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Fine Structure of the Nymphal Integument in the Camel Tick *Hyalomma (H) anatolicum anatolicum* (Ixoddoidea: Ixodidae)

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Abstract. The fine structure of the nymphal integument in the ixodid tick *Hyalomma (Hyalomma) anatolicum anatolicum* is compared for stages of development before feeding under electron microscope. The integument contains a cuticular layer and underlies epidermal cells.

The body cuticle consists of both sclerotized and non-sclerotized parts, and also is divided into an outer epicuticle, and an inner, thick fibrilar procuticle. Pore canals in the procuticle are continuous with wax canals, which traverse the epicuticle. The parallel, extensible epicuticular folds appear at this stage. By stretching of the cuticle, the pore canals, lose their parallel pattern and appear to have become deformed.

Keywords: Ixodid tick, body cuticle, electron microscope.

Introduction

The tick integument consists of single layer of epidermal cells which secrete the body cuticle. This layer extends to cover several specialized organs of the body, including respiratory[1], sensory[2], genital[3-8] organs and various glands including accessory, salivary, and foveal glands[9-14].

The studies by histochemical and histological examination revealed two principal cuticular layers: a thin, outer epicuticle consisting of lipid, cuticulin and polyphenol; and a thicker, inner procuticle composed of polysaccharides and chitin-protein[15].

The cuticle in *Haemophysalis leporispalustris* was described[16] at ultrastructural level by electron microscope. This study demonstrated that the cuticle consists of two distinct types: sclerotized cuticle serving as skeletal surface for musculature, and non-scleratized cuticle found elsewhere in the organism. The molting apolysis, is defined as the separation of "old" cuticle from epidermis[17]. The epidermal layer is responsible for secretion of some enzymes which digest the procuticle during the molting[18, 19].

The ultrastructure changes in the epidermis and its role during feeding and molting have received less attention than that of the cuticle.

Recent study has been examined[20] on the surface structure model and mechanism of an insect integument adapted to be damaged easily. The result showed haemolymph droplet acts as a feeding deterrent towards invertebrate predators. Moreover, that study described the cuticle surface, that the integument surface of several sawfly larvae of the *Tenthredinidae (Hymenoptera)* are called easy bleeders because their whole body integument, except the head capsule, disrupts very easily at a given spot, under a slight mechanical stress at this spot. Boeve *et al.*[20] conclude that the easy bleeders show spider-like microstructures on their cuticle surface. It is suggested that these microstructures may facilitate integument hydrophobic. This latter property would allows the exuding haemolymph to be maintained as a droplet at the integument surface.

The present study describes the fine structure of the integument, and the epidermis, of the unfed nymphal tick *Hyalomma (anatolicum) anatolicum.*

The genus *Hyalomma* contains 21 species, one of which is *Hyalomma (anatolicum) anatolicum* used in the present paper. This genus is distributed throughout the world, and nine species and subspecies occur in Saudi Arabia[21].

Materials and Methods

H. (H.) anatolicum anatolicum were collected mainly from abbatoirs and camel markets at Makkah in Saudi Arabia, particularly the Muna (Al Meassem) and Arafat areas. Specimens of *Hyalomma (anatolicum) anatolicum* were obtained from a colony maintained at 28ºC and 75% relative humidity, at the science department, Teacher college in Makkah, Saudi Arabia.

Domesticated rabbits were used as laboratory hosts. Unfed, 3-6 day-old nymphs were fixed in 2.5% glutarldehyde in sodium cacodylate buffer (pH 7.2) after excising legs at the level of the coxae. Feeding (1,3,5 and 7 days after attachment) and fully engorged (1 and 3 days after attachment) nymphs were dissected in insect physiological saline[22] under binocular microscope.

The dorsal and ventral body integument was gently removed from attached muscles and connective tissues, cut into longitudinally oriented pieces, and directly fixed in 2.5% glutarldehyde in 1% Na-cacodylate buffer (pH 7.2) for 6-12 h at 4ºC. The specimens were rinsed in 0.1M Na-cacodylate for 10 minutes at 4ºC, and postfixed in OsO_4 in 1% M Na-cacodylate buffer for 1h at 4ºC, dehydrated in a graded series ethanol and embedded in low viscosity resin[23]. Ultrathin sections were cut using glass knives on a Reichart OM 3 ultra-microtome, mounted on copper grids and stained for 20 min with 2% (W/V) uranylacetate in 70% alcohol and for 5 minutes in 0.3% lead citrate in 0.1M sodium cacodylate. Thin sections were observed in Philips 400 TEM. And photographic recordings taken on Ilford technical film[24-25].

Result

The nymphal integument of *H (Hyalomma) anatolicum* contains an external cuticle secreted by a single layer of epidermal cells that are separated from the hemolymph by a thin basal lamina (Fig. 1). Muscles are anchored to the cuticle through intervening epidermal cells.

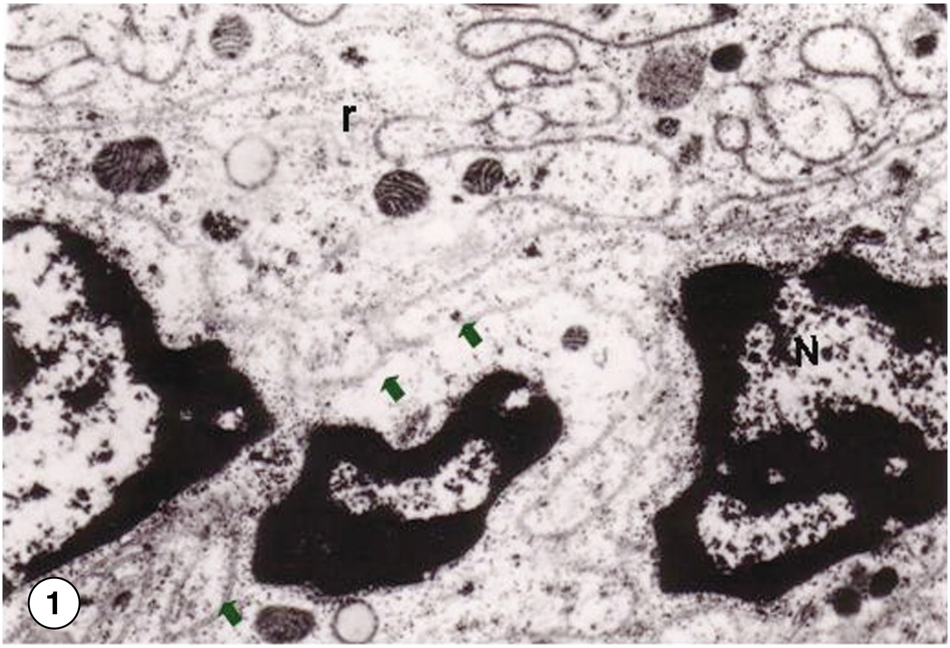

Fig. 1. Epidermal cell showing basal membrane infoldings (arrowheads). × 10.500.

The Cuticle

The cuticle consists of sclerotized (hard) and non-sclerotized (soft) cuticle, and is divided into two principal layers; 1) a thin (1-µm thick) outer complex epicuticle, and 2) a thick (2-100 µm) inner procuticle. The sclerotized cuticle has a smooth or slightly wavy epicuticle (Fig. 2) and is found in the scutum, capitulum, various sclerites and appendages, and does not stretch during the tick

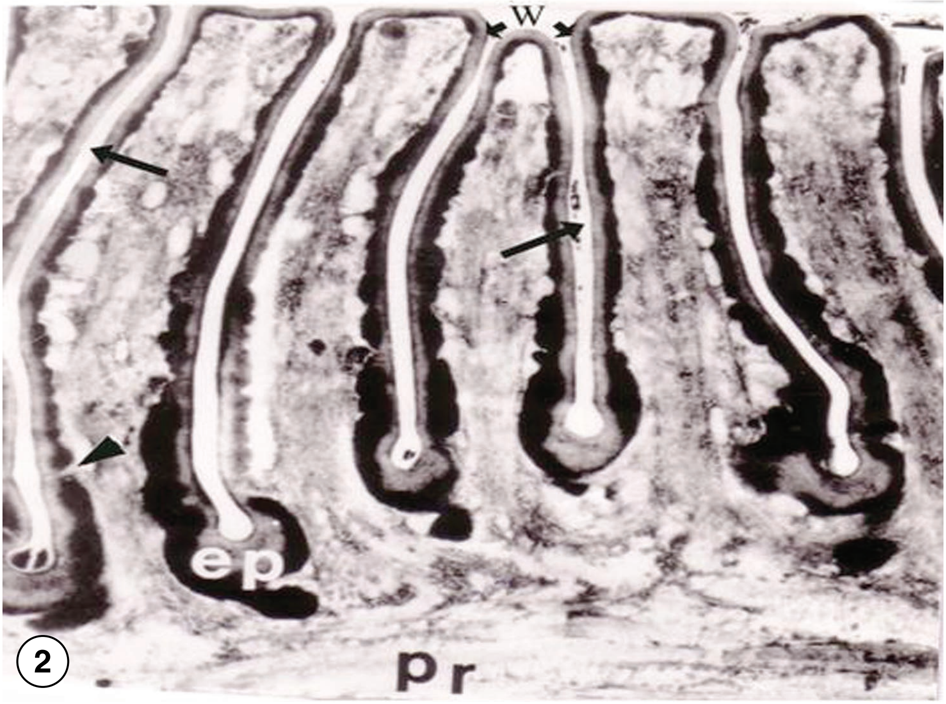

Fig. 2. Nonsclerotized cuticle showing extensible epicuticle (ep) traversed by wax canals (large extensible arrowheads) and covered by waxy layer (W). Pr, procuticle. Small arrowheads indicate to pore canals. × 15.500.

feeding. The non-sclerotized, or extensible cuticle of the alloscutum stretches where the epicuticle forms regular, parallel, deep folds (Fig. 2) covered by an outermost waxy layer (Fig. 2). The epicuticle is traversed by short waxy canal, which contains dense, homogeneous material transported from the pore canals (Fig. 2). These canals broaden and branch upward and terminate beneath the epicuticle where they join the waxy canals (Fig. 2).

The Epidermis

The cuboidal and flat cells (Fig. 3) forming the epidermis are joined together by long tortuous junctions (Fig. 4), while the basal cell membrane forms a few, narrow infoldings (Fig. 5). In the cytoplasm ill-defined mitochondria, rough endoplasmic reticulum, free ribosomes and microtubules are present (Fig. 3-5). The nucleus occupies most of the cell volume (Fig. 1-4).

Muscle attachment is richly endowed with microtubules (Fig. 3&4) oriented parallel with the long axis of the adjacent muscle fibers. The basal surface of the epidermal cell bears processes which interdigitate with matching projections of the muscle cell, and the apposed plasma membranes are linked by desmo-

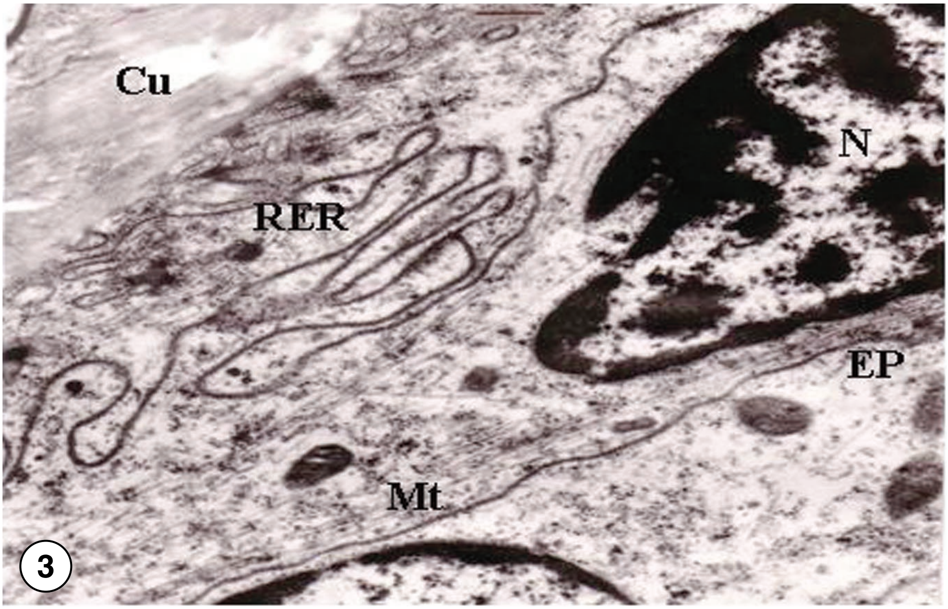

Fig. 3. Integument in unfed nymph showing sclerotized cuticle (cu), flat epidermal cells (Ep), Rough Endoplasmic Reticulum (RER), Nucleus (N) and Microtubules (MT). × 10.500.

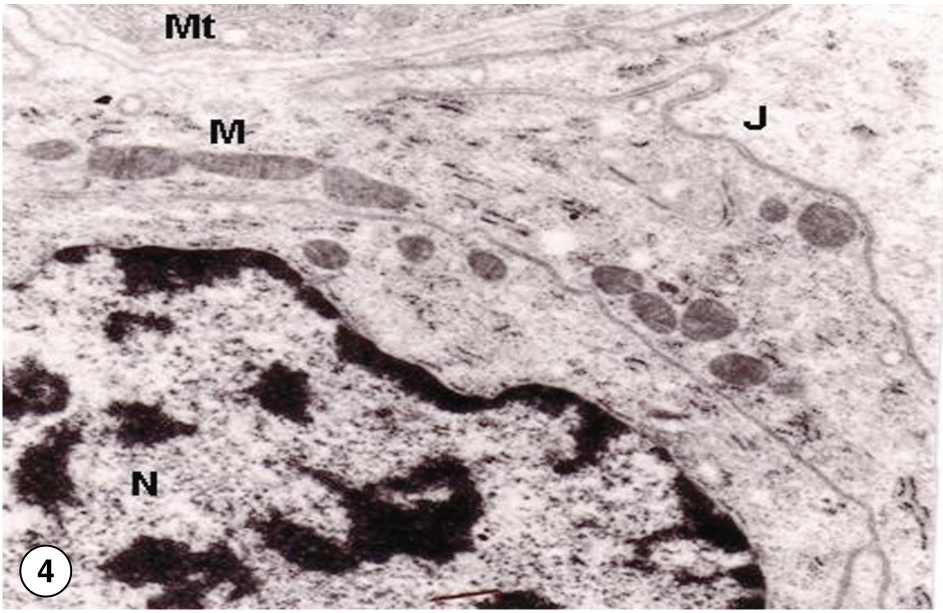

Fig. 4. T.S showing cuboidal epidermal cells involved in muscle attachment parallel micro-tubules (Mt) and mitochondria (M) are prominent in cytoplasm. J, septate junctions; N, nucleus. × 15.000.

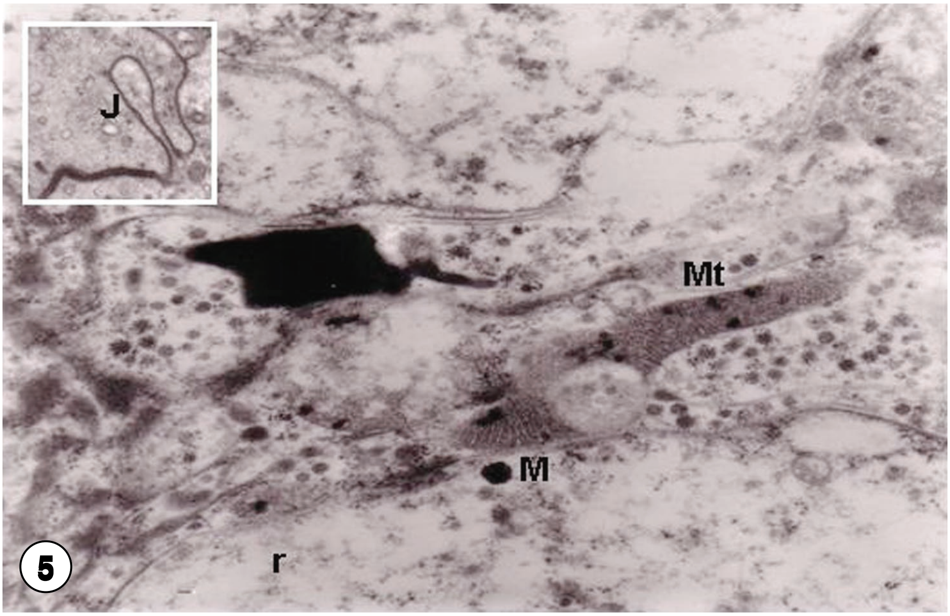

Fig. 5. Epidermal cells joined by septate junction (J) (Inset). The cytoplasm contains free ribosomes (R), mitochondria (M) and microtubules (MT). × 22.500.

some-like junctions (Fig. 6). Muscles are linked through the plasma membranes to bundles of microtubules which extend through the epidermal cells to points of anchorage on rod-like tonofibrillae within the cuticle (Fig. 7).

Discussion

The cuticle in the unfed nymphal *Hyalomma (H) anatolicum* is similar to that of other oxodid[18] and argasid tick[26].

The cuticle in this nymphal stage consists of sclerotized and non-sclerotized cuticular types and is divided into thin epicuticular types and thick procuticular layers. In this study, the presence of the deep extensible epicuticular folds are a common feature among ixodids, while the large amount of blood engorged during the final stage of feeding probably causes a rapid distension of the idiosoma and unfolding of the epicuticle. The epicuticle in argaside females and nymphs is tubercular-folded. In larvae there are regular rows of parallel folds similar to that in ixodids[15]. However, the ultrastructure of the epicuticle in *Hyalomma anatolicum* is similar to that observed in ixodids ticks *Haemaphsalis leporspalustris*[16&27] and *Hyalomma dromedrii*[6], *Boophilus microplus* and *Boophilus decoloratus*[28&29], and *Hyalomma asiaticum*[18], similar to that in insects and other arthropods[19&30], the wax canals are supposed to play an important role in transport of lipid from the epicuticle to the cuticular surface.

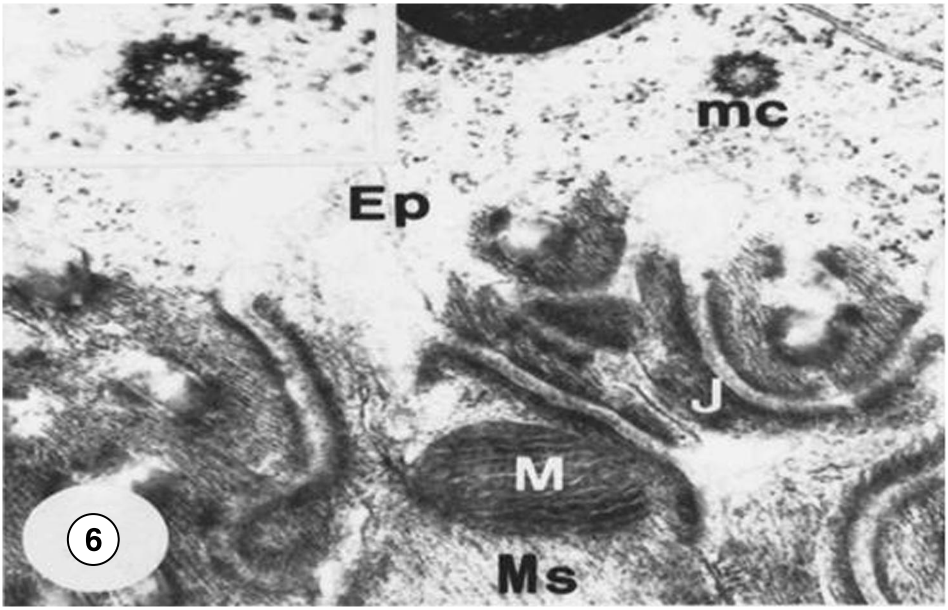

Fig. 6. Epidermis (Ep) attached to muscle fibers (Ms) by desmosome-like junctions (J). (mc) modified cilium with 10 peripheral microtubules (Inset). M, mitochondria. × 32.500.

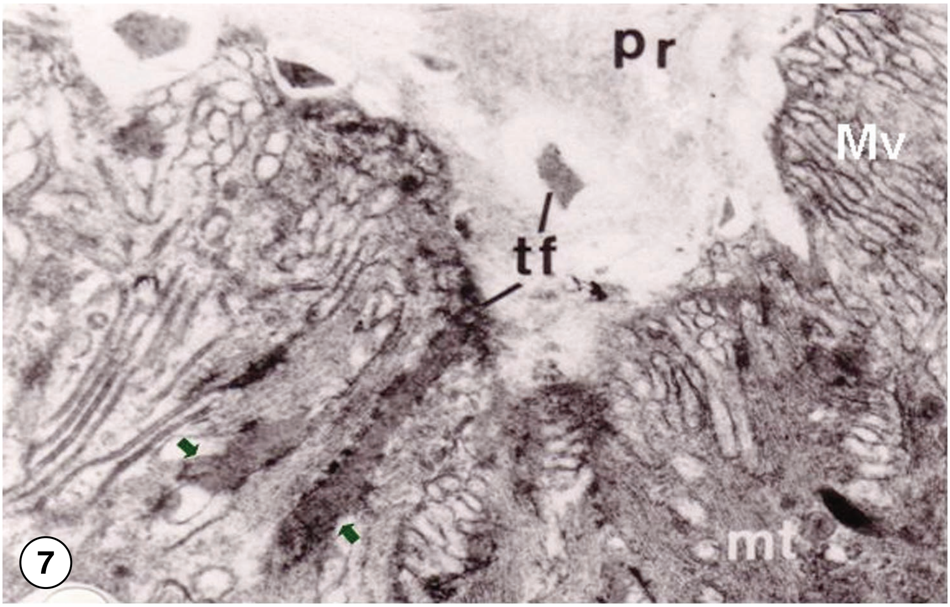

Fig. 7. Epidermal cells showing bundles of microtubules (Mt) inserted (arrowheads) on rod-like tonofibrillae (tf) extending inward from the procuticle (pr), Mv, microvilli. × 15.500.

In *Hyalomma asiaticum* epicuticle four layers differing in thickness and density are distinguished: a waxy layer, a lipid monolayer, a cuticulin layer; and an inner protein layer[18]. Furthermore, an additional cement layer is observed in the epicuticle of argaside tick *Ornithodrs moubata*[26].

As in other ixodids and other arthropods and insects, the growth of the cuticle is accompanied by considerable changes in the epidermis structure. The observations by light micrcoscope have shown that at the beginning of feeding, the epidermal cells greatly enlarge and rapidly proliferate[31].

The presence of numerous microtubcates in the cytoplasm may play an important role in the synthesis of nymphal integument. The function of these microtubules probably occurs after tick feeding or when are fully engorged. However, future study probably give more details.

The occurrence of septate desmosomes indicates a close functional relationship between the gland cells. However, these observations have been confirmed with other ultrastructural studies by transmission electron microscope for other ixodids ticks[18].

Rough endoplasmic reticulum detected in the cytoplasm appears to be responsible for the synthesis of protein and producing the epidermal secretion. This secretion is probably transported and released through extensive apical microvilli into the space between procuticle and epidermal cells. This observation is similar to that observed in other insects and ticks and thought to be ecdysis droplets containing proenzymes[18,32]. Also in insects, additional secretory granules released by epidermal cells, fusing into ecdysial droplet are a prelude to separation of “old” cuticle from the detected epidermis. The esterases in the adult female integument of organophosphate-resistant *Boophilus microplus* was detected[33]. They also reported that esterase activity was present in the integument female *Boophilus microplus (Canestrini)* ticks that are resistant to organophophates. Three esterases were purified from adult integument, which hydrolyze the substrates p-nitrophenylacetate and β-naphthyl acetate after comparison of OP-resistant strain and OP-susceptible strains. However, the detection of estarases probably plays an important role in detoxification mechanism in *B microplus* ticks at the integument. Also Villarino described a microblate biochemical assay for the detection of esterase activity in the tick integument, potentially a useful tool to detect esterase-mediated OP resistance in *B microplus* ticks.

Two secretory products responsible for delivering enzymes for procuticle lysis in *Hyalomma asiaticum* were described[18]: The first consists of the dense ecdysial droplets released at the preparatory time, the second of low-density granules secreted at the advanced stage of apolysis.

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التركيب الدقيق لغشاء حورية القراد الصلب هيالوما (هيالوما) أناتوليكوم، أناتوليكوم (إكسودويدي : إكسودودي)

فيصل عبد القادر بغدادي
قسم الأحياء ، كلية المعلمين ،
مكة المكرمة - المملكة العربية السعودية

المستخلص. قورن التركيب الدقيق لغشاء حورية القراد الصلب هيالوما (هيالوما) أناتوليكوم بمراحل التطور قبل التغذية باستخدام المجهر الإلكتروني.

يحتوي الغشاء على طبقة خلوية تحتها طبقة خلايا تحت بشرية (جلدية). يتكون جسم الطبقة الجلدية الخارجية من أجزاء صلبة وغير صلبة . وينقسم أيضًا إلى طبقة فوق جلدية وطبقة تحت جلدية مكونة من الليفات السميكة. القنوات المسامية في الطبقة التحت جلدية عبارة عن امتداد للقنوات الشمعية التي تفصل الطبقة الفوق جلدية . وتظهر خلال هذه المرحلة الامتدادات المتوازية للطيات (الانثناءات) الجلدية الخارجية والتي تؤدي إلى فقدان القنوات المسامية لتنظيمها المتوازي وتصبح على شكل حشوة .