Fine structure of the integument of *Argas* (*Persicargas*) *persicus* (Oken) (Ixodoidea: Argasidae)

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Montasser, A. & Amin, A. 2005: Fine structure of the integument of *Argas* (*Persicargas*) *persicus* (Oken) (Ixodoidea: Argasidae). — Entomol. Fennica 16: 193–200.

The integument of *Argas persicus* was investigated using light, scanning and transmission electron microscopy. The study revealed that two layers, viz. an outer epicuticle and an inner procuticle, form the cuticle. The epicuticle includes wax, cuticulin and protein epicuticular layers. The wax layer carries numerous crater-like deposits, oval or circular discs and numerous infoldings. The procuticle contains an exo-, endo- and a subcuticle. Underlining the cuticle, flattened epidermal cells are connected via desmosomes and contain rough endoplasmic reticulum, free ribosomes and mitochondria. Scattered dermal glands are located beneath the cuticle and are continuous with the outside through dermal ducts and surface pores.

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Received 23 September 2004, accepted 2 December 2004

1. Introduction

Of the ca. 800 known species of ticks, nearly 100 are capable of transmitting bacterial, viral, and protozoal agents to humans. Worldwide, ticks are important vectors of systemic diseases (Steen *et al*. 2004). Parasitism of hosts by ticks, and infection by tick-borne pathogens, constitute significant medical and veterinary problems, and the associated economic losses are considerable. During the last decade, some 400 cases of tick-borne rickettsioses have been reported both in humans and in animals (Jensenius *et al*. 2004).

Due to their immense medical and economical importance, argasid ticks are the focus of this report. These ticks transmit a great variety of microbial diseases both to domestic animals and humans. *Argas* species are also known to transmit the rickettsial agent, *Aegyptianella pullorum*, to chickens and geese in the tropics and subtropics of the old world, and the fowl spirochete, *Borrelia anserina*, to domestic poultry, canaries, guinea fowl and pigeons in South America (Saunders 1990). Infestation with *A. persicus* larvae has also been documented to cause fatal flaccid paralysis of chickens (Rosenstein 1976).

The integument of arthropods consists of a single-layered hypodermis and a multi-layered cuticle. These layers form the exoskeleton that offers mechanical protection against the environment, determines the size and colour of the animal, and can only grow to a certain extent (Condoulis & Locke 1966). The cuticle also plays an important role in regulating water balance and providing support (Amosova 1983). The cuticle itself is a fibre composite material in which the

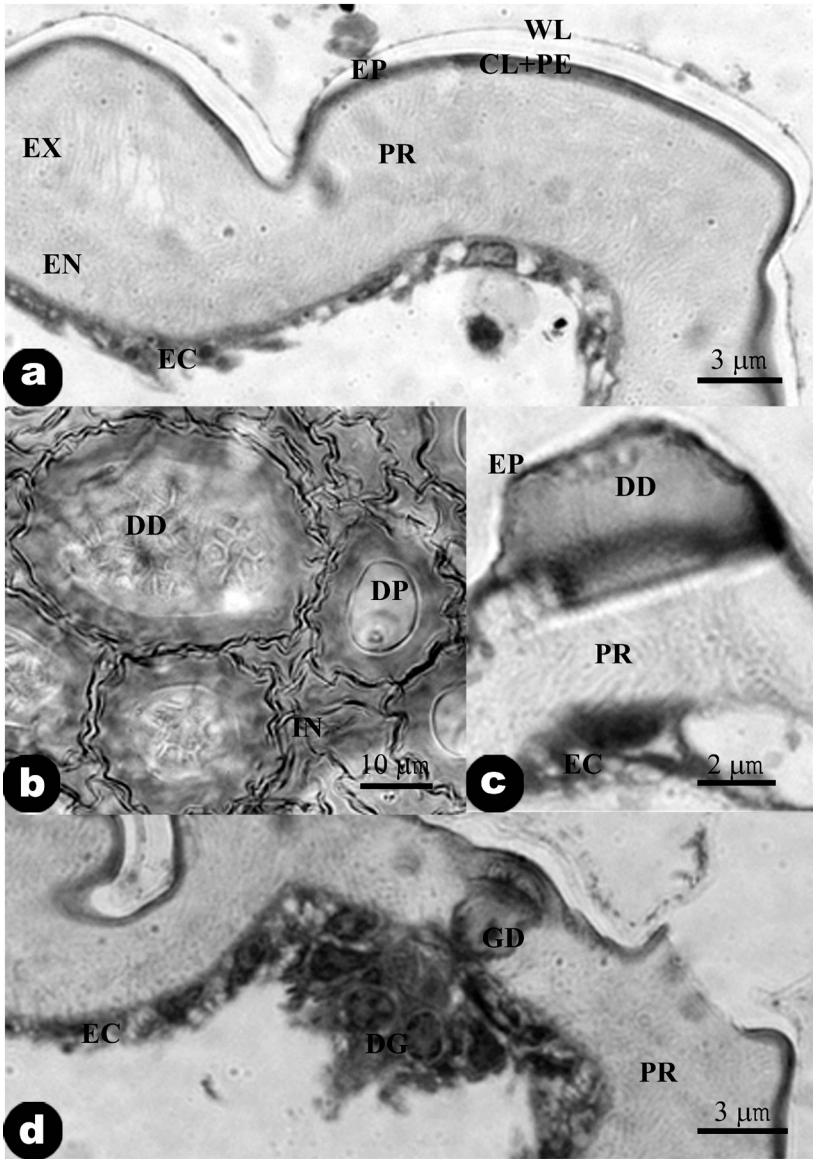

Fig. 1. Integument light micrographs of an unfed female *Argas persicus*. – a. Transverse section showing the different layers of the cuticle underlain by epidermal cells (EC). – b. Top view of the integument showing dense discs (DD) and dense deposits (DP) separated by extensive infoldings (IN). – c. Transverse section showing a dense disc that is continuous with the epicuticle. – d. Transverse section showing a dermal gland (DG) with ca. 7 cells between the flattened epidermal cells. The gland is connected to the outside through a gland duct (GD). CL = cuticulin; EN = endocuticle; EP = epicuticle; EX = exocuticle; PE = protein epicuticle; PR = procuticle; WL = wax layer.

chitin, in the form of microfibrils, is embedded in a protein-lipid matrix (Barth 1973).

Integuments of soft and hard ticks, as those of other arthropods, have been shown to consist of a cuticle and an underlining layer of epidermal cells that secrete the cuticle (Balashov 1972). While most electron microscopy (EM) studies of the tick integument have mainly focused on hard ticks (Ixodidae) (Nathanson 1967, 1970, Beadles *et al*. 1973, Beadle 1974, Filshie 1976, Amosova 1983, Walker *et al*. 1996a, b), little is known about the ultrastructure of the integument of soft ticks. Therefore, the main goal of this study is to shed light on the ultrastructure of the integument of soft ticks to unravel targets for developing potential anti-tick drugs and/or vaccines.

2. Materials and Methods

A. persicus was obtained from a colony maintained in the Biology Department laboratories, Faculty of Science, UAE University, UAE [grown at 28 (±3) °C and 75 (±5)% relative hu-

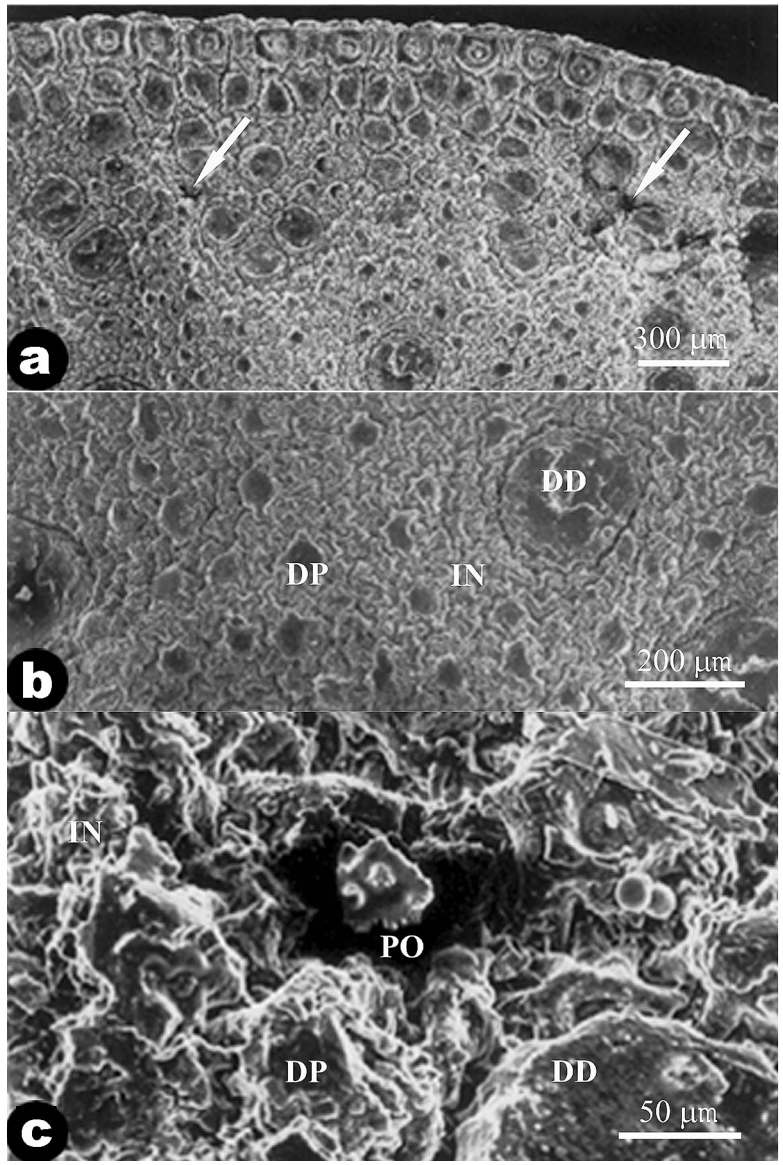

Fig. 2. Integument scanning electron micrographs of an unfed female *Argas persicus*. – a. Sector of the integument containing external pores (arrows) of the dermal glands. – b. Higher magnification of dense discs (DD) and deposits (DP) separated by infoldings (IN). – c. Higher magnification of the pore (PO) surrounded with numerous infoldings.

midity]. The domestic pigeon *Columba livia domestica* was used as a host.

Unfed female ticks were fixed in 3% buffered (pH 7.2) glutaraldehyde for scanning electron microscopy (SEM) and dehydrated in a graded series of ethanol. The prepared specimens were desiccated and then sputtered with gold using a sputter apparatus (BAL-TEC CPD 030). The surface topography was examined using a SEM (JOEL-JSM2).

The unfed female ticks were dissected in phosphate-buffered saline, pH 7.2. Dorsal integument was fixed with 3% and 1% buffered (pH 7.2) glutaraldehyde and buffered (pH 7.2) OsO4 respectively. Samples were then washed in phosphate buffer, dehydrated in a graded series of ethanol and embedded in epoxy resin. Semi-thin sections (1 μm) were treated with methylene blue and examined with a light microscope (LM; LEITZ DMRB). Ultrathin sections were stained with uranyl acetate and lead citrate (Venable & Coggeshall 1965) and were finally examined using a Philips transmission electron microscopy (TEM).

3. Results

Histological studies of the integument of unfed *A. persicus* showed that it consists of an overlying cuticle, underlain with epidermal cells. The cuticle is divided into two distinct layers, a thin outer epicuticle and a thicker inner procuticle.

The epicuticle is composed of wax, cuticulin and protein epicuticle layers (Fig. 1a). Nomenclature of these layers was according to Amosova (1983) and Walker *et al*. (1996a). The outermost wax layer is characteristically very thin (ca. 0.2 µm) (Fig. 1a). Numerous dense crater-like deposits (ca. 7.5 µm in height) are shown in the epicuticle (Figs. 1b, 2a–b, 3a). In addition to these deposits, multiple circular, semi-circular and oval discs (4–7 µm in height and 127–233 µm in diameter) are separated by extensive infoldings (Figs. 1b, 2b–c). These discs appear in the upper border of the procuticle and are traversed by small irregular canals (Fig. 3b). The cuticulin is a dense and thin (ca. 0.045 µm) layer followed by a less dense and thick (0.45–0.6 µm) protein layer (Fig. 4a). Numerous narrow (0.03–0.045 µm in diameter) canals appear to traverse the epicuticular protein layer (Fig. 4a).

The procuticle has a total thickness of ca. 3.7–9.2 µm that represents most of the integument thickness (Figs. 1a, 4). It is differentiated into three layers; an exo-, endo- and a subcuticular layer (Amosova 1983, Walker *et al*. 1996a). The exocuticle is traversed by fairly straight pore canals (Fig. 4a) and lacks any lamellae. The pore canals are 0.06–0.12 µm in diameter, and their lateral branches carry secretory materials probably from epidermal cells (Fig. 4b–c). Unlike the exocuticle, the endocuticle contains horizontal, electron-dense lamellae that are traversed by pore canals (Figs. 3b, 4c). A thin layer called the subcuticle lies on the border between the cuticle and epidermal cells (Fig. 4c). The subcuticle is morphologically similar to the basement membrane.

The epidermis consists of a single layer of flattened epidermal cells. They contain irregularly shaped nuclei (ca. 1.53 µm in diameter), some rough endoplasmic reticulum, free ribosomes and mitochondria (Fig. 5a–b). Desmosomes are often noticed between epidermal cells (Fig. 4c).

SEM clearly demonstrated a fair distribution of pores over the integument surface (Fig. 2a). These pores are guarded by the numerous infoldings (Fig. 2c) and are continuous with the dermal glands through dermal ducts (Fig. 1d). These dermal ducts are ca. 2.5 µm in height and ca. 1.7 µm in diameter. Each dermal gland consists of 7–10 cells (Fig. 1d), which are polygonal with oval or spherical nuclei of 1.1–1.48 µm in diameter. TEM shows numerous secretory vesicles,

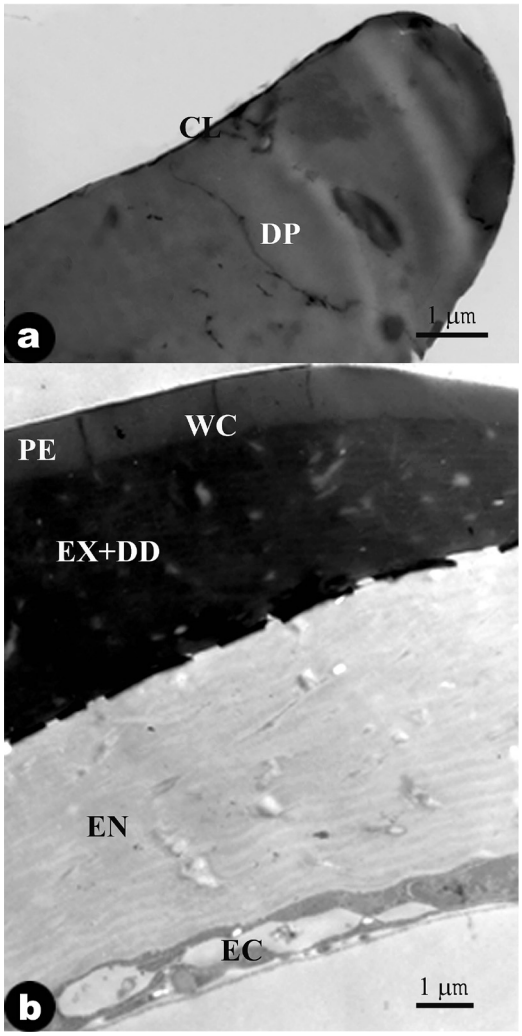

Fig. 3. Integument transmission electron micrographs of an unfed female *Argas persicus*. – a. Dense deposit (DP) surrounded by the cuticulin layer (CL). – b. Dense discs (DD) occurred in the exocuticle (EX). EC = epidermal cell; EN = endocuticle; PE = protein epicuticle; WC = Wax canal.

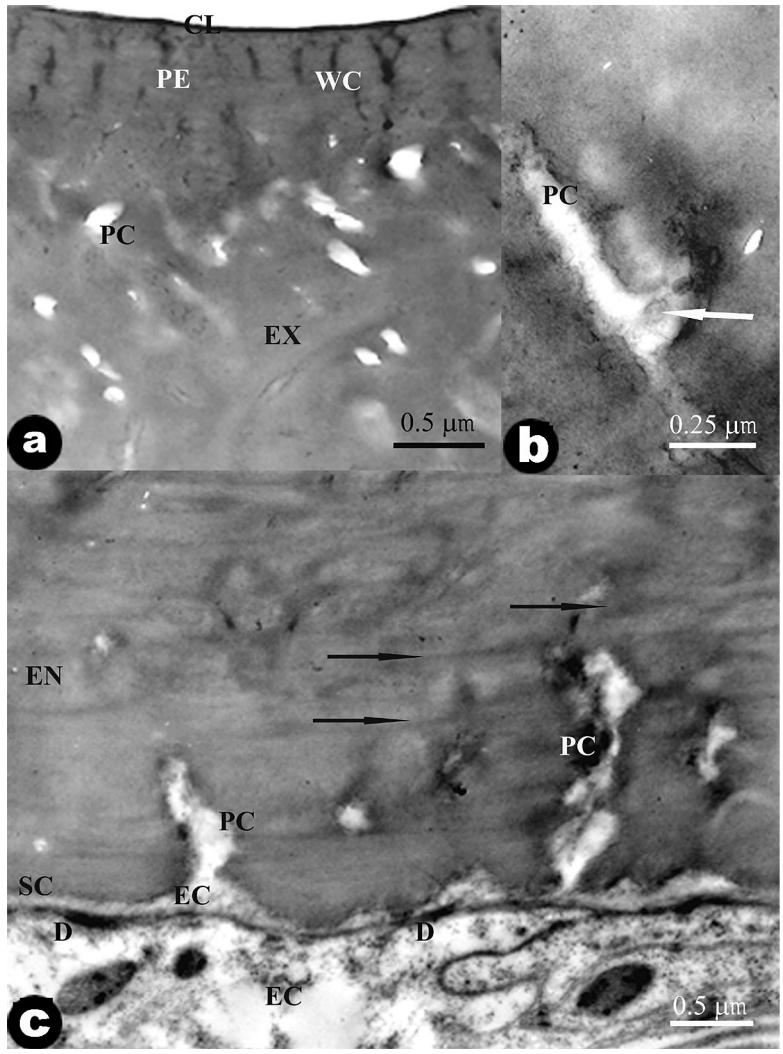

Fig. 4. Integument transmission electron micrographs of an unfed female *Argas persicus*. – a. Transverse section showing cuticulin (CL) and protein epicuticle (PE) traversed by wax canals (WC) and the exocuticle (EX) that contains some pore canals (PC). – b. Higher magnification of the pore canal showing its branching and enclosing secretory material (arrow). – c. Transverse section showing endocuticle (EN) with horizontal lamellae (arrows) and pore canals (PC) emerging from the epidermal cells (EC) that are connected together with desmosomes (D). The subcuticle (SC) is located between the endocuticle and epidermal cells.

rough endoplasmic reticulum and ribosomes in the cytoplasm of the dermal gland cells (Fig. 5c).

4. Discussion

The present study showed three layers, viz. wax, cuticulin and protein epicuticle layers, to make up the epicuticle of *A. persicus*. The light microscopic investigation of Balashov (1972) also showed three layers, designated as the cement, wax and cuticulin layers, for this same species. The present ultrastructural description replaces the cement layer that characterizes the integument of soft ticks only (Balashov 1972), with the deposits and discs superficial to wax layer. The present study also replaces the cuticulin layer of Balashov (1972) with two layers, namely the dense cuticulin and the less dense protein epicuticle. These conclusions are supported by similar ultrastructural cuticle studies on the hard ticks *Haemaphysalis leporispalustris* (Nathanson 1967, 1970), *Boophilus decoloratus* and *B. microplus* (Beadle 1974, Filshie 1976), *Hyalomma asciaticum* (Amosova 1983) and *Rhipicephalus appendiculatus* (Walker *et al*. 1996a).

Unlike the external openings of the dermal gland ducts, those for wax canals in the protein layer of the epicuticle of *A. persicus* are not visible in SEM. This is probably due to the termina-

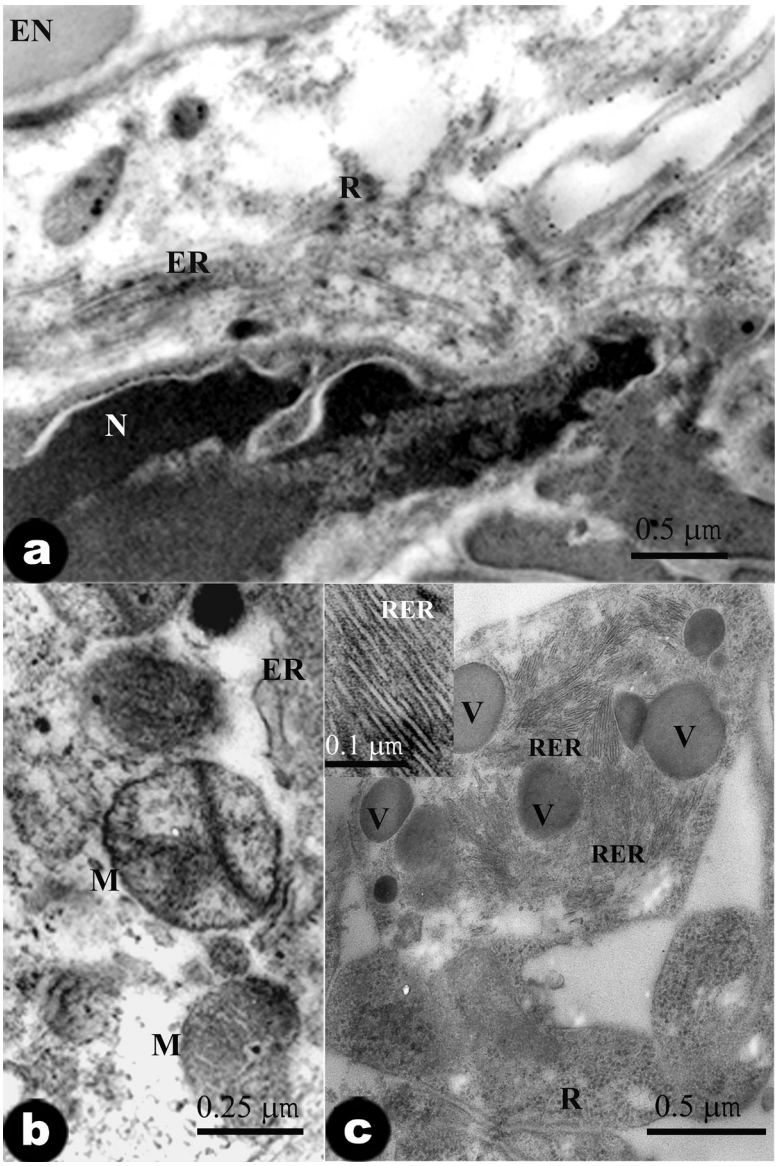

Fig. 5. Integument transmission electron micrographs of an unfed female *Argas persicus*. – a. Transverse section showing epidermal cell with an irregular shaped nucleus (N), few cisternae of rough endoplasmic reticulum (ER) and ribosomes (R). EN = endocuticle. – b. As in (a) but showing mitochondria (M) and endoplasmic reticulum (ER). – c. Transverse section of a secretory cell of the dermal gland showing numerous secretory vesicles (V), rough endoplasmic reticulum (RER) and ribosomes (R); the inset shows higher magnification of rough endoplasmic reticulum (RER).

tion of wax canals with the cuticulin and wax layers. No wax canal openings have previously been reported for hard ticks (Amosova 1983, Walker *et al*. 1996a), but they have been noticed on the surface of the scorpion *Hadrurus arizonensis* (Hadley & Filshie 1979). Wax canals and pore canals are believed to have an important role in the transportation of lipids from the epidermis to the cuticular surface (Bruck & Stockem 1972).

The procuticle of the studied ticks represents the thickest part of the cuticle. Its endocuticle contains horizontal lamellae. These lamellae have previously been reported for *B. microplus* nymphs (Hackman & Filshie 1982). The lamellated procuticle may provide the cuticle of the studied species with a significant capacity to expand and stretch while engorging during a blood meal. Villarino *et al*. (2001) have characterized the localization of esterases in the procuticle, and particularly within the endocuticle of *B. microplus* females. They have also reported that the procuticle represents a considerable physical barrier for organophosphate compounds.

Pore canals of the procuticle of *A. persicus*

contain secretory materials. Using HPLC analysis, the secretory material of epidermal cells of *Ornithodoros parkeri* has been identified as ecdyson (Zhu *et al*. 1991). Procuticle pore canals may branch into narrower and numerous wax canals in the epicuticle in order to pass the secretions of epidermal cells into the cuticulin layer and external structures. Hackman (1982) and Amosova (1983) have earlier reported some continuity between these canals.

The light microscope examinations of Balashov (1972) and the present study showed numerous scattered amorphous dense discs and deposits in the *A. persicus* integument. The present TEM study showed the inclusion of these discs in the upper parts of the procuticle. Accordingly, these structures may bear a resemblance to the scuta of hard ticks or the sclerites of other arachnids, and might provide partial sclerotization to the leathery integument of argasid ticks.

Therefore, these structures probably support the underlying layers, decrease transpiration rates and/or protect against drought in soft ticks. The exoskeleton of hard ticks mainly functions in supporting and protecting these ticks and has an important role in regulation of water balance (Amosova 1983).

The dermal glands of the studied tick contain secretory materials, and the pores on the integument are probably homologous to type 1 or 2 glands in the integument of the hard ticks *H. asiaticum* (Amosova 1983) or *R. appendiculatus* (Walker *et al*. 1996b), respectively. Dermal glands have been implicated in the production of pheromones in *Amblyomma* ticks (Diehl *et al*. 1991), and an allomone used by *Dermacentor* and *Amblyomma* as a defense against ants (Yoder *et al*. 1993, Pavis *et al*. 1994). In *R. appendiculatus*, these glands might also secrete semiochemicals (Walker *et al*. 1996b).

The complex construction of the cuticle of *Argas persicus* underpins the highly specialized parasitic lifestyle of this species and illustrates the importance of the cuticular skeleton for the enormous evolutionary success of arthropods. Further molecular and biochemical studies are underway to isolate and characterize components of the integument of the soft tick. If successful, these further studies might identify potential anti-tick drug targets.

Acknowledgements. Our special thanks and deep gratitude go to Dr. R. Padmanaphan, Mr. S. Tariq and Mr. A. Gbewonyo, Electron Microscope Unit, Faculty of Medicine, United Arab Emirates University, for providing lab facilities and outstanding technical support throughout the study. We also thank Dr. Michael Buratovich, Spring Arbor University, U. S. A., for proofreading the manuscript.

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