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

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Acini in the salivary glands of female tick specimens of Amblyomma cajennense *unfed at both postnymphal and adult phases, were studied. The salivary glands are consisted by three acini, one agranular and two granular. The agranular acini are directly attached to the anterior portion of the main salivary duct, consisting of cells without valve. A relatively large, clear, central cell occupies most of the alveolar midsection. The central cell is in contact with the acini lumen. Granular acini consist of approximately seven to fourteen cells (type II acini) or seven to sixteen (type III acini). The type II acini have three types of granular cells ("a", "b" and "c") and valve; the type III acini have another three types of granular cells ("d", "e" and "f") also presenting a valve.*

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

The histological structure of tick salivary glands has been studied by several authors (Till, 1959; Balashov, 1968; Kirkland, 1971; Coons & Roshdy, 1973; Binnington, 1978; Megaw & Beadle, 1979; Fawcett et al., 1981; Krolak et al., 1982). However, the new evidence suggests that the structure of ixodid salivary glands may be more complex than was originally appreciated. The glands were considered to contain two functionally distinct types of acini: one concerned with the elimination of fluid, the other with the secretion of granular materials. Acini that appeared to be secreted with both fluid and granular material were observed in the salivary glands of partially fed female *Dermacentor andersoni* (Meredith & Kaufmann, 1973) and *Boophilus microplus* (Megaw, 1976); the acini were initially concerned with the granular secretion but become involved with fluid secretion as feeding progresses.

MATERIALS AND METHODS

Unfed adult female tick of *Amblyomma cajannense*, 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 removed by cirurgical method and fixed in Zenker solution (Behmer et al., 1976), dehydrated in an alcohol series and embedded in parafin wax, individualy. For histological studies by light microscopy 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 structure of the salivary gland was achieved according to the methodology of Till (1959, 1961).

RESULTS

The paired racemore salivary glands are in the anteriolateral region of the body and extend along ventrolateral portion of both sides of the sagital plane of body from salivarium until the fourth coxa. Anteriorly, the main salivary ducts enter the salivarium and posteriorly, branch into secundary and terciary ducts that end in eferent acini ducts. Aproximately 66% of the salivary glands were covered by midgut ramification and Malphigian tubes. The glandular mass showed a distinct tendency to divide into separate clusters; they are spheroids acini that can be grouped into three types: the non-glandular (type I) acini, all of these acini were attached to the main salivary ducts at the anterior and mid regions of the gland, and at the first quarter of secundary ducts; granular type II acini were attached to the

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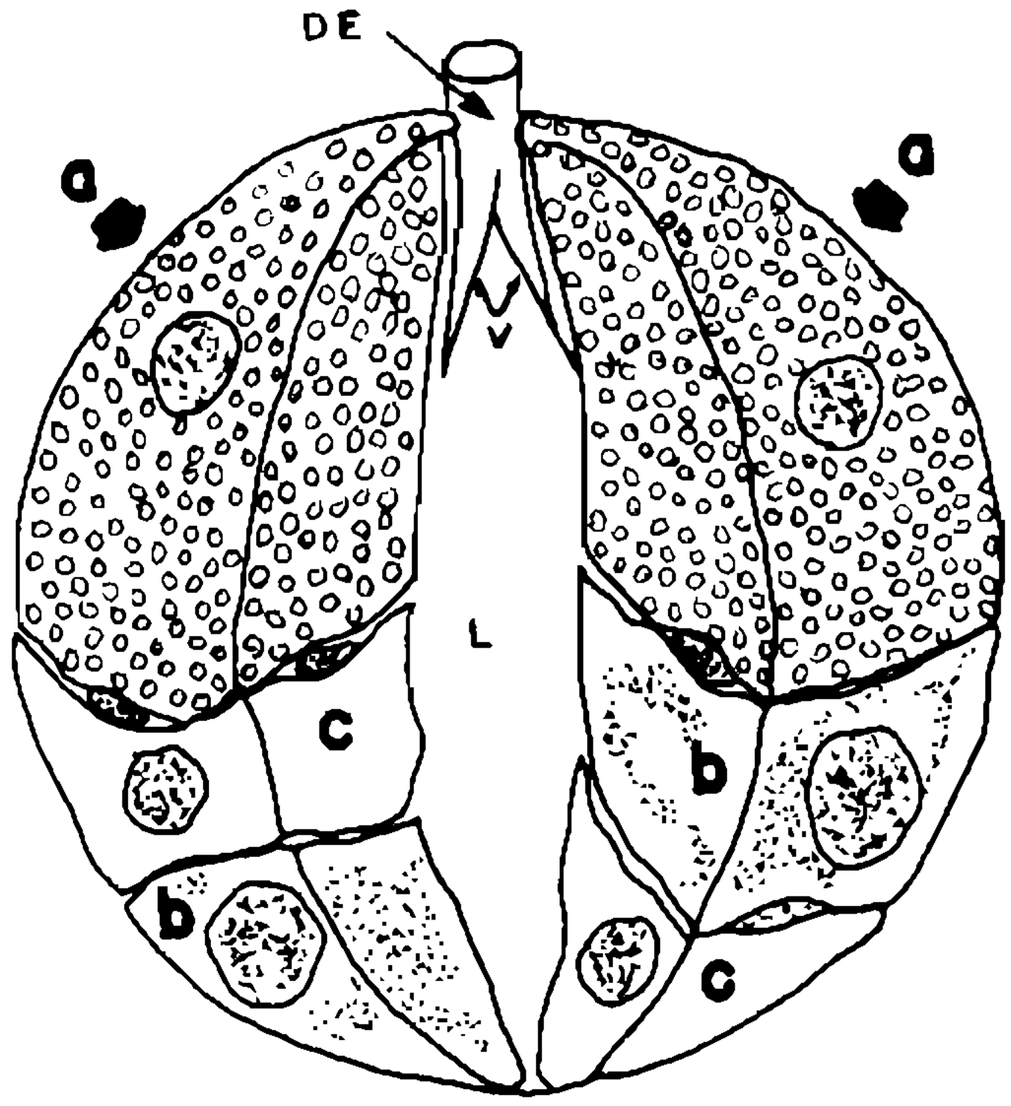

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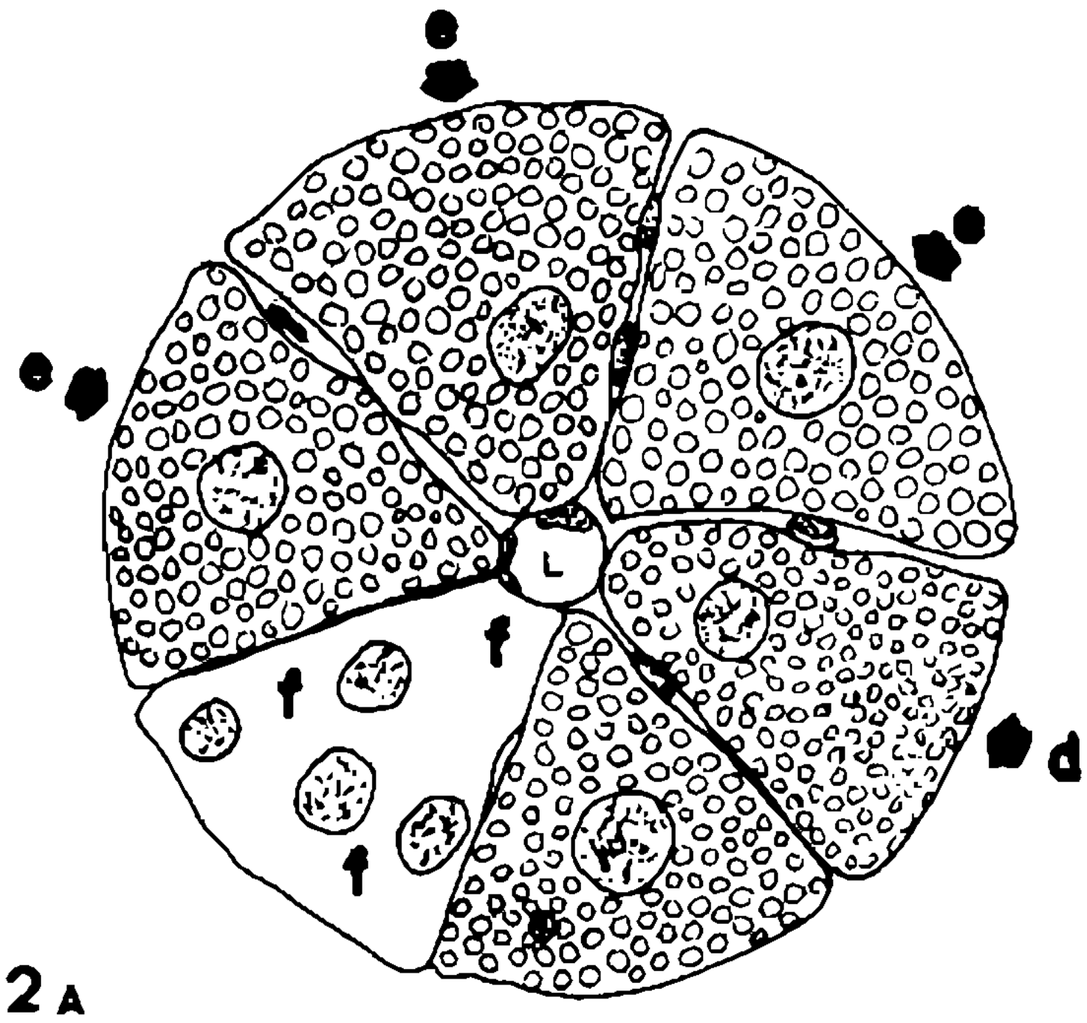

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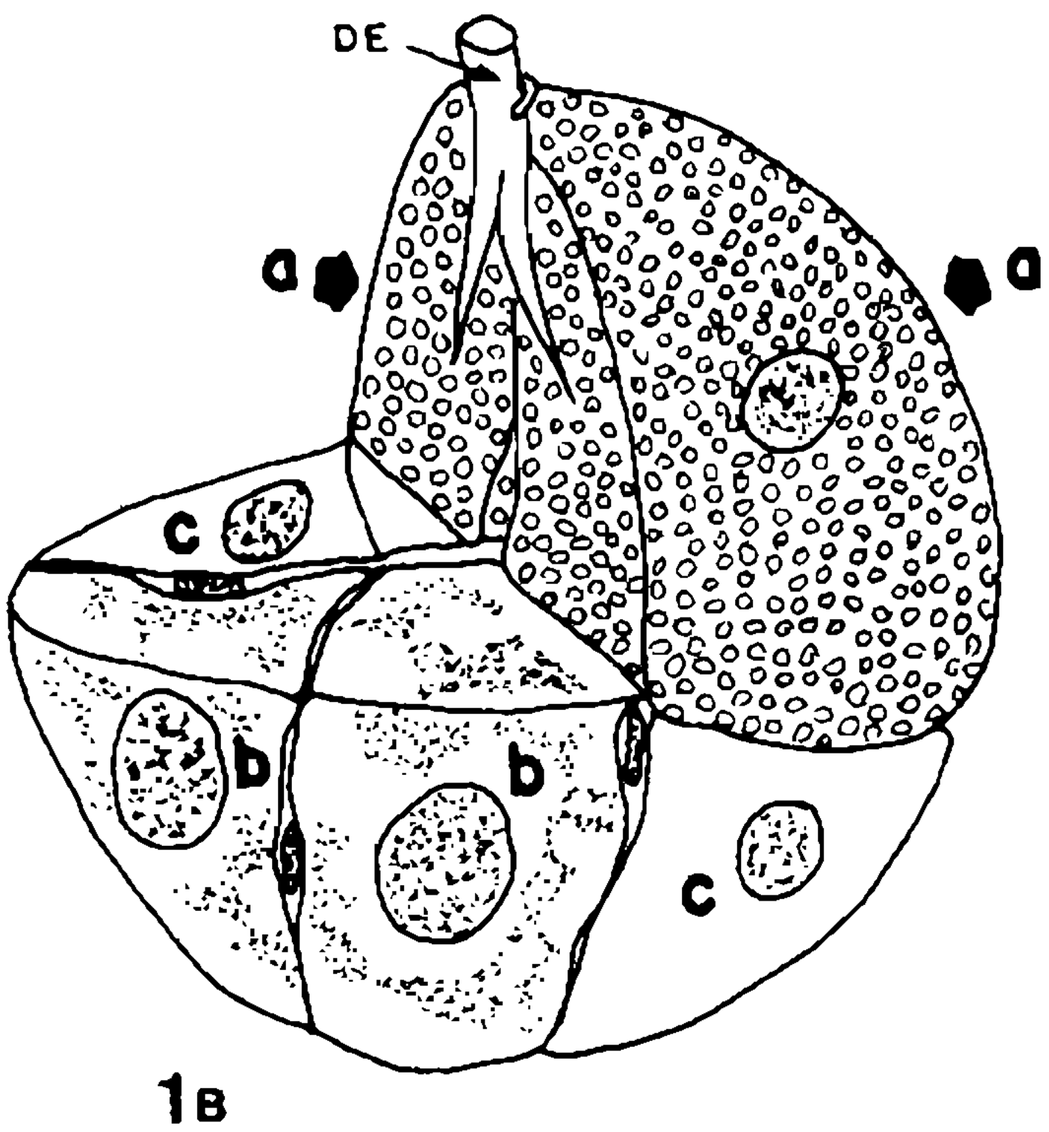

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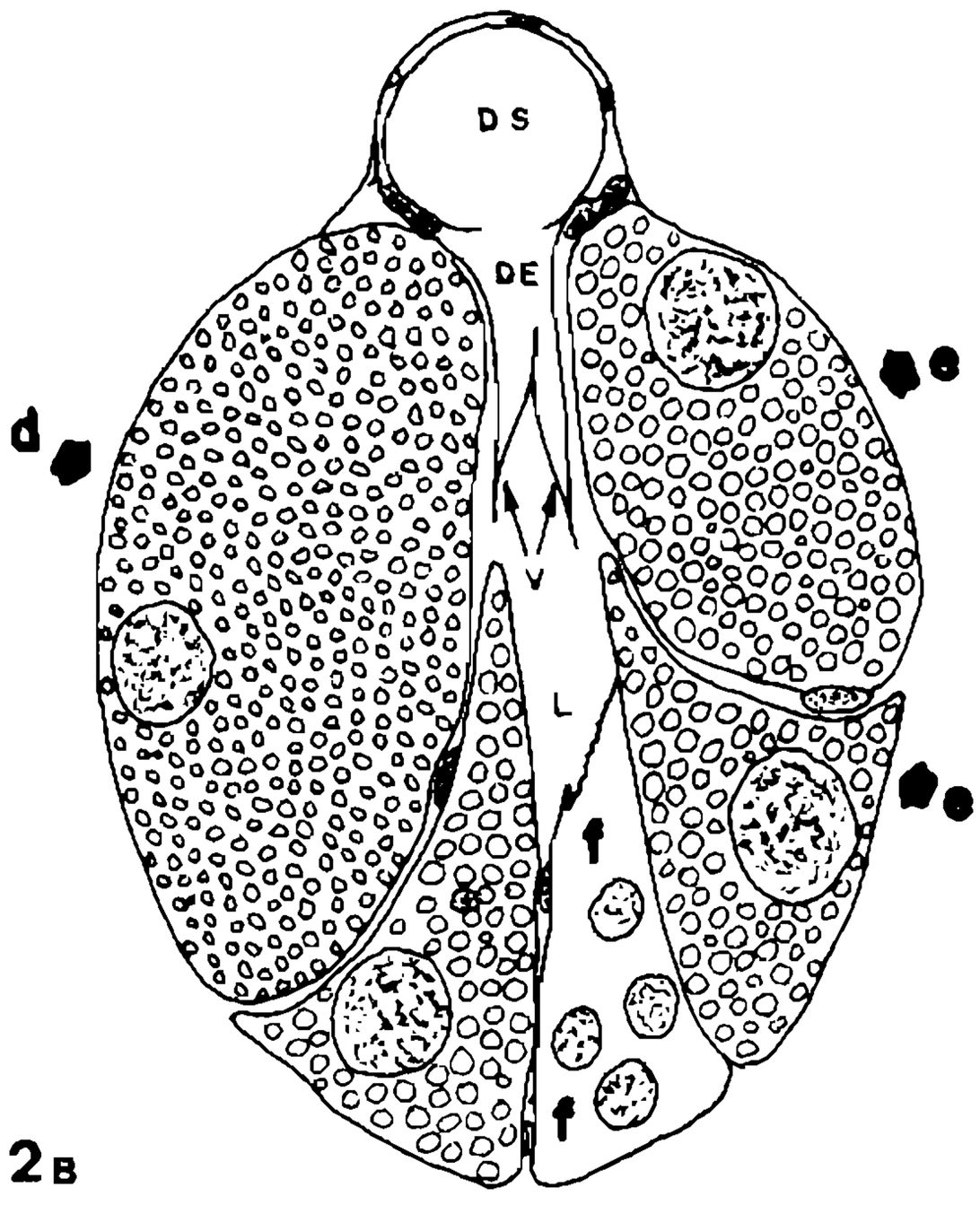

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Fig. 1 A-B: drawing of type II acinus unfed female *Amblyomma cajennense:* granule secreting cells type "a" (a), type "b" (b) and type "c" (c); note presence of a valve (v) at the apherent acinar ductus (DE) guarding acinus lumen (L). A. longitudinal quadrant removed; B. transversal quadrant or dial removed.

Fig. 2 A-B: drawing of type III acinus unfed female *Amblyomma cajennense:* granule secreting cells type "d" (d), type "e" (e) and type "f" (f); note presence of a valve (v) at the epherent acinar ductus (DE) guarding acinus lumen (L) and secondary salivary ductus (DS). A. transversal section; B. longitudinal section.

secundary and terciary ducts and are located in the anterior and mid regions of the gland. Adjacent granular type III acini were attached to the terciary ducts and are located mainly in the posterior region of the gland. Adjacent granular secreting cells in the type II and type III acini are separated by thin epithelial cells. A diagrammatic representation on type II and III acini is showed in Figs 1-2.

The salivary gland ducts, which are an epidermal derivative, are formed by a single layer of epithelial cells that rests on a basement lamina and is lined by cuticle on the

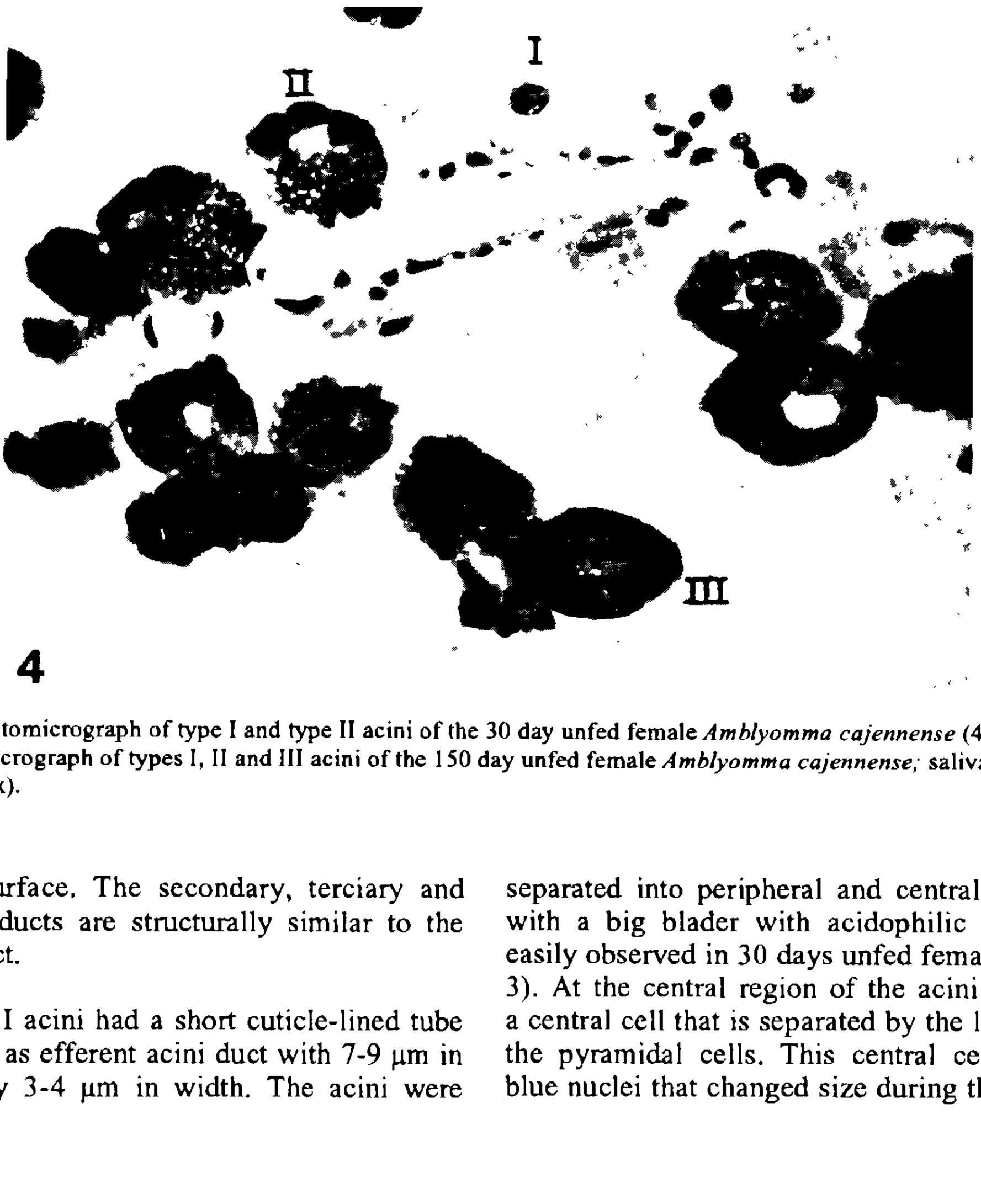
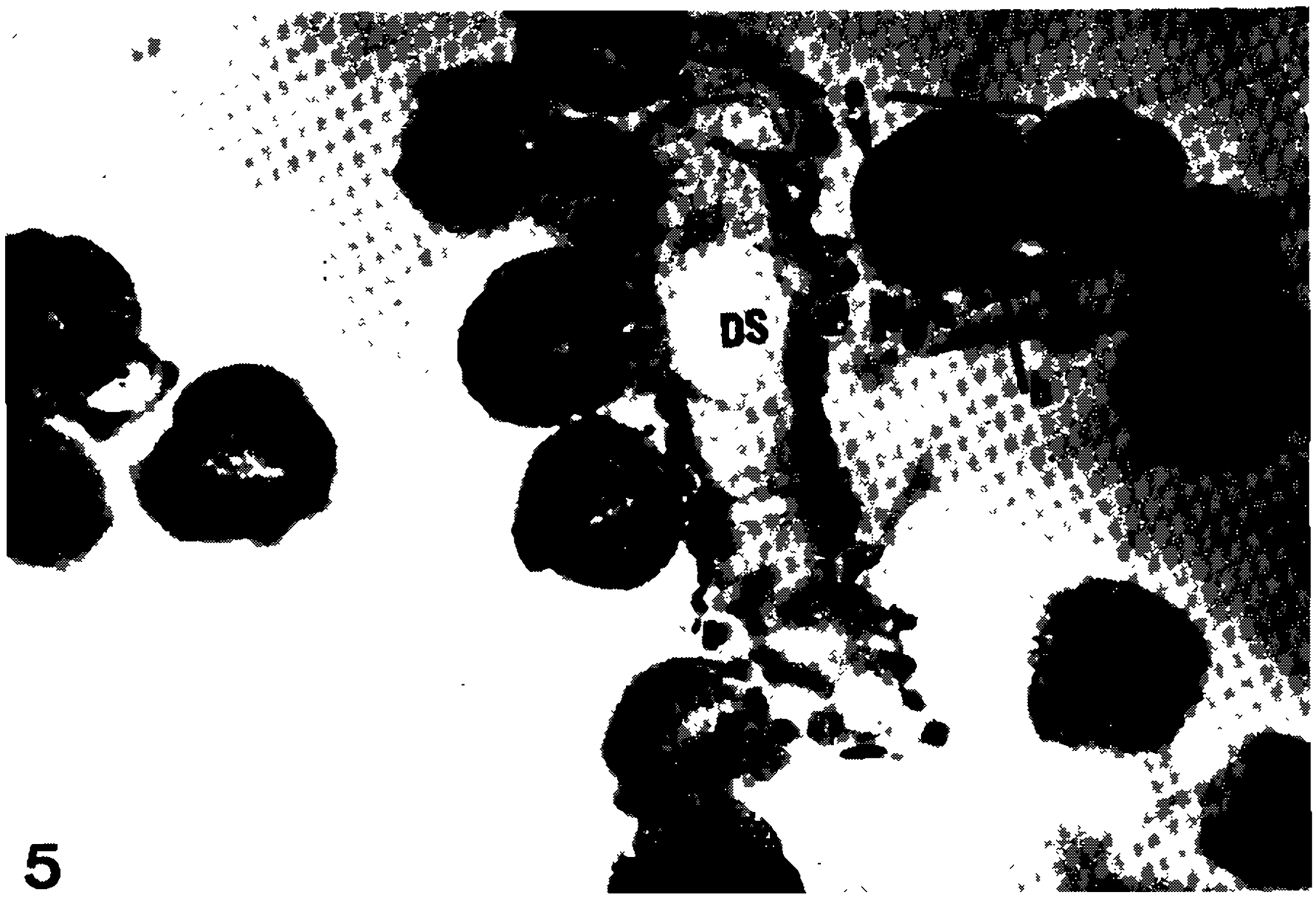


Fig. 3: photomicrograph of type I and type II acini of the 30 day unfed female *Amblyomma cajennense* (400x). Fig. 4: photomicrograph of types I, II and III acini of the 150 day unfed female *Amblyomma cajennense;* salivary ductus (DS) (160x).

apical surface. The secondary, terciary and efferent ducts are structurally similar to the main duct.

Type I acini had a short cuticle-lined tube designed as efferent acini duct with 7-9 µm in length by 3-4 µm in width. The acini were separated into peripheral and central regions with a big blader with acidophilic material easily observed in 30 days unfed females (Fig. 3). At the central region of the acini there is a central cell that is separated by the lumen of the pyramidal cells. This central cell has a blue nuclei that changed size during the unfed



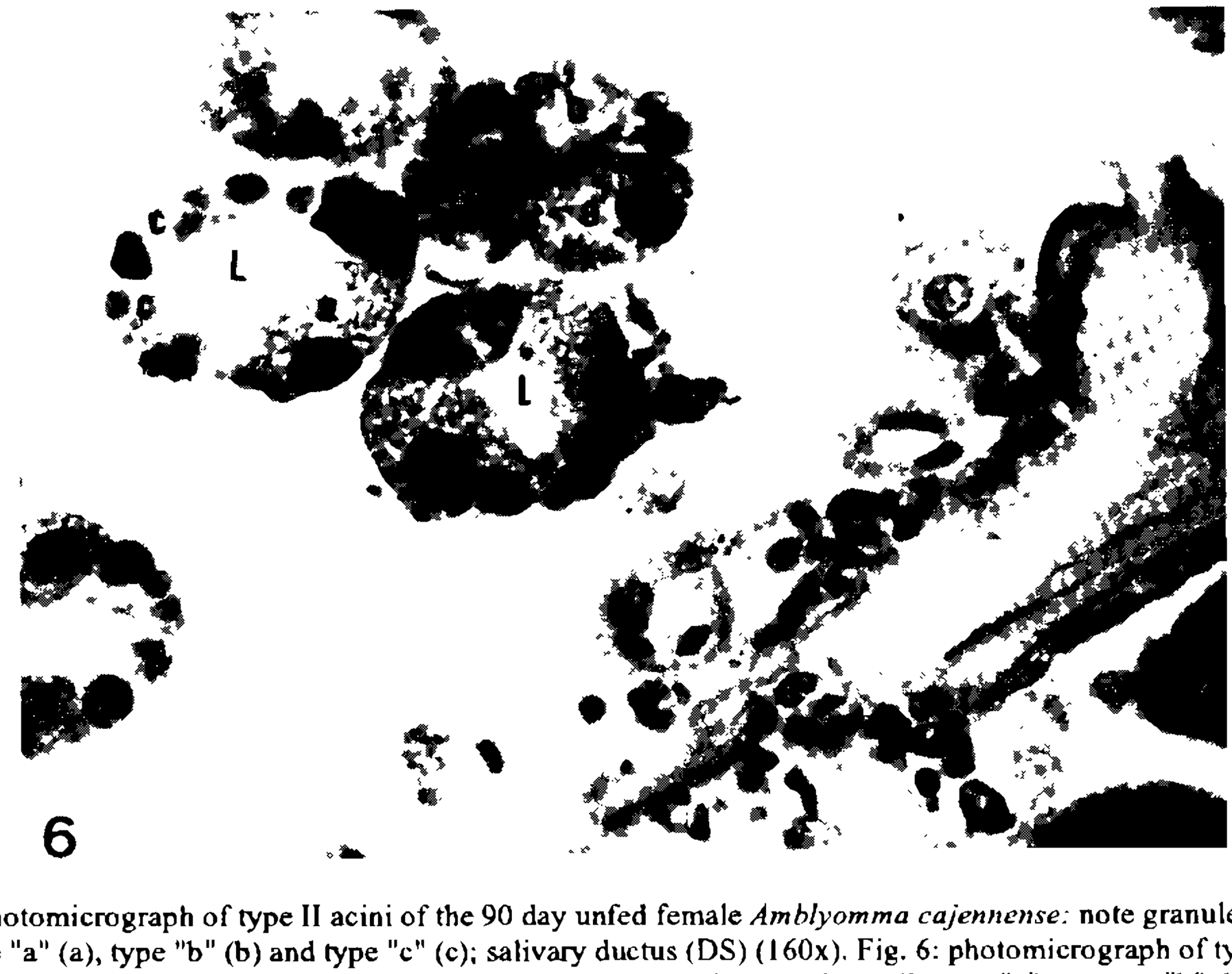


Fig. 5: photomicrograph of type II acini of the 90 day unfed female *Amblyomma cajennense:* note granule-secreting cells type "a" (a), type "b" (b) and type "c" (c); salivary ductus (DS) (160x). Fig. 6: photomicrograph of type II acini of the 150 day unfed female *Amblyomma cajennense:* note granule-secreting cells type "a" (a), type "b" (b) and type "c" (c); lumen (L) (200x).

period; the biggest size were 13 x 9 μm, at its the 150 unfed day. Other two to six small nuclei were present closed to the valvular region of acini.

The type I acini increased in size from the 30 to the 150 day unfed, and had four pyramidal cells (Figs 3-4).

Type II acini contain three types of granule-secreting cells that surround central lumen and are separated from the salivary duct by a

Fig. 7: photomicrograph of type III acini of the 30 day unfed female *Amblyomma cajennense:* note granule-secreting cells type "d" (d), type "e" (e) and type "f" (f) (160x): Fig. 8: photomicrograph of type III acini of the 150 day unfed female *Amblyomma cajennense:* note granule-secreting cells type "d" (d), type "e" (e) and type "f" (f); secundary salivary ductus (DS) and lumen (L) (200).

cuticle-lined tube. The entrance in the lumen of the acinus is guarded by a valve and extensions of the epithelial cells bordering the acinar duct. The efferent acini duct is 6 µm long by 3-4 µm wide, and each valvular arm of the valve is 3-5 µm. Just closed to the end of efferent acini duct there are two to four small kidney-like or round shaped nuclei.

The type II acini increased in size from 25 x 24 µm at 30 unfed day to 42 x 33 µm at 150 unfed day, and had one or two type "a" cells,

Fig. 9: photomicrograph of type II acini of the 90 day unfed female *Amblyomma cajennense:* note presence of a valve at the epherent acinar ductus.

two to six type "b" cells and four to six type "c" cells (Figs 5-6).

The type "a" cells are found at the valvular area of the acinus and are characterized by a full granular citoplasm, with numerous aggregations of purple granules stained by H/E. Each granule was about 3-4 μm in diameter. The nuclei of type "a" cells were round or egg shaped sometimes with an irregular form; the size were 4-6 μm in diameter and had a dark blue colour with uniform disperse cromatin. Along the unfed period, there were no changes in the characteristics of cells "a".

The type "b" cells are found between valvular and basal area and have a pyramidal form with the top near the central area of acinus. The citoplasm is filamentous and with a shinning pink colour shaded with blue around the 30 unfed day. From the 90 to the 150 unfed day the citoplasm changed to violet blue colour with a few shine. The nuclei were rounded, dark blue with a compact cormatin. The type "b" cells lie between type "a" and type "c" and showed distinct amorphous opaque granules.

The type "c" cells are found at the basal area of the acinus, but in a few cases this "c" cells were found at the valvular area. The citoplasm stained in strong pink or in pale violet, and had a fibrilar aspect. The nuclei were round or egg-shaped with 4-6 μm in diameter stained in blue. No change in this aspect along the unfed period was observed.

Branches in all three types of granule-secreting cells were simple and thin extensions of epithelial cells (Figs 1-2).

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 the valve is the same of the arms into type II acini. The type III acini increased in size from 26 x 24 μm at the 30 unfed day to 42 x 32 μm at the 150 unfed day, and had one or two type "d" cells, two to six type "e" cells and four to eight type "f" cells (Figs 7-8). Adjacent to and between the granular cells there are thin agranular cells extending from the basal lamina to an agranular cell lining the acinar lumen.

The type "d" cells are found at the valvular area of the acinus occupying a quarter of area; this cells are similar in position and granular composition to type "a" cells. During the unfed period, histological studies show various granu-

lar substructures probably representing stages of granular formation.

The type "e" cells were large pyramidal occupying most of the acinar volume and readily distinguishable by large granular inclusions containing orthochromatic granules and reacting faintly with PAS. From the 30 to the 150 unfed day there were no changes on the nuclei size (7 to 9 μm) that were dark blue with a compact cromatin. The orthochromatic granules were also found in the acinar lumen.

The type "f" cells from a group in the acinar fundus and the light microscopy could not show granules into the citoplasm of those thin cells extending from the basal lamina to a lumen with a pink colour; the nuclei were blue with 4 to 6 μm diameter (Figs. 7-8).

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). In some ticks the salivary glands secrete paralytic toxins (Gregson, 1967; Serra Freire, 1983; Magalhães et al., 1987).

The examination of the morphology of the salivary glands of unfed female *A. cajennense* using light microscopy ractifies previous findings regarding other species of ixodid ticks. It has also provided new insights related to the salivary gland structure in female ticks, with different periods of unfeeding.

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) and Megaw & Beadle (1979). These observations differ from those of Kirkland (1971) who considered this type I acini in nymphal *Haemaphysalis leporispalustris* as unicellular, from Till (1961) who states that the nuclei protrude into the lumen in *Rhipicephalus appendiculatus* and Krolak et al. (1982) who described a "constrictor cell" just anterior to the central cell and posterior to the alvelar duct that was not observed now. The present findings for long unfed period suggested that in unfed female *A. cajennense* the large central cell communicating with the nonvalvular acinar duct may collect fluids from adjacent cells and passes them to the lumen even though in the non attached tick.

The finding of two granular-secreting acini types in female *A. cajennense* is similar to that observed in other species (Till, 1961; Balashov, 1968; Meredith & Kaufman, 1973; Binnington, 1978; Krolak et al., 1982); however Kirkland (1971) described only one type of granule-secreting acinus in salivary glands of nymphal *H. leporispalustris*.

Type II acini is a concentric arrangement of seven 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 acini however an alternative or additional function may be reflected by any inherent resistence of the valve (Fig. 9).

The type III acini is 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 is also identified.

Types "a" and "d" cell in unfed female *A. cajannense* are similar between them and to those of *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 presentation. 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 female *A. americanum* and agree with those of Megaw & Beadle (1979). According to these studies these are two complex granular cells in type II acine 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 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 unfed female *A. cajennense* accord with those of Binnington (1978), except for the number of cell types in type II acini, mainly considering that the applied methodology was identical.

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