A. hermanni* (X 1385) showing (5) interstitial cell (arrow) (osmic acid) (6) valve-like structure (arrow) at the junction with the alveolar duct, (7) cells type a<sub>1</sub>, a<sub>2</sub>, b and e<sub>2</sub>, (8) cells type a<sub>2</sub>, b and d, and (9) cells type c, d, e<sub>1</sub> and e<sub>2</sub>.

Fig. 10: Section in A. hermanni type II alveolus after feeding showing vacuoles in type a2 and d cells (X 1385).

Table2
Changes in striated zone (SZ) thickness and nuclear dimensions in type I salivary alveoli during Argas hermanni development

	Mean SZ thickne	ess	Mean nuclear dimensions (um) ± S.E. (range)		
Stage	(um)±SE range	Small			
N <sub>1</sub> *	8.0±2.58 a **	5.0±1.15 c	6.2±1.47x11.1±1.68 d		
•	(2.0-11.5)	(4.0-9.3)	(4.0-9.9x8.1-12.0)		
N <sub>2</sub>	7.5±3.16 a	5.0±1.41 c	6.3±1.67x11.8±1.67 d		
-	(2.0-12.1)	(4.1-8.2)	(4.1-10.0x9.8-13.8)		
Male	11.3+3.16 b	4.9±1.00 c	6.7±1.50x12.3±1.38 d		
	(4.2-16.9)	(4.0-7.9)	(4.5-9.8x10.1-14.2)		
Female	11.2+3.85 b	5.1±1.71 c	7.3±1.63x12.3±1.67 d		
	(4.1-17.7)	(4.2-8.1)	(6.0-10.0x10.0-15.7)		

<sup>\*</sup> N<sub>1</sub>=first-instar nymph, N<sub>1</sub>=second-instar nymph

**Type a<sub>1</sub> cells:** Type  $a_1$  cells do not change (P>0.05) in size during  $N_1$  starvation period but decrease (P<0.01) in size after  $N_1$  feeds (Table 3). No change (P>0.05) occurs in cell size during  $N_1$  postfeeding period and in  $2WN_2$ . However, these cells enlarge (P<0.01) in  $6WN_2$  (Table 4), do not change (P>0.05) in size after  $N_2$  feeds or in 1M males (Table 5), and enlarge in 2 M males. Type  $a_1$  cells are larger (P<0.01) in 1M females than in fed  $N_2$  but they do not change is size in 2 M females (Table 6). In both males and females, no change (P>0.05) occurs in cell size after feeding. Also, no change in shape or average type  $a_1$  cell number in each alveolus, or in secretory globule appearance occurs throughout nymphal and adult development. The mean diameter of their nuclei is  $6.1\pm0.02$  um (range 5.8 - 6.4 um).

**Type a<sub>2</sub> cells:** Type  $a_2$  cells increase (P<0.01) in size only during  $N_1$  prefeeding period (Table 3) and no change (P>0.05) in size occurs after  $N_1,N_2$  and adults feed (Tables 3-6). However, these cells are larger (P<0.01) in 1M females than in  $N_2$  (Tables 4,6). In all stages, the average number of these cells in each alveolus remains unchanged after feeding. Also, their globules do not exhibit a change in appearance before or after feeding but a large vacuole may appear in these cells after feeding (Fig. 10). The mean diameter of their nuclei is  $5.9\pm0.01$  um (range 5.8-6.0 um).

**Type b cells:** Type b cells do not change significantly (P>0.05) in size during development within each stage (Tables 3-6). However, they are larger (P<0.01) in  $N_2$  than in  $N_1$  and in females than in  $N_2$ , but their size is similar (P>0.05) in males and  $N_2$ . Although no change in average cell number per alveolus or in globule appearance occurs after nymphs and adults feed, a vacuole may appear in some of these cells (Fig. 10). The mean diameter of their nuclei is  $5.3\pm1.03$  um (range 4.0-6.1 um).

**Type c cells:** Type c cells vary greatly in size within each stage examined. However, their mean size does not change during  $N_1$  and  $N_2$  starvation period (Tables 3, 4), but they are larger (P<0.01) in  $N_2$  than in  $N_1$ . Their size is similar (P>0.05) in 1 M adults and  $N_2$  but is greater in 2M than in 1M adults (Tables 5, 6). After nymphs and adults feed, only one type c

cell may be observed in a few alveoli. However, in  $N_1$  and  $N_2$ , the discernible type c cells do not exhibit a significant change (P>0.05) in size (Tables 3, 4), while in adults they decrease (P<0.01) in size after feeding (Tables 5,6). In all fed stages, the globules in the discernible cells do not change in appearance. The mean diameter of their nuclei is  $6.6\pm1.2$  um (range 4.0-8.1 um).

Type d cells: Type d cells do not change (P>0.05) in size during the nymphal and adult starvation period (Tables 3-6). In unfed  $N_1$ ,  $N_2$  and males, their size is similar (P>0.05) but is smaller (P<0.01) than in females. After nymphs and adults feed, a few smaller (P>0.05) cells of this type are discernible in only few alveoli.

While in  $2WN_1$  the globule content appears nearly colourless and homogeneous, weakly basophilic granules may appear inside and between the globules in some cells in  $6WN_1$ ,  $2WN_2$ , and 1M and 2M adults (Fig. 11). However, in fed ticks the few discernible cells do not contain such granules but may contain a large vacuole. The mean diameter of their nuclei is  $5.3 \pm 1.03$  um (range 4.1 - 5.9 um).

Type e<sub>1</sub> cells: Type e<sub>1</sub> cells do not change (P>0.05) in size and their globules do not exhibit marked changes in appearance during the starvation period of any of the examined stages (Tables 3-6). Also, these cells are similar in size (P>0.05) in all unfed stages. However, after nymphal and adult feeding they decrease (P<0.01) in size. A vacuole or coarse granules may replace most of the globules (Figs. 12, 13) and only 1 or 2 cells of this type may be observed in most alveoli. The mean nuclear diameter of this type is 4.7±1.03 um (4.1-6.2 um).

Type  $e_2$  cells: Type  $e_2$  cells do not change (P>0.05) in size during the starvation period in all stages (Tables 3-6). However, those in  $N_2$  are larger (P<0.01) than those in  $N_2$ , similar (P>0.05) in size to those in males, and smaller (P<0.01) than those in females.

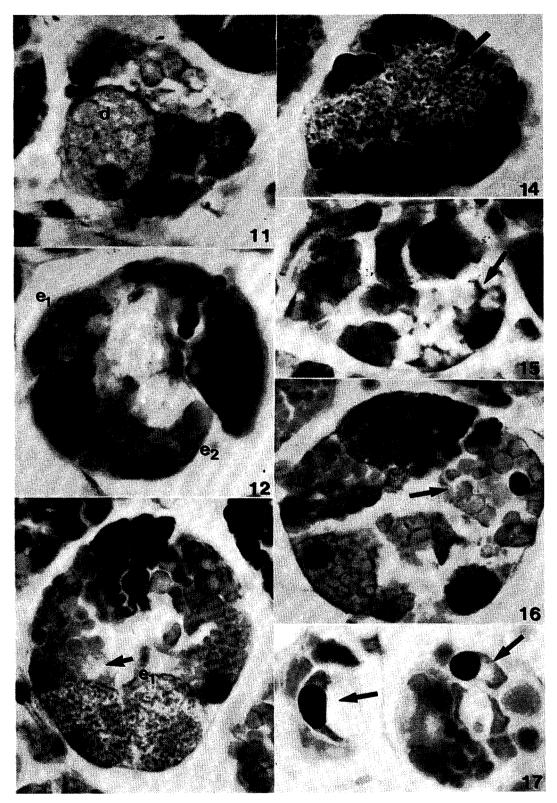
In  $2WN_1$ , type  $e_2$  cells contain only globules, while in  $6WN_1$ , coarse granules may replace most of the globules in some cells, and may occur inside the globules in others (Fig. 14). After feeding, the granules replace most or all of the globules in numerous cells. Other type  $e_2$  cells may contain only few typical globules and a large vacuole (Fig. 12) or empty-looking globules (Fig. 15). However, all these cells as well as those containing typical globules are similar in size to those in unfed  $N_1$ .

In  $2WN_2$  and in 1M adults, most type  $e_2$  cells contain typical globules, while a few cells contain the empty-looking globules or coarse granules inside and between the globules. Such cells are not observed in  $6WN_2$  and 2M adults in which all type  $e_2$  cells contain only typical globules. However, the globules may stain differently in the same or different cells in unfed  $N_2$  and adults, being light purple or bluish purple (Fig. 16).

After  $N_2$  and adults feed, many type  $e_2$  cells similar to those observed in fed  $N_1$  are observed with no change (P>0.05) in cell size after feeding (Tables 4-6). However, in all examined stages, only 1-3 cells may be observed in each alveolus. The mean nuclear diameter in this type is  $6.9 \pm 1.07$  um (range 6.1 - 10.1 um).

In both nymphs and adults, many small cells measuring  $10.1 \pm 1.27 \times 16.3 \pm 1.97$  um in their largest dimensions (range

<sup>\*\*</sup> Figures followed by similar letters are not significantly different (P>0.05); those followed by different letters are statistically different (P<0.05).



Figs. 11.17: Sections in *A. hermanni* type II alveoli (X 1385) in (11) 6WN<sub>1</sub> showing type d cells with basophilic granules inside the globules, (12) N<sub>2</sub> after feeding showing a vacuole in type e<sub>1</sub> and e<sub>2</sub> cells, (13) N<sub>1</sub> after feeding showing a vacuole (arrow) and granules in type e<sub>1</sub> cells, (14) 6WN<sub>1</sub> showing coarse granules in type e<sub>2</sub> cells, (15) N<sub>1</sub> after feeding showing empty - looking globules in a type e<sub>2</sub> cell (arrow), (16) 2WN<sub>1</sub> showing globules with different shades of purple in type e<sub>2</sub> cell (arrow), and (17) N<sub>1</sub> after feeding showing cells lacking the globules (arrows).

Mean cell dimensions (um) $\pm$ SE (range)								
Stage	al	a <sub>2</sub>	b	c	d	e <sub>1</sub>	e <sub>2</sub>	
2-week-unfed	14.7±1.03x21.0±4.15 a*	14.1±2.77x21.4±2.98 c	15.0±3.57x21.3±2.79 e	11.1±4.33x19.4±5.54 f	16.4±2.07x21.4±3.53 g	16.7±2.10x22.0±4.15 i	18.0±2.97x24.4±2.34 k	
	(14.0-16.0x16.0-26.0)	(10.0-20.0x16.0-26.0)	(10.0-22.0x18.0-26.0)	(6.0-24.0x12.0-36.0)	(14.0-20.0x16.0-26.0)	(12.0-22.0x16.0-30.0)	(12.0-22.0x20.0-28.0)	
5-week-unfed	14.7±1.78x21.7±4.81 a	17.8±3.49x23.5±3.54 d	14.6±2.23x22.9±3.44 e	12.2±3.16x19.6±4.16 f	17.6±2.85x21.4±3.40 g	14.8±3.20x22.5±6.82 i	18.7±4.00x22.4±3.84 k	
	(10.0-16.0x14.0-30.0)	(12.0-24.0x20.0-30.0)	(12.0-18.0x16.0-26.0)	(6,0-13.0x10.0-28.0)	(12.0-22.0x16.0-26.0)	(10.0,20.0x20,0-36.0)	(14.0-24.0x18.0-30.0)	
Fed	11.4±1.70x16.6±1.97 b	17.7±2.61x23.3±2.94 d	13.5±2.54x20.2±2.89 e	12.7±3.89x19.1±3.90 f**	15.8±2.11x18.5±2.79h**	13.7±3.02x20.7±3.02 j	19.7±1.59x23.3±2.48 k	
	(8.0-14.0x14.0-20.0)	(10.0-20.0x1626.0)	(10.0-18.0x16.0-26.0)	(6.0-22.0x10.0-28.0)	(12.0-18.0x16.0-22.0)	(6.0-18.0x16.0-28.0)	(18.0-22.0x20.0-28.0)	

<sup>\*</sup> Figures followed by similar letters in the same column are not significantly different (P>0.05); those followed by different letters are statistically different. (P<0.05-P<0.01).

Table 4
Changes in dimensions of the cell types in the granular salivary alveoli during development of Argas hermanni second-instar nymph.

	Mean cell dimensions (um) ± SE (range)								
Stage	al	a <sub>2</sub>	b	,c	d	el	e <sub>2</sub>		
2-week-unfed	11.7±2.09x17.1±3.20 a*	18.1±2.96x25.9±4.35 c	17.3±3.35x24.7±4.58 d	13.5±4.22x19.0±5.60 e	16.0±3.58x20.0x3.80 f	16.9±2.66x22.5±4.41 h	20.2±3.40x27.5±2.66 j		
	(8.0-14.0x12.0-24.0)	(14.0-26.0x20.0-34.0)	(12.0-24.0x16.0-32.0)	(8.0-20.0x10.0-28.0)	(10.020.0x14.0-26.0)	(12.0-20.0x 16.0-30.0)	(16.0-26.0x24.0-32.0)		
6-week-unfed	15.7±2.62x19.8±2.71 b	16.4±2.07x23.6±4.09 c	17.1±2.52x22.9±3.65 d	15.2±6.76x20.9±5.91 e	15.0±2.76x20.0±1.79 f	14.4±4.55x22.4±2.95 h	23.3±4.50x26.6±4.48 j		
	(12.0-20x14.0-22.0)	(14.0-20.0x18.0-30.0)	(10.0-21.0x14.0-30.0)	(8.0-30.0x12.0-32.0)	(12.0-18.0x18.0-22.0)	(16.0-22.0x18.0-24.0)	(18.0-30.0x20.0-32.0)		
Fed	13.0±2.16x18.6-1.65 b	16.5±3.50x25.1±4.10 c	16.7±2.69x22.5±3.81 d	15.4±5.25x19.3±5.88 e**	13.3±2.46x17.8±2.48 g**	13.7±1.73x18.9±2.69 i	20.3±2.30x25.6±3.74 j		
	(10.0-16.0x16.0-22.0)	(10.0-24.0X26.0-32.0)	(10.0-22.0X26.0-30.0)	(6.0-24.0X12.0-28.0)	(12.0-20.0X14.0-22.0)	(12.0-16.0X16.0-22.0)	(16.0-24.0X20.0-30.0)		

<sup>\*</sup> Figures followed by similar letters in the same column are not significantly different (P>0.05); those followed by different letters are statistically different. (P<0.05-P<0.01).

<sup>\*\*</sup> Only few cells are discernible.

<sup>\*\*</sup> Only few cells are discernible.

Table 5
Changes in dimensions of the cell types in the granular salivary alveoli during development of male Argas hermanni

Mean cell dimensions (um) ± SE (range)								
Stage	aı	a <sub>2</sub>	. b	c	d	e <sub>1</sub>	e <sub>2</sub>	
-month-unfed	13.5±2.84x19.1±2.25 a*	16.6±2.63x23.1±3.88 c	16.2±2.74x22.0±1.63 d	12.0±2.00x19.5±4.20 e	15.7±2.43x19.4±1.90 f	14.4±3.57x21.4±3.64 h	18.6±2.12x26.6±2.95 j	
	(10.0-18.0x14.0-22.0)	(12.0-22.0x 18.0-30.0)	(12.0-20.0x20.0-24.0)	(10.0-16.0x12.0-26.0)	(10.0-20.0x16.0-22.0)	(10.0-20.0x14.0-28.0)	(16.0-22.0x18.0-32.0)	
2-month-unfed	15.3±2.10x22.7±3.20 b	15,4±3.67x25.9±4.05 c	15.7±2.15x24.5±5.46 d	15.4±4.77x22.4±7.87 f	16.3±4.59x20.0±3.02 f	16.8±3.90x21.2±2.28 h	21.5±2.98x26.5±3.82	
	(10.0-20.0x18.0-30.0)	(10.0-24.0x20.0-34.0)	(12.0-20.0x16.0-32.0)	(10.0-24.0x12:0-32.0)	(10.0-22.0x16.0-24.0)	(14.0-22.0x18.0-24.0)	(18.0-28.0x22.0-32.0)	
Fed	15.3±3.50x20.4±3.20 b	16.2±3.28x27.2±3.87 c	16.0±2.56x23.2±3.76 d	12.5±2.60x17.1±4.01 e**	14.5±4.44x17.5±6.19 g**	14.5±3.71x18.2±2.73 i	18.9±2.66x28.6±5.74 j	
	(10.0-22.0x14.0-24.0)	(10.0-22.0x14.0-35.0)	(12.0-20.0x20.0-32.0)	(10.0-18.0x12.0-28.0)	(12.0-22.0x14.0-26.0)	(10.0-20.0x 16.0-22.0)	(16.0-24.0x22.0-38.0)	

<sup>\*</sup> Figures followed by similar letters in the same column are not significantly different (P>0.05); those followed by different letters are statistically different. (P<0.05-P<0.01).

Mean cell dimensions (um) ± SE (range)								
Stage	aı	a <sub>2</sub>	b	··· c	d	e <sub>1</sub>	e <sub>2</sub>	
l-month-unfed	14.9±2.80x23.7±4.07 a*	20.0±4.81x29.0±4.92 b	17.7±3.15x29.2±7.68 c	12.6±2.99x18.9±4.87 d	23.0±3.21x28.3±4.83 f	18.7±3.93x26,0±2.53 h	23.3±5.24x30.4±4.72 j	
	(12.0-20.0x20.0-32.0)	(12.0-30.0x22.0-36.0)	(12.0-22.0x18.0-46.0)	(8.0-20.0x12.0-30.0)	(18.0-28.0x22.0-38.0)	(12.0-22.0x22.0-28.0)	(20.0-38.0x22.0-40.0)	
2-month-unfed	13.2±2.71x24.0±4.14 a	17.1±2.43x25.3±4.58 b	17.6±2.95x26.0±3.65 c	17.5±3.80x14.6±3.36 e	20.5±3.21x25.7±3.39 f	16.0±4.90x24.0±1.63 h	22.3±4.83x29.3±4.65 j	
	(10.0-18.0x18.0-30.0)	(14.0-20.0x20.0-32.0)	(14.0-22.0x20.0-30.0)	(10.0-22.0x20.0-30.0)	(16.0-26.0x18.0-30.0)	(10.0-20.0x22,0-26.0)	(16.0-30.0x24.0-36.00	
Fed	13.8±2.73x20.7±5.13 a	16.8±2.48x26.4±3.56 b	17.1±3.02x26.4±3.44 c	13.8±4.49x21.1±7.82 d**	15.7±3.45x21.7±4.27 g**	14.7±2.34x20.5±2.96 i	22.9±2.61x28.5±4.01 j	
	(8.0-18.0x14.0-30.0)	(12.0-22.0x22.0-32.0)	(14.0-22.0x22.0-34.0)	(8.0-21.0x10.0-30.0)	(12.0-20.x16.0-28.0)	(10.0-18.0x18.0-28.0)	(17.0-24.0x18.0-38.0)	

<sup>\*</sup> Figures followed by similar letters in the same column are not significantly different (P>0.05); those followed by different letters are statistically different. (P<0.05-P<0.01).

<sup>\*\*</sup> Only few cells are discernible.

<sup>\*\*</sup> Only few cells are discernible.

8.0 - 12.1 x 14.2 - 18.0 um) are observed after feeding (Fig. 17). These cells are devoid of globules, their cytoplasm is basophilic, and the nucleus occupies most of the cell; these cells may be type c, d, e<sub>1</sub> or e<sub>2</sub> cells exhausted during feeding.

#### **DISCUSSION**

The general structure of A. hermanni salivary glands and ducts conform to that of other argasid ticks (Roshdy, 1966; 1972; Balashov, 1968; Sonenshine and Gregson, 1970; Guirgis, 1971; Chinery, 1974; El Shoura 1985). The cuticular spiral thickening probably aid dilation and collapse due to pharyngeal movements and thus assists the flow of saliva. A valve-like structure similar to that in A. hermanni type II alveoli was suggested to control the movement of secretory material from the alveolar lumen into the duct in Boophilus microplus (Megaw and Beadle, 1979) and Amblyomma americanum (Krolak et al., 1982).

Type 1 alveoli in A. hermanni are histologically similar to those of other argasid (Balashov, 1968; Sonenshine and Gregson, 1970; Chinery, 1974; Roshdy, 1972; Roshdy and Coons, 1975) and ixodid species (Till, 1961; Balashov, 1968; Chinery, 1965; Coons and Roshdy, 1973; Megaw and Beadle; 1979; Meredith and Kaufman, 1973; Binnington, 1978; Binnington et al., 1983). Kirkland (1971) considered this type in Haemaphysalis leporispalustris to be unicellular. On the other hand, in A. arboreus a central cell with a clear cytoplasm was described to be connected directly with the alveolar duct (Roshdy and Coons, 1975). In Am. americanum, the central cell made contact with the duct lumen through an opening of a "constrictor cell" which was considered to store fluid prior to secretion (Krolak et al., 1982). The peripheral cells, with their basal membrane infoldings, were considered to resemble the avian salt cells (Balashov, 1968) and to be associated with osmoregulation and fluid transport by the salivary glands (Sauer et al., 1974; Hsu and Sauer, 1975; Needham and Sauer, 1975; Binnington, 1978).

Type I alveoli in A. hermanni probably participate in fluid secretion during the feeding process, since they decrease in size after feeding, but with no discernible structural changes. Guirgis (1971) and Khalil (1972) did not observe postfeeding changes in A. arboreus type I alveoli but Chinery (1974) observed histological changes in A. persicus alveoli. Similarly, while El Shoura (1985) reported no postfeeding ultrastructural changes Dzhafarov (1965) reported extensive changes in Ornithororos moubata alveoli. Also, while type I alveoli in B. microplus (Binnington, 1978) and Haem. leporispalustris (Kirkland, 1971) showed no postfeeding histological or ultrastructural changes, those in H. asiaticum (Dzhafarov, 1965), Am. americanum (Barker et al., 1984 and Haem, longicornis (Yanagawa et al., 1987) exhibited extensive changes.

The increase in type I alveolar size in unfed  $N_2$  and adult A. hermanni is probably associated with the general growth of the tick body as well as storage of secretory material. In adults, the increase in alveolar size is associated with elongation of the membrane infoldings which probably contributes to the functional efficiency of these alveoli.

In A. hermanni, 7 cell types and subtypes are observed, a number greater than that previously described in other argasids. This difference may be attributed to variation among the species or the use of different fixatives and staining techniques. Also cells containing globules of different sizes and exhibiting the same staining reaction were considered to be of the same type. In support of the latter assumptions,

Robinson and Davidson (1913) described only one type in A. persicus alveoli, while Chinery (1974) observed 2 types and Roshdy (1972) observed 3 types. Chinery (1974) considered cells with granules of 3 different sizes, but staining (with haematoxylin-eosin) more or less similarly, as well as others containing colloid-like masses to belong to a single cell type. This author suggested that these cells represented different stages in salivary secretion elaboration. On the other hand, Roshdy (1972) considered these cells to belong to 3 distinct types as they showed different staining reactions with Mallory Histochemical studies in A. triple and Giemsa stains. to ascertain the chemical hermanni are underway characteristics of the 7 cells types and subtypes.

The interstitial cells in A. hermanni type II alveoli were observed in many tick species. In O. moubata (El Shoura, 1985), these cells developed during feeding to form extensively branched canaliculi similar to those of transporting epithelial cells and were considered responsible for elimination of excess water in the blood meal (Meredith and Kaufman, 1973; Coons and Roshdy, 1979, 1981; Binnington, 1978; Megaw and Beadle, 1979). However, Coons and Roshdy (1973) suggested that these cells may have a supportive function or may differentiate into new secretory cells.

A. hermanni type II alveoli do not exhibit a significant change in size during the starvation period except in females in which they increase in size, probably owing to increased storage of secretory material. The postfeeding decrease in size is probably associated with loss of the globules of many cells. Guirgis (1971) and Khalil (1972) observed numerous globule-free cells of greatly reduced size after A. arboreus fed. In contrast to these findings, Chinery (1974) reported no change in granule appearance or staining properties during or after A. persicus feeding.

Type II alveoli in A. hermanni increase in size in unfed N<sub>2</sub> and females. This is porbably due to globule restoration in cells depleted during feeding, and/or formation of new cells. In nymphal A. arboreus, Khalil (1972) observed formation of new globules 3 days after feeding in 2 cell types which lost their globules during feeding. These cells reached a maximum size 5 days after moulting to the following stage. Globule formation may take place in the Golgi cisternae and adjacent rough endoplasmic reticulum as described in A. arboreus (Coons and Roshdy, 1981) and O. mubata (El Shoura, 1985). Delay of alveolar growth in A. hermanni males may be related to the male sexual activity since the gonads develop considerably in male-producing nymphs (Balashov, 1968); argasid males are engaged in mating shortly after moulting which probably divert the greatest part of the body anabolic activity to the gonads.

Type  $a_1$  cells do not exhibit a significant change throughout development except for the decrease in size after the  $N_1$  feeds. It may be presumed that this type store a precursor secretory material to replace a certain cell type(s) depleted during feeding. Further investigations are required to varify this assumption.

In  $6WN_1$  and females only, type  $a_2$  cells change in size during development. Also, type b cells increase in size is limited to unfed  $N_2$  and females. Both types do not exhibit a change after feeding except for the presence of a vacuole in some cells. These vacuoles may represent soluble metabolic products which are washed out during fixation and dehydration, or they may represent extracellular spaces surrounded by a thin membrane similar to those formed in some cells after A. arboreus feeding owing to apocrine

secretion (Coons and Roshdy, 1981). Such assumptions require further investigation.

Changes in type c and d cells strongly suggest that most of these cells release all of their globules during nymphal and adult feeding and some release part of their globules during adult feeding. Basophilic granules appearing inside and between the globules in type d cells in unfed ticks and disappearing after feeding may represent a change in the chemical structure well before the secretory material is released during feeding.

Changes in type e<sub>1</sub> cells suggest that the globules change into coarse granules while most of the cells release all or most of their content during feeding. However, changes in type e<sub>1</sub> cells may represent globule degradation into coarse granules during the starvation period as well as during feeding when most of these cells release all of their secretory content. Some cells may release these granules afterwards which appear in the salivary duct lumen in unfed ticks. Difference in the globule staining properties in type e<sub>2</sub> cells may represent different steps in the synthesis or degradation of their contents.

Replacement by new cells or replenishment of depleted type c, d, e<sub>1</sub> and e<sub>2</sub> cells with new globules occurs during the nymphal postfeeding period. In type c and e<sub>2</sub> cells, secretory material storage probably continues during the prolonged starvation period.

Cell types c, d,  $e_1$  and  $e_2$  in A. hermanni may release their globules by apocrine or merocrine secretion. During A. arboreus (Coons and Roshdy, 1981) and O. moubata (El Shoura, 1985) feeding, the granules of some cell types are released by an apocrine secretion while those of other types are released by exocytosis. Merocrine and apocrine secretions are of significant adaptive value for the rapid and repeated feeding of argasid ticks since cells remain sufficiently intact to synthesize new granules for the next feeding.

During the different phases of development, no change in size of nuclei in type I and type II alveoli. This may reflect a continuous cell activity throughout *A. hermanni* development.

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#### **REFERENCES**

- Balashov, Yu. S., 1968. Bloodsucking ticks (Ixodoidea) vectors of diseases of man and animals. Leningrad, Akad. Nauk SSSR. Zool. Inst. Leningrad 319 pp (In Russian) [In English, Misc. Publ. Entomol. Soc. Am. 8: 161-376 (1972)].
- Barker, D. M., C. L. Ownby, J. M. Krolak, L.P. Claypool, and J. R. Sauer, 1984. The effect of attachment, feeding and mating on the morpohology of type I alveolus of salivary glands of the lone star tick., *Amblyomma americanum* (L.). J. Parasitol. 70: 99-113.

- Bennington, K. C., 1978. Sequential changes in salivary gland structure during attachment and feeding of the cattle tick, *Boophilus microplus*. Intern. J. Parasitol. 8: 97-115.
- Bennington, K. C., A. S. Young and F. D. Obenchain, 1983.

  Morphology of normal and *Theileria parva* infected salivary glands of *Rhipicephlus appendiculatus*. J. Parasitol. 9: 421-424.
- **Chinery, W. A., 1965.** Studies on the various glands of the tick *Haemaphsalis spinigera* Neumann 1897, Part III. The salivary glands. Acta Trop. 22: 321-349.
- Chinery, W. A., 1974. Studies on the salivary glands of *Argas persicus* (Oken, 1818), J. Med. Entomol. 11: 480-487.
- Coons, L.B. and M.A. Roshdy, 1973. Fine structure of the salivary glands of unfed male *Dermacentor variabilis* (Say) (Ixodoidea) J. Parasitol. 59: 900-912.
- Coons, L. B. and M. A. Roshdy, 1979. Functional morphology and cytochemical localization of chloride ions and ouabain sensitive phosphatase activity in the salivary gland transport epithelia of four species of ticks. *In. J. G. Rodriguez (Ed.): Recent Adv. Acarol. 1:* 427-434. Academic Press: New York, San Francisco and London.
- Coons, L. B. and M. A. Roshdy, 1981. Ultrastructure of granule secretion in salivary glands of *Argas* (*Persicargas*) arboreus during feeding. Z. Parasitenk. 65: 225-234.
- **Dzhafarov, T. E., 1965.** An electron microscope investigation of the salivary gland pyramidal alveoli in certain ticks. *In:* An electron and fluorescent microscope study of the cell. Izd. Akad. Nauk SSSR, Leningrad (1964). pp 31-37 (In Russian) (English translation: NAMRU3-T1233).
- El Shoura, S. M., 1985. Ultrastructure of salivary glands of *Ornithodoros (Ornithodoros) moubata* (Ixodoidea: Argasidae). J. Morphol. 186: 45-52.
- Guirgis, S. S., 1971. The subgenus *Persicargas* (Ixodoidea: *Argas*). 13. Histological studies on *A.* (*P.*) *arboreus* Kaiser, Hoogstraal and Kohls. J. Med. Entomol. 8: 648-667.
- Hsu, M. H. and J. R. Sauer, 1975. Ion and water balance in the feeding lone star tick. Comp. Biochem. Physiol. 52A: 269-276.
- **Khalil, G. M., 1972.** Biochemical and physiological studies of certain ticks (Ixodoidea). Incorporation of tritiated tyrosine in the salivary glands of nymphal *Argas* (*Persicargas*) arboreus. Ann. Entomol. Soc. Am. 65: 746-750.
- **Kirkland. W. L., 1971.** Ultrastructural changes in the nymphal salivary glands of the rabbit tick. *Haemaphysalis leporispalustris* during feeding. J. Insect Physiol. 17: 1933-1946.
- Krolak, J. M., C. L. Ownby and J. R. Sauer, 1982. Alveolar structure of salivary glands of the lone star tick. *Amblyomma americanum* (L.): Unfed females. J. Parasitol. 68: 61-82.

- Marzouk, A. S., G. M. Khalil and Z. E. A. Darwish, 1987.

  Neurosecretory cell types and distribution in unfed female *Hyalomma dromedari* (Acari: Ixodoidea: Ixodidae) synganglion. J. Egyp. Soc. Parasitol. 17: 41-61.
- Megaw, M. W. J. and D. J. Beadle, 1979. Structure and function of the salivary glands of the tick (*Boophilus microplus* Canestrini (Acarina: Ixodidae). Intern. J. Insect Morphol. Embryol. 8: 67-83.
- Meredith, J. and W. R. Kaufman, 1973. A proposed site of fluid secretion in the salivary gland of the ixodid tick *Dermacentor andersoni*. Parasitology 67: 205-217.
- Needham, G. R. and J. R. Sauer, 1975. Control of fluid secretion by isolated salivary glands of the lone star tick. J. Insect Physiol. 21: 1893-1898.
- **Robinson, L. E.** and **J. Davidson, 1913.** The anatomy of *Argas persicus* (Oken, 1818). Part II. Parasitology, 6: 217-256.
- Roshdy, M. A., 1966. Comparative internal morphology of subgenera of *Argas* ticks (Ixodoidea, Argsidae). 4. Subgenus *Ogadenus: Argas brumpti* Neumann, 1907. J. Parasitol. 52: 776-782.
- **Roshdy, M. A., 1972.** The subgenus *Persicargas* (Ixodoidea, Argasidae, *Argas*). 15. Histology and histochemistry of the salivary glands of *A. (P.) persicus* Oken. J. Med. Entomol. 9: 143-148.

- Roshdy, M. A. and L. B. Coons, 1975. The subgenus *Persicargas* (Ixodoidea: *Argas*). 23. Fine structure of the salivary glands of unfed *A.* (*P.*) *arboreus* Kaiser, Hoogstraal, and Kohls. J. Parasitol. 61: 743-752.
- Sauer. R. J., J. H. Frick and J. A. Hair, 1974. Control of <sup>36</sup>Cl uptake by isolated salivary glands of the lone star tick. J. Insect Physiol. 20: 1771-1778.
- Sonenshine, D. E. and J. D. Gregson, 1970. A contribution to the internal anatomy and histology of the bat tick *Ornithodoros kelleyi* Cooley and Kohls, 1941, 1. The alimentary system, with notes on the food channel in *Ornithodoros denmarki* Kohls, Sonenshine and Clifford, 1965. J. Med. Entomol. 7: 46-64.
- **Till, W. M., 1961.** A contribution to the anatomy and histology of the brown ear tick *Rhipicephalus appendiculatus* Neumann. Mem. Entomol. Soc. S. Afr. 6: 1-124.
- Yanagawa, H., T. Mori, S. Shiraishi and T. A. Uchida, 1987. Ultrastructural changes in the salivary gland of the female cattle tick, *Haemaphysalis longicornis*, during feeding, J. Zool. 212: 283-301.