

ULTRASTRUCTURE OF THE OVARY OF *DERMATOBIA HOMINIS* (DIPTERA: CUTEREBRIDAE). I. DEVELOPMENT DURING THE 3RD LARVAL INSTAR

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The ultrastructure and distribution of gonial and somatic cells in the ovary of Dermatobia hominis was studied during the 3rd larval instar. In larvae weighing between 400 and 500 mg, the ovary is partially divided into basal and apical regions by oblong somatic cells that penetrate from the periphery; these cells show ovoid nucleus and cytoplasm full of microtubules. In both regions, gonial cells with regular outlines, large nucleus and low electron-density cytoplasm are scattered among the interstitial somatic cells. These later cells have small nucleus and electron-dense cytoplasm. Clear somatic cells with small nucleus and cytoplasm of very low electron-density are restrict to the apical region of the gonad. Degenerating interstitial somatic cells are seen in the basal portion close to the ovary peduncle. During all this larval period the morphological features of the ovary remain almost the same. At the end of the period there is a gradual deposition of glycogen in the cytoplasm of the somatic cells, increase in the number and density of their mitochondria plus nuclear modification as membrane wrinkling and chromatin condensation in masses.

Key words: ovary – bot-fly – Diptera – *Dermatobia hominis*

Histology of post-embryonic development of the gonads in *Dermatobia hominis* was studied by Lello (1979) and Lello et al. (1984, 1985). The authors verified that when the larva changes to the 3rd and last instar it weighs around 50 mg, and when it leaves the host to pupate its weight varies from 700 to more than 900 mg. They also showed that sexual morphological differentiation of the gonads occurs in larvae weighing around 400 mg. In these the ovaries are two egg-shaped structures measuring 200 to 250 μm of diameter with a groove in their median portion. Each gonad has a peduncle through which it is fastened to a fibrous ring that surrounds a traqueal branch. This branch is a tertiary ramification of the main lateral one that penetrates into the interior of the larval body at its third posterior region. Histologically, the ovary is surrounded by an acellular tunica externa and in its interior somatic and gonial cells are scattered without a definite arrangement. The exceptions are the oblong somatic cells located in the region of the groove, dividing the ovary in two regions: apical and basal. No great variation is observed

in the ovarian structure until the end of the larval period.

Ultrastructural studies of the development of the ovary, in insects, give special attention to the relationship between gonial and nurse cells during the germarium formation (King et al., 1956, 1968; Koch & King, 1966; Koch et al., 1967; King, 1975; Rousset, 1978; Buning, 1979 and others). The studies in earlier phases of development to our knowledge have not been published.

In this paper we describe the ultrastructure of ovarian development in *D. hominis* during the final larval period.

MATERIALS AND METHODS

Third-instar larvae weighing over 400 mg were obtained from natural infested cattle in the region of Botucatu, Sao Paulo State, Brazil. They were dissected in insect saline solution and the ovaries fixed in 2.5% glutaraldehyde in 0.1 M, pH 7.3 phosphate buffer during 3 h. After post-fixation in 0.1% OsO_4 in the same buffer, they were dehydrated and embedded in Araldite. Ultrathin sections were observed in a EM-301 Philips.

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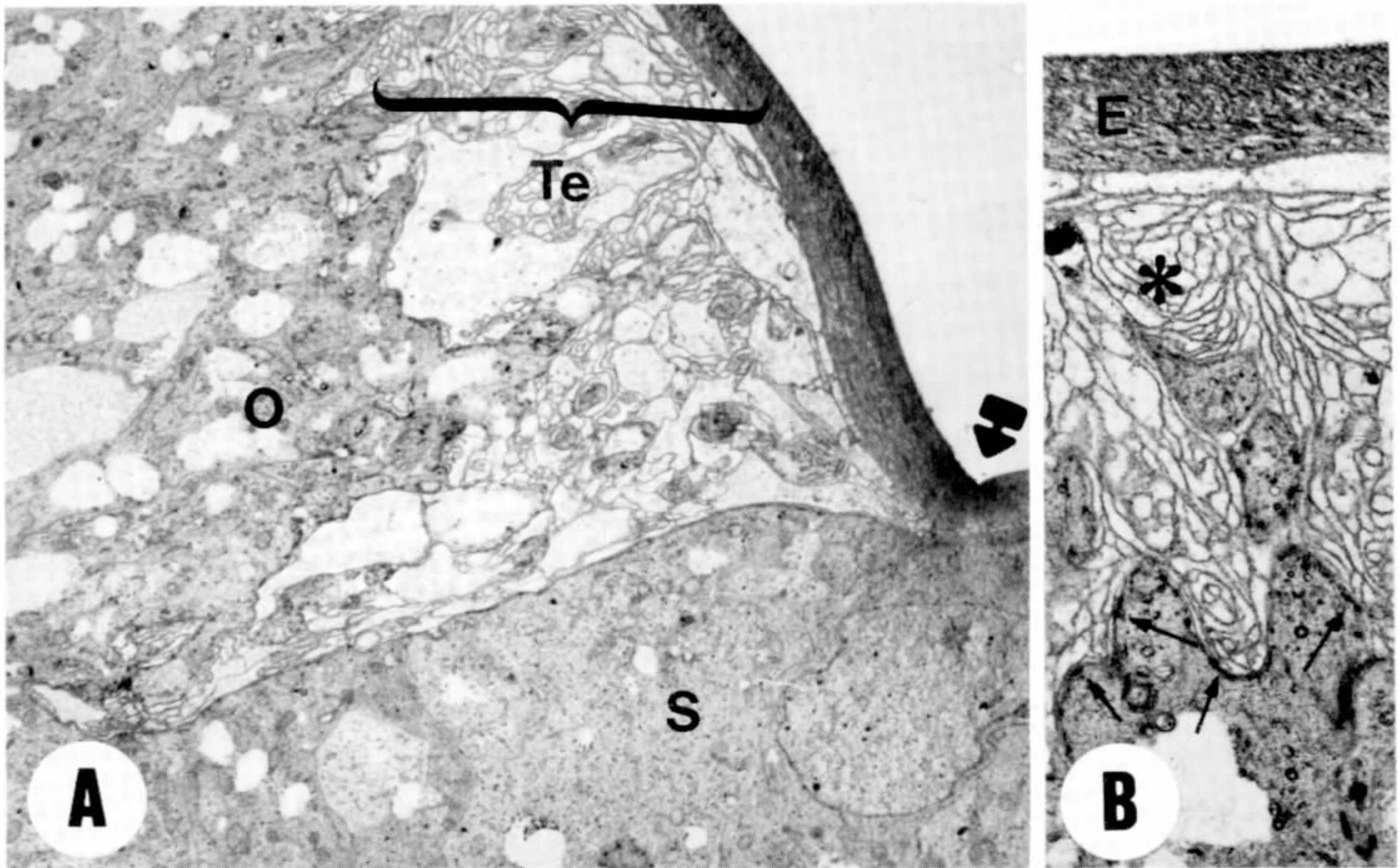


Fig. 1: ovary in 400-500 mg larva. A: peripheric and median region. Groove region (arrow), interstitial somatic cells (S), oblong somatic cells (O), tunica externa (Te). X 5000. B: detail of the tunica externa. Condensed external portion (E) and the loose framework pattern of the internal one (*). Oblong somatic cells (O); hemidesmosomes (arrows). X 11000.

RESULTS

Ovaries of larvae weighing between 400 and 500 mg are surrounded by a thick acellular filamentous tunica. This tunica externa has two portions: one external, with a dense arrangement of fibrils and one internal that shows a network pattern of loose fibrils. In the region of the groove the tunica presents a slight depression where its internal portion penetrates partially into the gonad (Fig. 1A, B). At the basal portion of the ovary, the tunica continues surrounding the peduncle and emits a thin layer of material separating the gonad from the basal peduncle.

There are two kinds of cells in the ovary: gonial and somatic recognized by nuclear and cytoplasmic characteristics. The gonial cells are large with regular outlines, their cytoplasm showing low electron-density, scarce rough endoplasmic reticulum, large round nucleus with loose chromatin and prominent nucleolus (Fig. 2). Three kinds of somatic cells are identifiable: a) *Oblong somatic cells*: these cells are spindle shaped, having a large number of

microtubules, free ribosomes, scant rough endoplasmic reticulum and a few small mitochondria; the nucleus is oval chromatin loose and nucleolus well defined (Fig. 3A, B); b) *Interstitial somatic cells*: they are small, with irregular nuclear and cellular shape; its cytoplasm is denser than the gonial ones with few and scattered organelles (Fig. 2); c) *Clear somatic cells*: they are prismatic with round nucleus, chromatin loose, large low-density cytoplasm with few organelles, giving them the characteristic features of clear cells (Fig. 3C).

The location of these cells is characteristic: covering the ovary internally and accompanying the depression formed by the tunica externa, there is a layer of oblong somatic cells (Figs. 1A, 3A) that penetrates parallel to the groove dividing incompletely the gonad in two regions: apical and basal. No junctions were observed between these cells; only the peripheric ones have hemidesmosomes in contact with the internal portion of the tunica externa (Fig. 1B). Enlarged intercellular spaces among these cells give, initially, the erroneous impression of intense vacuolization (Figs 1A, 3A). In

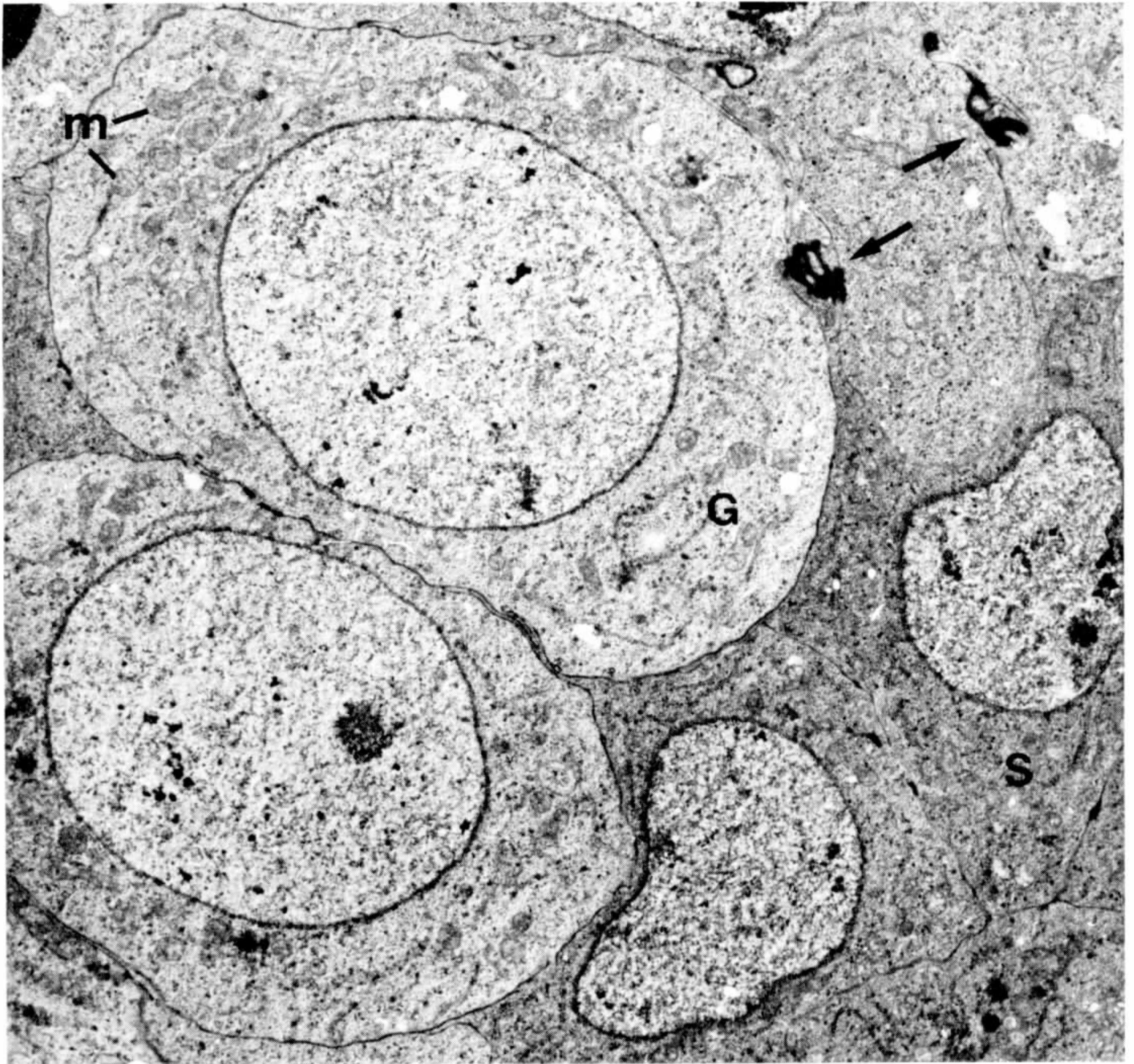


Fig. 2: apical region of the ovary in 400-500 mg larva. Gonial cells (G); interstitial somatic cells (S); deposit of amorphous material (arrows); mitochondria (m). X 9000.

the apical region, immediately under the tunica externa, there is one layer of clear somatic cells. Between this layer and the groove region, as well as in the basal region of the ovary, there are gonial and interstitial somatic cells without a definite arrangement. The gonial cells are found isolated or in pairs. Desmosome-like junctions were found between gonial cells as well as between the interstitial ones. Among all kinds of cells there are interstitial deposits of amorphous electron-dense material (Fig. 2).

Close to the peduncle some interstitial somatic cells show degenerating features as denser cytoplasm, vacuoles of variable contents,

dilated rough endoplasmic reticulum and nucleus with condensed chromatin (Fig. 4A).

This cellular arrangement is maintained until the end of the larval period. In larvae weighing between 500 and 600 mg the gonial cells show irregular condensation of nuclear chromatin; the cytoplasm, that has a poor vacuolar system, shows an increase in its mitochondria electron-density (Fig. 4B). From this time on, the somatic cells whatever they are, show nuclei with irregular outlines, more evident in the interstitial ones and scattered chromatin masses along with the nucleolus; the cytoplasm contains a variable amount of glycogen (Figs 4B, 5B).

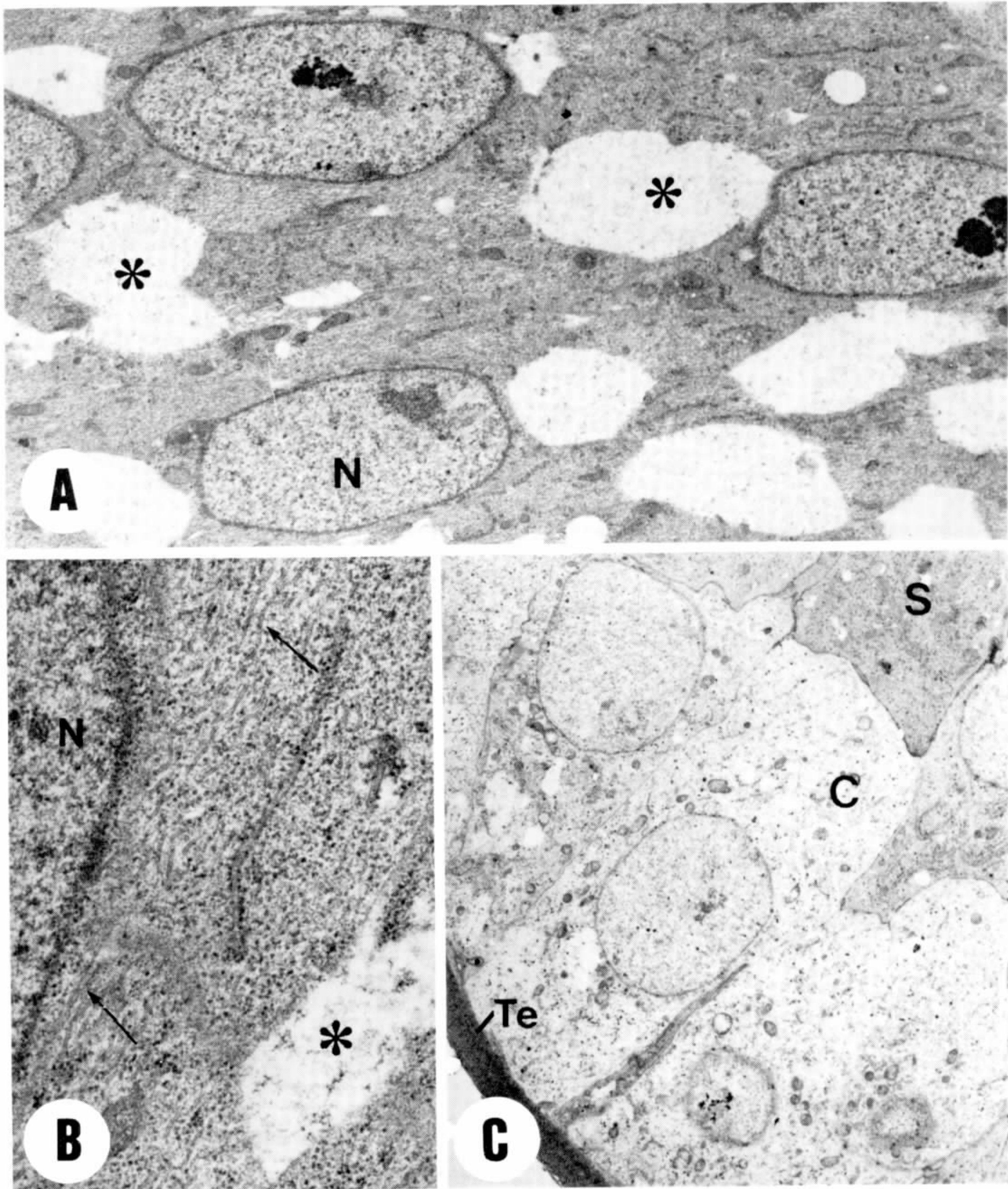


Fig. 3: ovary in 400-500 mg larva. A: oblong somatic cells in the median region. Nucleus (N); intercellular spaces (*). X 4200. B: detail of the oblong somatic cells. Microtubules (arrows); nucleus (N); intercellular space (*). X 10500. C: apical region. Clear somatic cells (C); interstitial somatic cells (S); Tunica externa (Te). X 6300.

In larvae weighing between 600 and 700 mg, the former clear somatic cells in the ovary apical region, now are denser and present somewhat irregular nuclei with few blocks of chromatin and prominent nucleolus; small deposits of glycogen and condensed mitochondria are seen in the cytoplasm (Fig. 6A). In

the basal region of the ovary the cells are loosely arranged (Fig. 5A) and it is possible to distinguish somatic from gonial cells because the former show large accumulations of glycogen (Fig. 5B). Both gonial and somatic cells show great number of electron-dense mitochondria (Figs 5B, 6A).

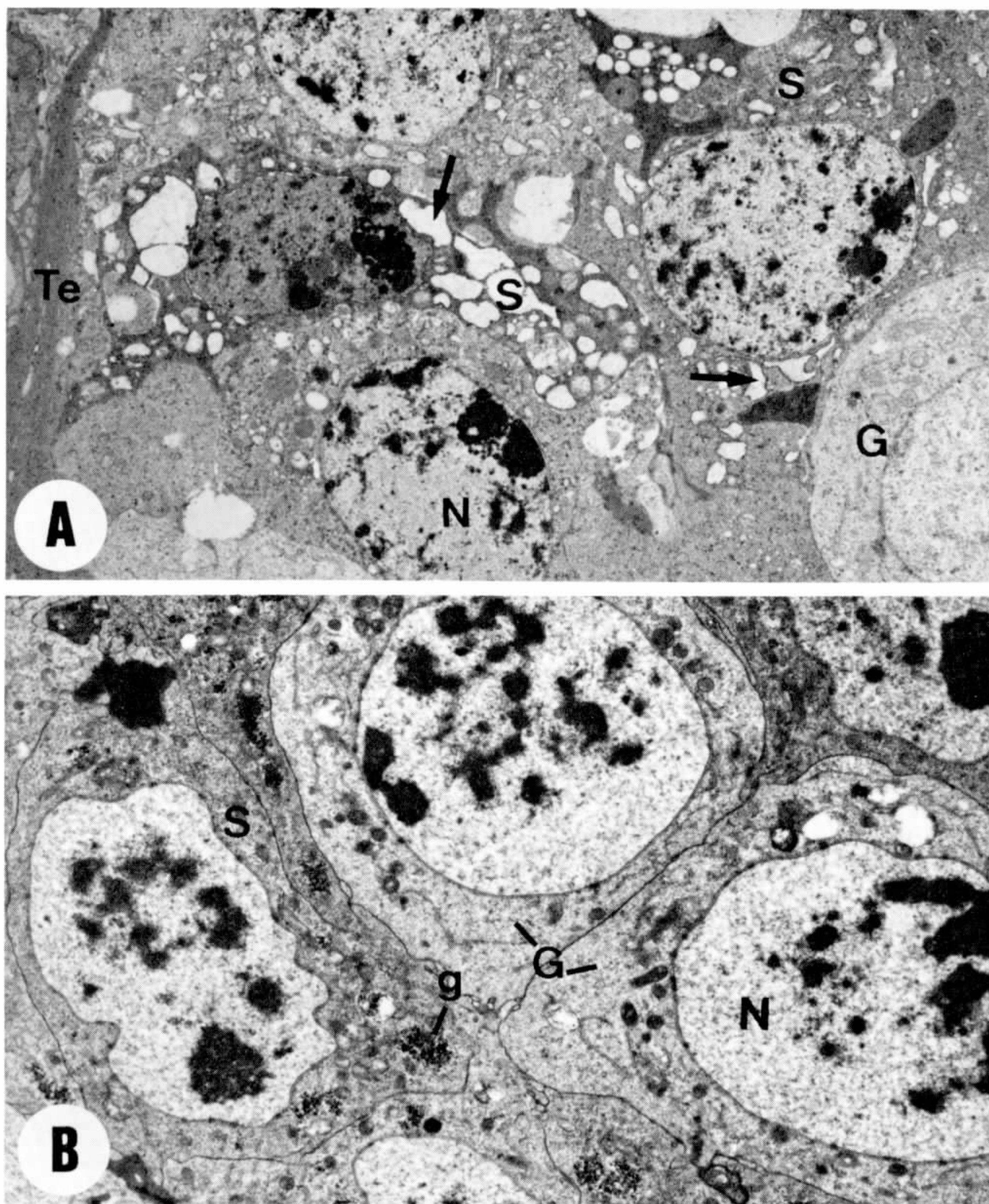


Fig. 4: A: basal region of the ovary in 400-500 mg larva. Tunica externa (Te); gonial cells (G) degenerating interstitial somatic cells (S); nucleus (N); dilated rough endoplasmic reticulum (arrows). X 6500. B: basal region of the ovary in 500-600 mg larva. Gonial cells (G); interstitial somatic cells (S); nucleus (N); glycogen (g). X 10000.

In larvae weighing more than 700 mg up to the end of the 3rd instar, the same features of the ovaries are maintained. Nevertheless there is an increase in the chromatin condensation and in the irregularity of the somatic cells nuclear membrane; some of the interstitial somatic cells are in mitosis. (Fig. 6B).

DISCUSSION

Since ovarian development occurs during pupation, the majority of papers on ultrastructure of insect ovaries cover this period (King & Vanoucek, 1960; King & Koch, 1963; Brown & King, 1964; Koch & King, 1966;

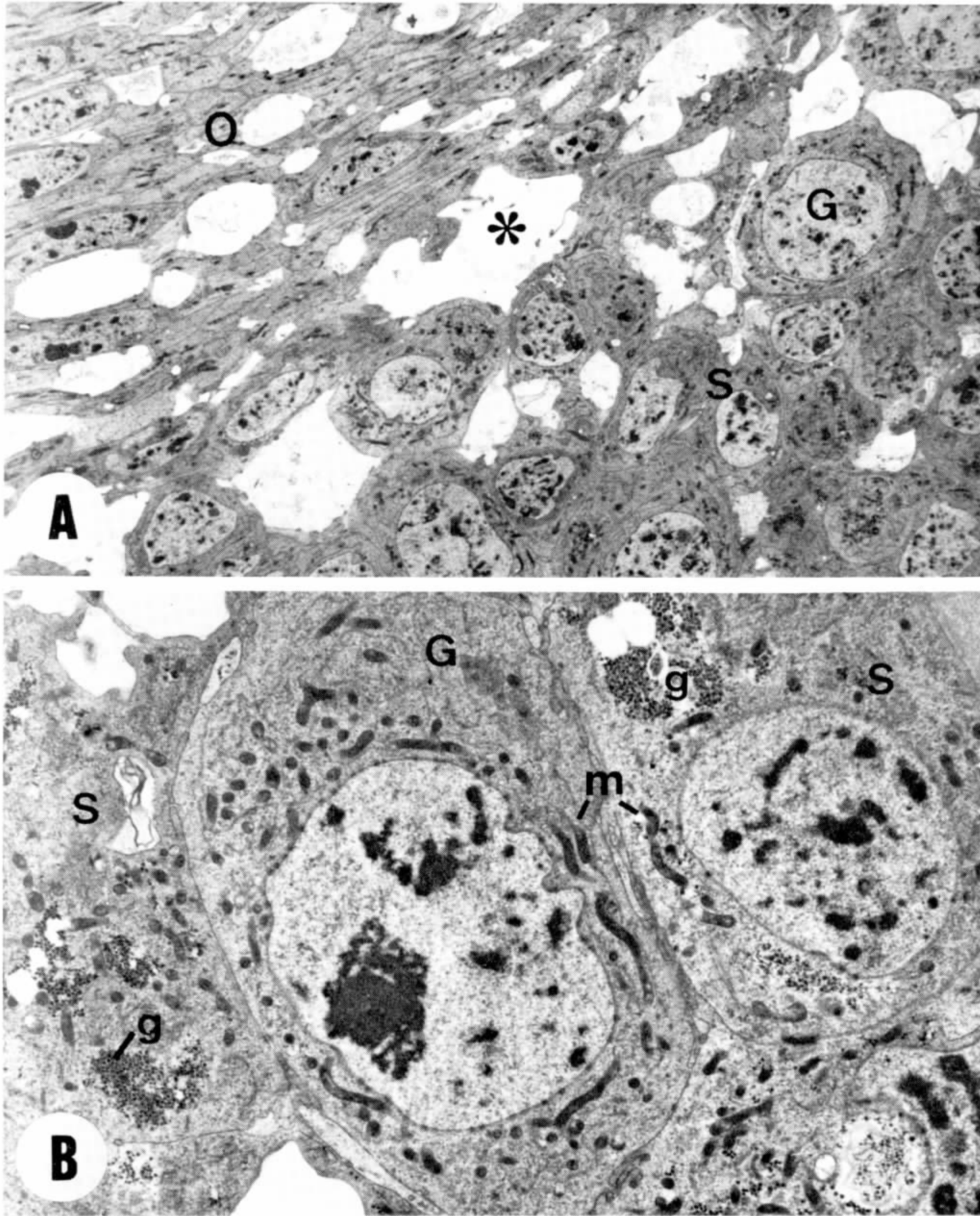


Fig. 5: ovary in 600-700 mg larva. A: basal region. Oblong somatic cells (O); intersitial somatic cells (S); gonial cells (G); intercellular spaces (*). X 2800. B: basal region. Gonial cells (G); interstitial somatic cells (S); glycogen (g); mitochondria (m). X 10000.

Koch et al., 1967; Sharma, 1967; King et al., 1968, 1982; Koch & King, 1969; Mahowald, 1972; King, 1975; Mathew & Rai, 1976). Nevertheless, the knowledge of ovary morphology in the larval instars is very important to the understanding of the differentiation and development of the ovarioles.

There is no ultrastructural description of the ovary of *D. hominis*. The histology of the

ovarian development in larvae of *D. hominis* was described by Lello (1979) and Lello et al. (1984) and show that until the end of this period there is no evidence of ovariole formation. The female gonad differentiates in larvae weighing 400 mg; gonial and somatic cells are scattered in both apical and basal regions, separated by oblong somatic cells. The authors call the attention to the presence of vacuolated somatic cells in the cortical portion of the apical

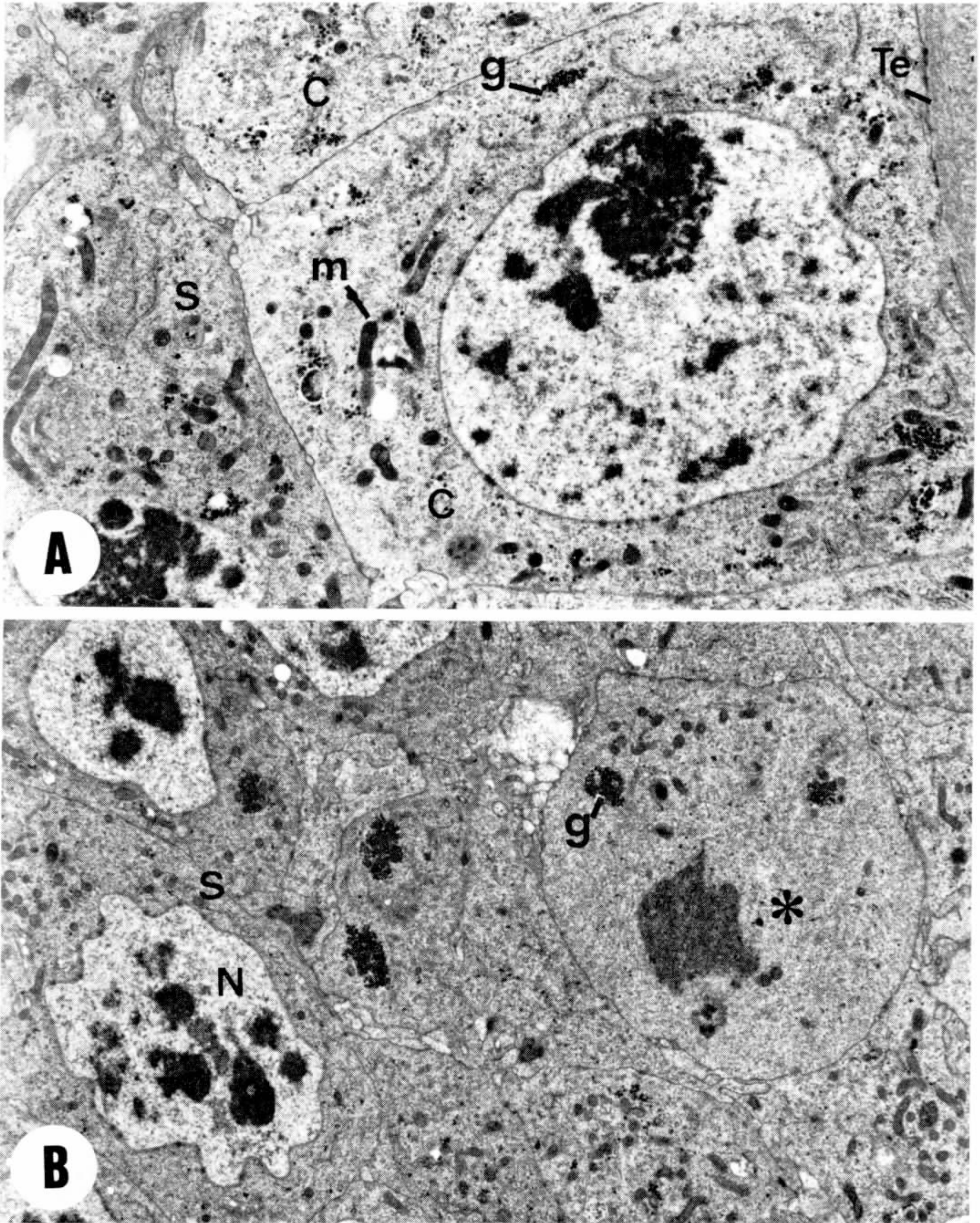


Fig. 6: A: apical region of the ovary in 600-700 mg larva. Clear somatic cells (C); interstitial somatic cells (S); tunica externa (Te); mitochondria (m); glycogen (g). X 15400. B: ovary in larva weighing more than 700 mg. Interstitial somatic cells (S) with irregular nuclear outlines or in mitoses (*); mitochondria (m); glycogen (g). X 11500.

region of the ovary. Our results agree with the majority of these descriptions. We observed that the gonad is surrounded by a thick acellular electron-dense tunica of filamentous material. This coat was observed in the larval gonads of *D. hominis* since the 1st instar (Lello, 1979).

Its thickness is constant during the larval period independently of the enlargement of the gonad. This is an indication that there is a constant addition of material, the nature and origin of which we were unable to determine. The cortical cells which would probably be

related to this function do not show ultrastructural features of secretion. In the ovary of *Drosophila melanogaster*, King et al. (1968) suggest a blood cell, the lamelocyte, as the responsible for the secretion of the tunica externa. In *D. hominis* this tunica is similar to the ones surrounding other organs of the insect (Gregório et al., 1981). So, a common origin from blood cells for the covering of the organs can not be discarded.

The gonial cells are characteristically different from the somatic ones as they have a larger nucleus-cytoplasmic relation. Although appearing frequently in pairs, we did not observe cytoplasmic continuity between them; such continuity is defined as "ring canals", and it is the result of incomplete cytokinesis indicating the differentiation of the oogonia into oocyte (King et al., 1968; King, 1975; Buning, 1979). This observation permits to come to the conclusion that until the end of the 3rd larval instar, the oogonia in *D. hominis* are just multiplying. The only noticeable modification observed in these cells was an increase in the density of their mitochondria. This event should not be considered as specific of gonial differentiation because was also observed in all the somatic cells.

The observation of great amount of microtubules in the cytoplasm of the oblong somatic cells lead us to believe that they are related to conformational modifications that occurs in the gonad as described by Lello (1979). At the groove region these cells appear to be dragging fibrillar material from the tunica externa to the interior of the organ.

The clear somatic cells observed in larvae weighing between 400 and 500 mg correspond to the vacuolated cells described by Lello (1979). Actually these cells do not present any vacuoles and along the larval development their cytoplasm become more electron-dense and store glycogen. According to the above author they will degenerate at the beginning of pupation.

The interstitial somatic cells present the same ultrastructural aspects wherever they are located. They accumulate glycogen and change their nuclear morphology at the end of the larval period. These nuclear modifications are the inicial preparation for the great number of mitoses, that will occur during pupation, as

observed by Lello et al. (1985). Probably they will originate all kinds of somatic cells of the full grown ovary, but the nurse ones.

One of the sharp differences between somatic and gonial cells during this period is that the former progressively accumulate glycogen in their cytoplasm and there is an increase in the number and density of their mitochondria. These modifications are obviously related to the increase of the metabolism that will occur from this time on, as the larvae are preparing themselves to pupation. During pupation there will be important phases of cellular degeneration. Actually its initiation in the basal cells can be observed at the end of the larval period.

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