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# STRUCTURE OF THE SALIVARY GLANDS OF THE UNFED MALE TICK *AMBLYOMMA CAJENNENSE* (FABRICIUS) (ACARINA: IXODIDAE)

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*Acini in the salivary glands of unfed male* Amblyomma cajennense *of different ages, were studied. The salivary glands consist of one agranular and three granular acini types. The agranular acini are directly attached to the medial and anterior portion of the main salivary duct, and to some branches of secondary ducts. A large, clear, central cell occupies the centre and this cell is in contact with the acinar lumen. There is no valve to the lumen. Granular acini consist of approximately six to fourteen cells (type II acini) or eight to thirteen (type III acini). The type II acini have three types of granular cells ("a", "b" and "c") and a valve; the type III acini have three types of granular cells ("d", "e" and "f") and a valve.*

Key words: *Amblyomma cajennense* – salivary glands – unfed male tick – Ixodidae

The histological structure of tick salivary glands has been studied by many authors (Till, 1959; Balashov, 1968; Kirkland, 1971; Coons & Roshdy, 1973; Binnington, 1978; Megaw & Beadle, 1979; Fawcett et al., 1981; Krolak et al., 1982; Shoura, 1987; Schumaker & Serra-Freire, 1991). The glands are considered to contain two functionally distinct types of acini: one concerned with the elimination of fluid, the other with the secretion of granular materials (Ribeiro & Spielman, 1986; Ribeiro et al., 1988, 1990; Fonseca, 1990). Acini that appeared to secrete both fluid and granular material were observed in the salivary glands of the partially fed females of *Dermacentor andersoni* (Meredith & Kaufmann, 1973), *Boophilus microplus* (Megaw, 1976) and *A. cajennense* (Olivieri & Serra-Freire, 1992); the acini are initially concerned with the granular secretion but become involved with fluid secretion as feeding progresses.

## MATERIALS AND METHODS

Unfed adult male *A. cajennense,* supplied by the W. O. Neitz, Parasitological Research Station of Universidade Federal Rural do Rio de Janeiro, were 30, 90 and 150 days old. Salivary glands were dissected out and were processed according to Olivieri & Serra-Freire (1992). For histological studies by light microscopy, serial 5 μm sections were mounted on glass slides and stained with hematoxilin and eosin, periodic acid-Schiff (PAS) and alcian blue (Michalany, 1980). The histological interpretation of the structure of the salivary gland was according to Fawcett et al. (1981) and Olivieri & Serra Freire (1992). All measurements were based repeated hundred times for each character.

## RESULTS

The paired racemose salivary glands are in the anteriolateral region of the body from the salivarium until the end of the podosoma. Anteriorly, the main salivary ducts enter the salivarium and posteriorly, they branch into secondary and tertiary ducts that end in efferent acini ducts. Most of the salivary glands were covered by midgut ramifications and Malphigian tubes. The glandular mass showed a distinct tendency to divide into separate clusters: the non-granular (type I) acini, which were attached to the main salivary ducts at the anterior and mid regions of the gland, and at the first quater of secondary ducts; granular type II acini which were attached to the secondary and tertiary ducts and were located in the anterior and mid regions of the gland; granular type III acini which were attached to the tertiary ducts and were located mainly in the

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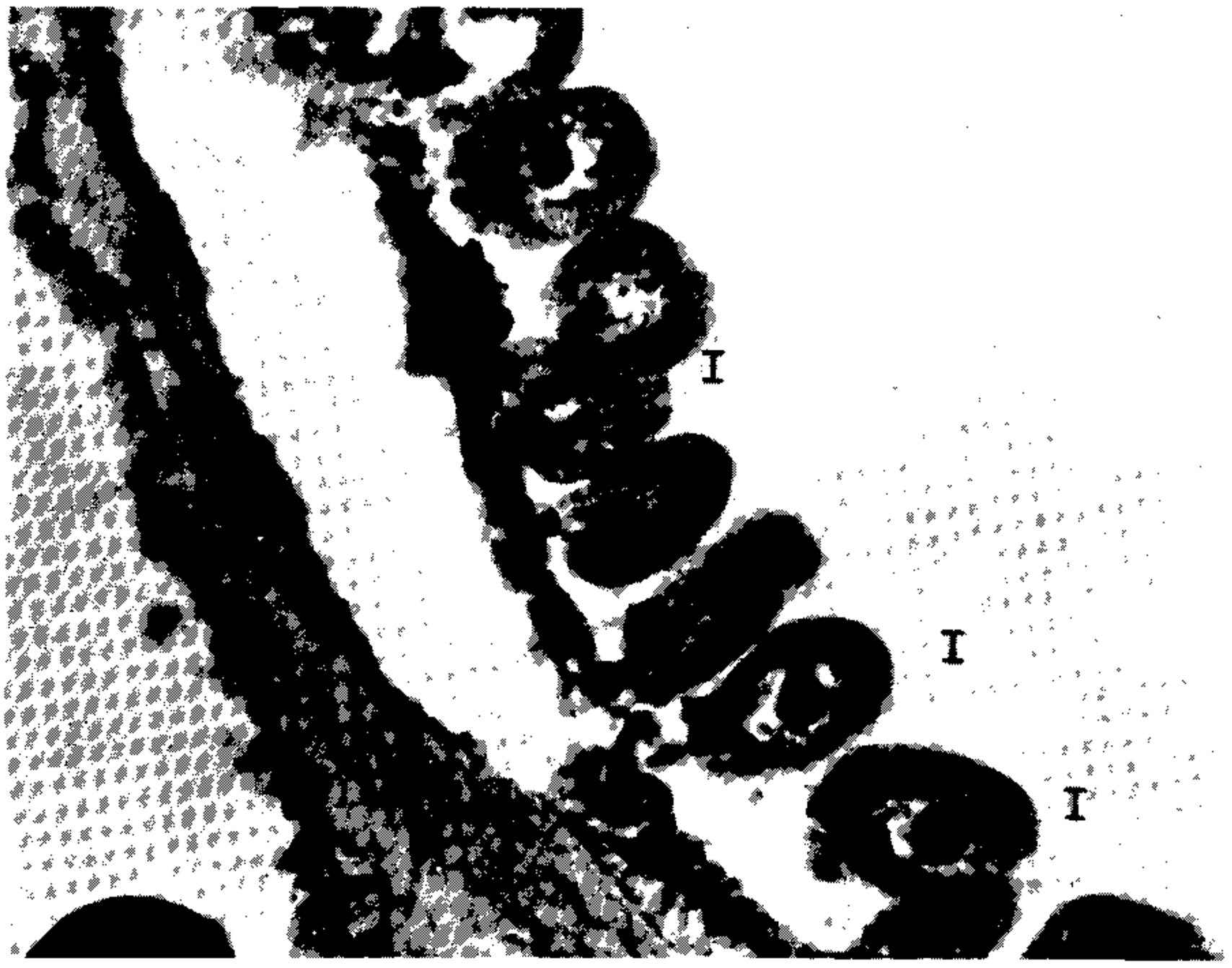


Fig. 1: photomicrograph of type I acini of the 30th day unfed male *Amblyomma cajennense* (400x).

posterior region of the gland. Adjacent granular secreting cells in the type III acini were separated by thin epithelial cells.

The salivary gland ducts, which are an epidermal derivative, were formed by a single layer of epithelial cells that rest on a basement lamina and is lined by cuticle on the apical surface. The secondary, tertiary and efferent ducts are structurally similar to the main duct.

Type I acini had a short cuticle-lined efferent duct 6-9 μm in length per 3 μm in width. The acini were separated into peripheral and central regions with a central lumen with acidophilic material easily observed in 30-day old unfed males (Fig. 1). At the central region of the acini there was a central cell, which has a nucleus that changed size during the unfed period; the biggest size was 7 x 11 μm. Other two to six small nuclei were present close to the central region of the acini; another two reniform nuclei were present at the base of the efferent duct.

The type I acini increased in size from the 30th to the 150th day unfed, and had four pyramidal cells (Figs 1-2).

Type II acini contained three types of granule-secreting cells that surround a central lumen and were separated from the salivary duct by a cuticle-lined tube. The entrance in the lumen of the acinus was guarded by a valve and extensions of the epithelial cells bordering the acinar duct. The efferent acini duct was 5-6 μm long per 3-4 μm wide, and each valvular arm of the valve was 4-6 μm length. Close to the end of efferent acini ducts, there were two small reniform of round shaped nuclei.

The type II acini increase in size from 22 x 21 μm to 31 x 24 μm at the 30th unfed day to 38 x 33 μm at the 150th day, and had one or two type "a" cells, two to six type "b" cells and three to six type "c" cells (Figs 3-4).

The type "a" cells were found at the valvular area of the acinus and are characterized by a full granular cytoplasm, with numerous aggregations of purple granules stained by H/E or PAS positive. Each granule was about 5-6 μm in diameter. The nuclei of type "a" cells were round sometimes with an irregular form; the size was 5-6 μm in diameter and had a dark blue colour with uniform disperse chromatin. There were no changes in the characteristics of cells "a" in ticks of different ages.

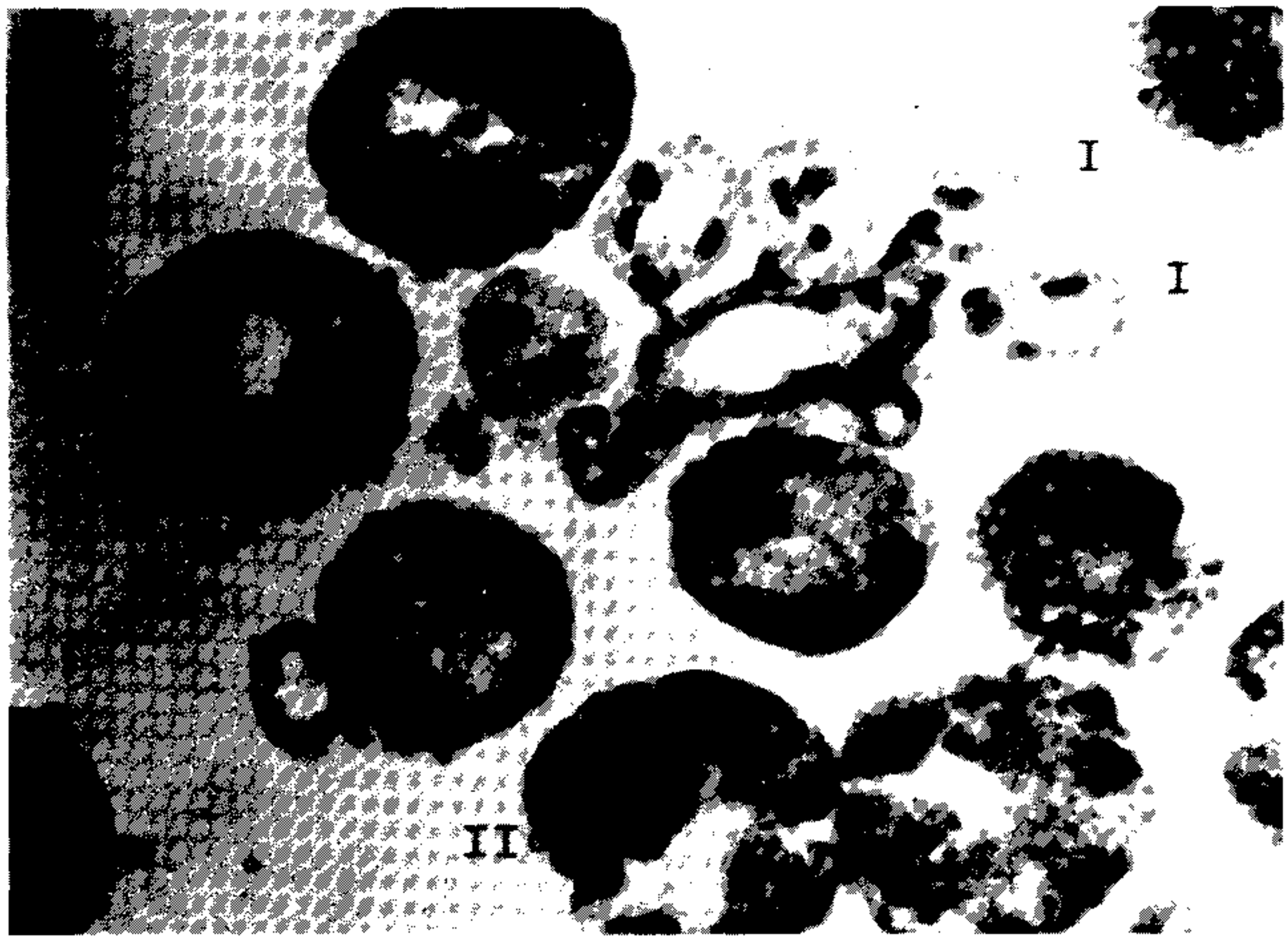


Fig. 2: photomicrograph of type I and type II acini of the 90th day unfed male *Amblyomma cajennense* (400x).

Fig. 3: photomicrograph of type II acini of the 30th day unfed male Amblyomma cajennense: note granule-secreting cells type "a" (a), type "b" (b) and type "c" (c); salivary ductus (DS); valve (v) (160x).

Type "b" cells were found between the valvular and the basal area and had a pyramidal form. The cytoplasm were filamentous. the nuclei were rounded, with a compact chromatin, and increased in size from 6, to 7 and to 8 μm from the 30th, to the 90th and to the

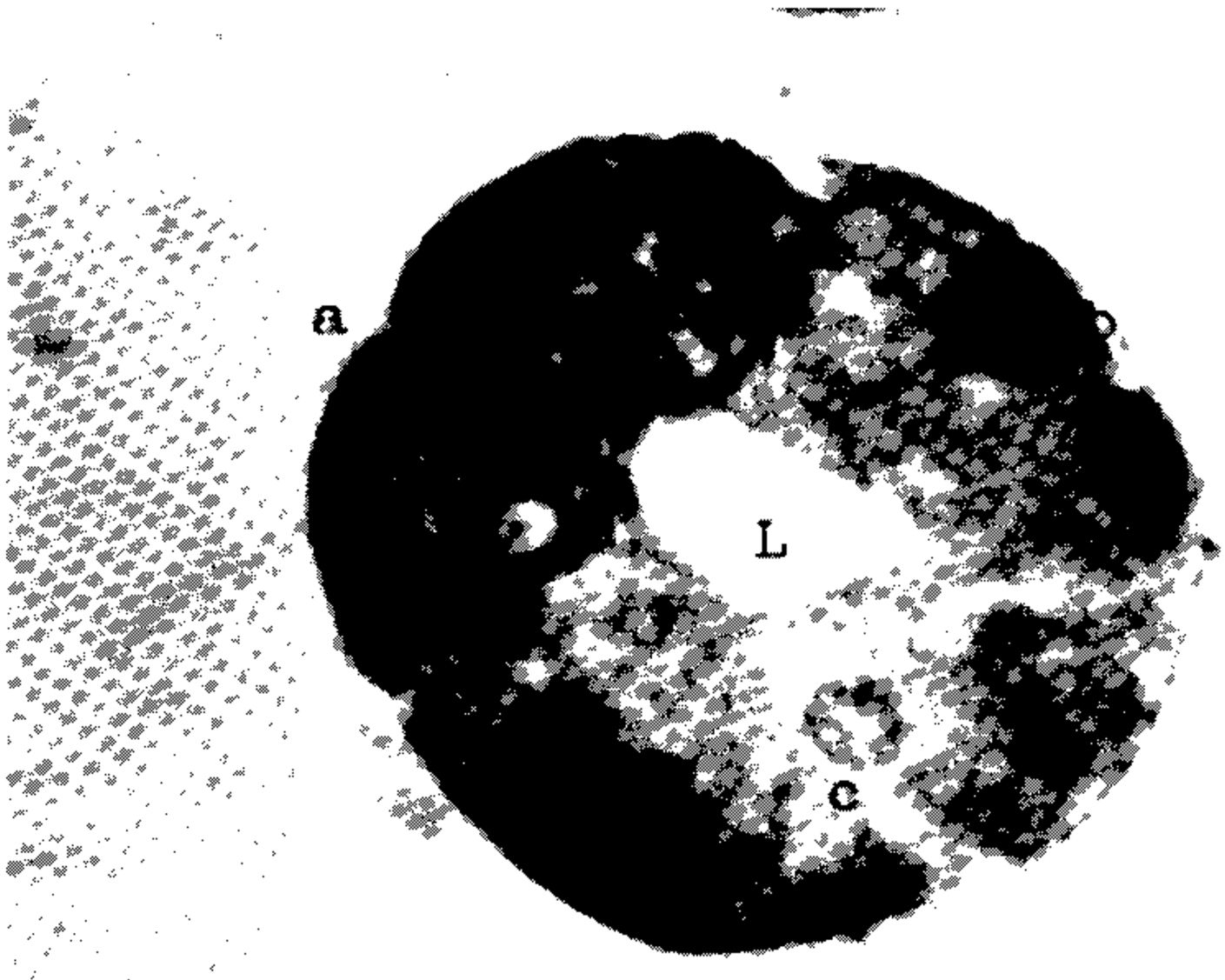


Fig. 4: photomicrograph of type II acini of the 150th day unfed male *Amblyomma cajennense:* note granule-secreting cells type "a" (a), type "b" (b) and type "c" (c); lumen (L) (200x).

150th unfed day. The type "b" cells lay between type "a" and type "c" and showed distinct amorphous opaque granules.

The type "c" cells were found at the basal area of the acinus, but in a few cases "c" cells were found at the valvular area. At day 30 it was hard to distinguish type "c" from type "b", because both were small. The cytoplasm had a fibrillar aspect. The nuclei were rounded, with 6-7 μm in diameter, and no change in size with increasing age was observed.

Branches in all three types of granule-secreting cells were simple and thin extensions of epithelial cells (Fig. 3).

The type III acini were similar or with more granular cells than were seen in type II acini. The type III acini also contain three types of granule-secreting cells. The attachment of type III acini to the duct system is identical to that described for type II acini. The size of each arm of valve was 4-6 μm length. The type III acini increased in size from 30 x 29 μm on day 30 to 37 x 33 μm on day 150, and had one or two type "d" cells, two to seven type "e" cells and three to five type "f" cells (Figs. 5-6). Adjacent to and between the granular cells there were thin agranular cells extending from the basal lamina to an agranular cell lining the acinar lumen.

One or two type "d" cells were found at the valvular area of acinus occupying a quater of the area; these cells were similar in position and granular composition to type "a" cells but each granule was about 3-4 μm in diameter. During the unfed period, histological studies showed various granular substructure probably representing stages of granular formation.

Two to seven "e" cells were present; they were large and had pyramidal format, occupying most of the acinar volume and readily distinguishable by large granular inclusions which were eosinophilic and reacting faintly with PAS. From day 30 to day 150 there were no changes on the nuclei size (6-9 μm), they were with a compact chromatin. The eosinophilic granules were also found in the acinar lumen.

The type "f" cells formed a group of three to five cells in the acinar fundus and the light microscopy could not show granules in the cytoplasm of those thin cells extending from the basal lamina to a lumen. The nuclei were 5-7 μm diameter (Fig. 6).

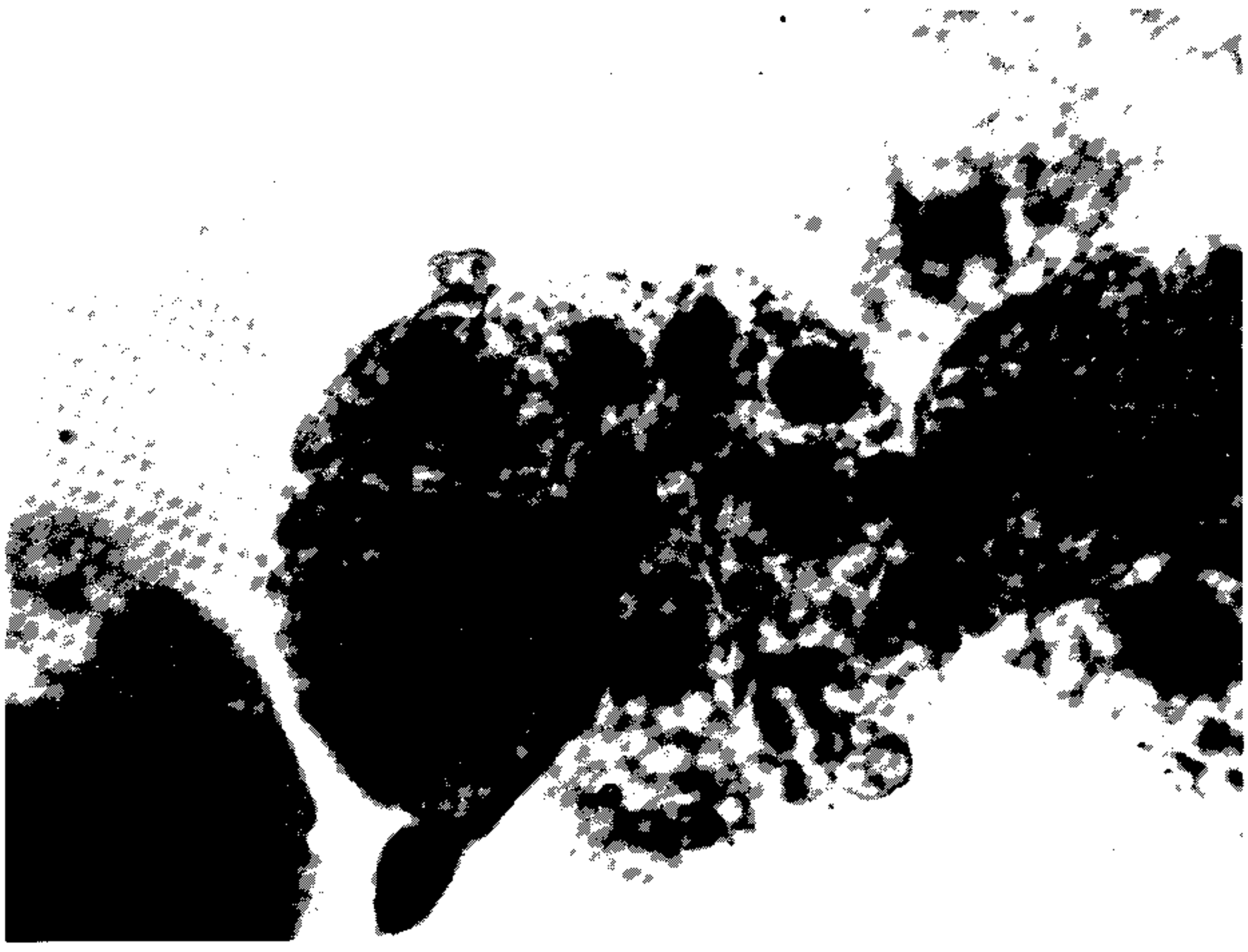

Fig. 5: photomicrograph of type III acini of the 30th day unfed male *Amblyomma cajennense:* note granule-secreting cells type "d" (d), type "e" (e) and type "f" (f) (160x).

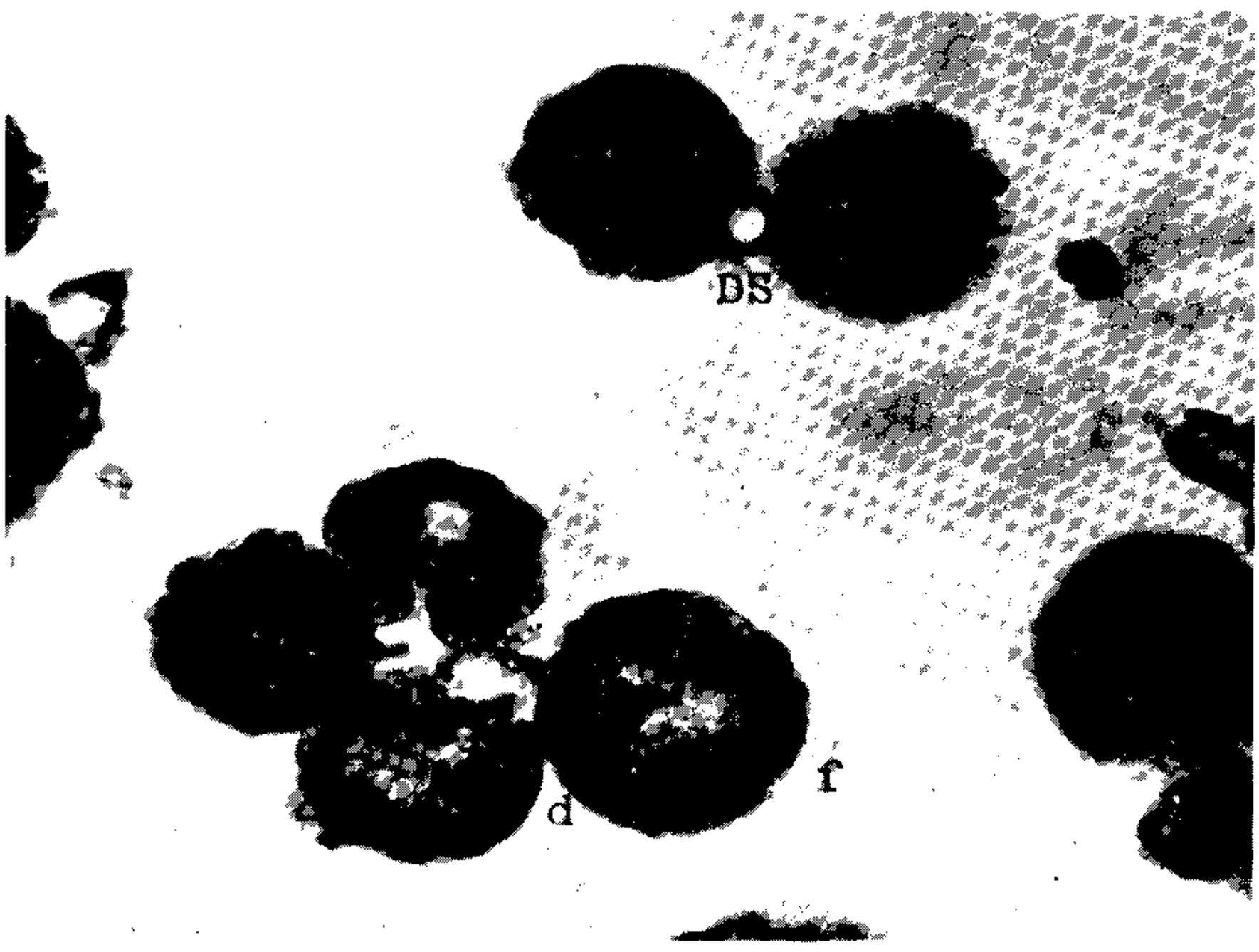


Fig. 6: photomicrograph of type III acini of the 90th day unfed male *Amblyomma cajennense:* note granule-secreting cells type "d" (d), type "e" (e) and type "f" (f); secondary salivary ductus (DS) (200x).

The type IV acini were hard to distinguish because the size was less than 25 x 22 μm.

## DISCUSSION

Various functions have been assigned to ixodid salivary gland; a cement substance secreted serves to strengthen the attachment of the tick to the host (Gregson, 1960; Moorhouse & Tatchell, 1966; Balashov, 1968; Tatchell & Moorhouse, 1968) or anaphylatoxin inactivating activity (Ribeiro & Spielman, 1986). In some ticks the salivary glands secrete paralytic toxins (Gregson, 1967; Serra-Freire, 1983; Magalhães et al., 1987), anti-complement toxin (Ribeiro, 1987) or prostacyclin (Ribeiro et al., 1988).

The examination of the morphology of the salivary glands of the *A. cajennense* unfed male using light microscopy ratifies previous findings regarding other species of ixodid ticks. It has also provided new insights related to the salivary gland structure in unfed male ticks of different ages.

The histological observations on the localization and cellular organization of nongranular acini (type I) in unfed *A. cajennense* salivary glands corroborate those of Chinery (1965), Balashov (1965), Coons & Roshdy (1973), Megaw & Beadle (1979) and Olivieri & Serra-Freire (1992). These observations differ from those of Kirkland (1971) who considered this type I acini in nymphal *Haemaphysalis leporispalustris* as unicelular, from Till (1961) who states that the nuclei protude into lumen in *Rhipicephalus appendiculatus.*

Considering the fact that all observations were achieved by light microscopy, we were unable to confirm previous data according to Krolak et al. (1982) related to the description of a "constrictor cell".

The present finding for long period suggested that in the *A. cajennense* unfed male the large central cell communicating with the nonvalvular acinar duct may collect fluids from adjacent cells and pass them to the lumen even though ticks were not feeding.

The increased size of the type I acini, from the 30th to the 150th unfed day, may be related to the process of water regulation, during a long unfed period, according Needham & Coons (1984).

The finding of two granular-secreting acini types in *A. cajennense* unfed male is similar to that observed in females of the same species (Olivieri & Serra-Freire, 1992).

Type II acinus is a concentric arrangement of six to fourteen granular cells around a central lumen interrupted by a cuticular valve. The valves would prevent fluid, already flowing along the duct, from entering subsequent acinus.

Type III acini was similar to the type II except for the number of cells. The same concentric arrangement of granular cells around a centrally located lumen and an apparent cuticle-lined valve was also identified.

Types "a" and "d" cell in *A. cajennense* unfed male were similar between them and to those of females (Olivieri & Serra-Freire, 1992), and *H. spinigera* (Chinery, 1965) and *Dermacentor variabilis* (Coons & Roshdy, 1973). Binnington (1978) and Megaw & Beadle (1979) described the structure of salivary glands and acini from *Boophilus microplus.* These two studies differ considerably in their interpretation of cell types. Binnington (1978) used histochemical staining methods with light microscopy and Megaw & Beadle (1979) used electron microscopy. Krolak et al. (1982) reported results based on a study of the *A. americanum* female and agree with those of Megaw & Beadle (1979). According to these studies there are two complex granular cells in type II acini and only one in type III. Binnington (1978) described a larger number of granular cell types (six) in type II acini based mostly on the intensity of granular reactions to chemical stain, and three different granular cell types in the type III acini. As a consequence of these differences in interpretation of cell types presentation the results based on a histological light microscopy of the *A. cajennense* unfed male are in agreement with those of Binnington (1978), except for the number of cell types in type II acinus, considering mainly that the applied methodology was identical.

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