

ULTRASTRUCTURE OF THE OVARY OF *DERMATOBIA HOMINIS* (DIPTERA: CUTEREBRIDAE). II. ORIGIN OF THE TUNICA PROPRIA IN OVARIOLES

E. A. GREGÓRIO; V. N. D. P. SECCO; L. A. TOLEDO & E. LELLO

Instituto de Biociências, UNESP, Campus de Botucatu, 18600, Botucatu, SP, Brasil

Ovaries up to the 8th day pupae of Dermatobia hominis were studied by transmission electron microscopy. Ovarioles were recognized in ovaries of 4-day old pre-pupae, surrounded by a thin tunica propria of acellular fibrillar material similar in structure to the internal portion of the external tunica of the ovary. There is continuity of the tunica propria and the ovarian tunica, indicating that the former structure originates from the tunica externa. In 5 to 7-day pupae the interstitial somatic cells from the apical region of the ovary, close to the ovarioles, show delicate filamentous material inside of their rough endoplasmic reticulum cisternae; similar material is seen among these cells. Our observations suggest that interstitial somatic cells do not originate the tunica propria but contribute to its final composition.

Key words: tunica propria – ovarioles – *Dermatobia hominis* – Diptera

Histology of post-embryonic development of the ovary of *Dermatobia hominis* was described by Lello (1979) and Lello et al. (1984, 1985); ultrastructural studies of the 3rd larval instar were carried out by Gregorio et al. (1990). Lello (1979) described the formation of characteristic ovarioles surrounded by a thin membrane called tunica propria around the 8th day after the beginning of pupation. The author was unable to define which cells were responsible for the formation of this membrane, but suggested that the tunica propria originates from invagination of the external tunica of the ovary.

In *Drosophila melanogaster* the origin of the tunica propria was analyzed by electron microscopy by King et al. (1968) and King (1970). They proposed that it was secreted by the apical ovarian cells. The same origin was indicated by Boulard (1967) in *Hipoderma*.

In this paper we present our results concerning the formation of the tunica propria in *D. hominis* studied by transmission electron microscopy.

MATERIALS AND METHODS

Pre-pupae and pupae of *Dermatobia hominis* up to the 7th day of development were dissected in insect saline solution under stereomicroscope. After anatomical identification the ovaries were transferred to 2.5% glutaraldehyde in 0.1 M buffer phosphate pH 7.3, post-fixed in 1.0% OsO₄ in the same buffer, dehydrated and embedded in Araldite. The ultrathin sections were analyzed under EM-301 Philips.

RESULTS

Ovaries of 2-day old pre-pupae are ultra-structurally similar to those of larvae at the end of the 3rd instar, however a larger number mitosis in somatic and gonial cells are observed; ovarioles are not defined yet.

In 3-day old pre-pupae there is an increase of the internal portion of the ovarian tunica which penetrates among the oblong somatic cells at the groove region (Fig. 1A). The somatic interstitial cells of the median region start to pile up. Close to them, there is a fibrillar material similar to the internal portion of the tunica externa (Fig. 1B).

Ovarioles can be defined in 4-day-old pre-pupae. They are recognized at the median region of the ovary by the terminal filament formed by stacking of cells (Fig. 2). They are

Supported by FAPESP (Proc. 84/2979-0 and 84/1229-7), CNPq (Proc. 300755/85-BM) and FIPIC (Proc 1483/4).

Received 25 April 1990.
Accepted 13 June 1990.

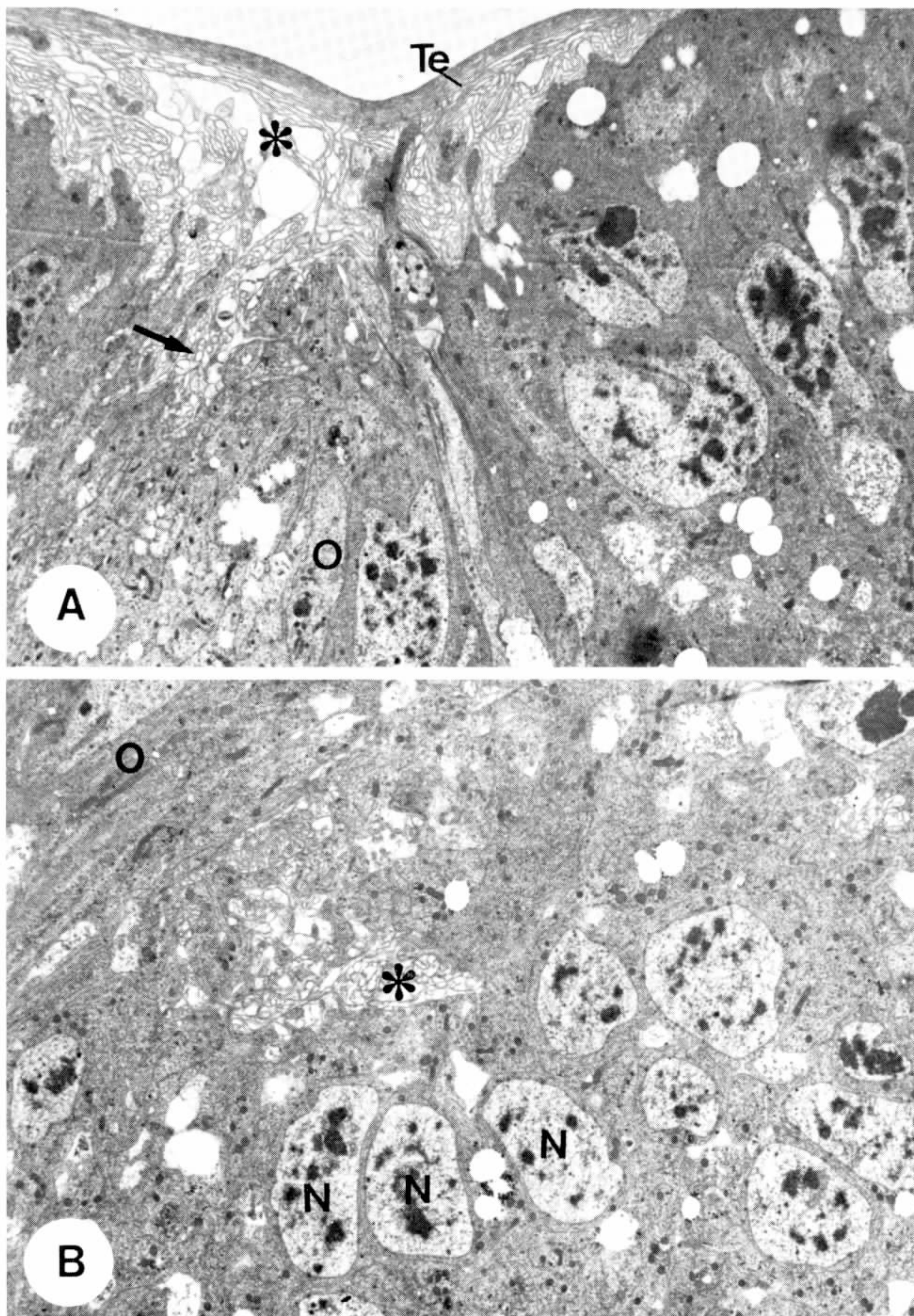


Fig. 1: ovary in 3-day old pre-pupa. A: groove region. Internal fibrillar portion (*) of the external ovarian tunica penetrating among the oblong somatic cells (arrow). Tunica externa (Te); oblong somatic cells (O). X 5000. B: median region. Nucleus (N) of interstitial somatic cells piled up. Fibrillar material (*); oblong somatic cells (O). X 6500.

surrounded by a thin tunica propria of acellular fibrillar material, similar to the internal portion of the external tunica. At the groove region there is continuity between this tunica propria

and the internal fibrillar portion of the tunica externa (Fig. 3). In this stage, as in the previous, there is no morphological evidence suggesting that cells near to the ovarioles are secreting.

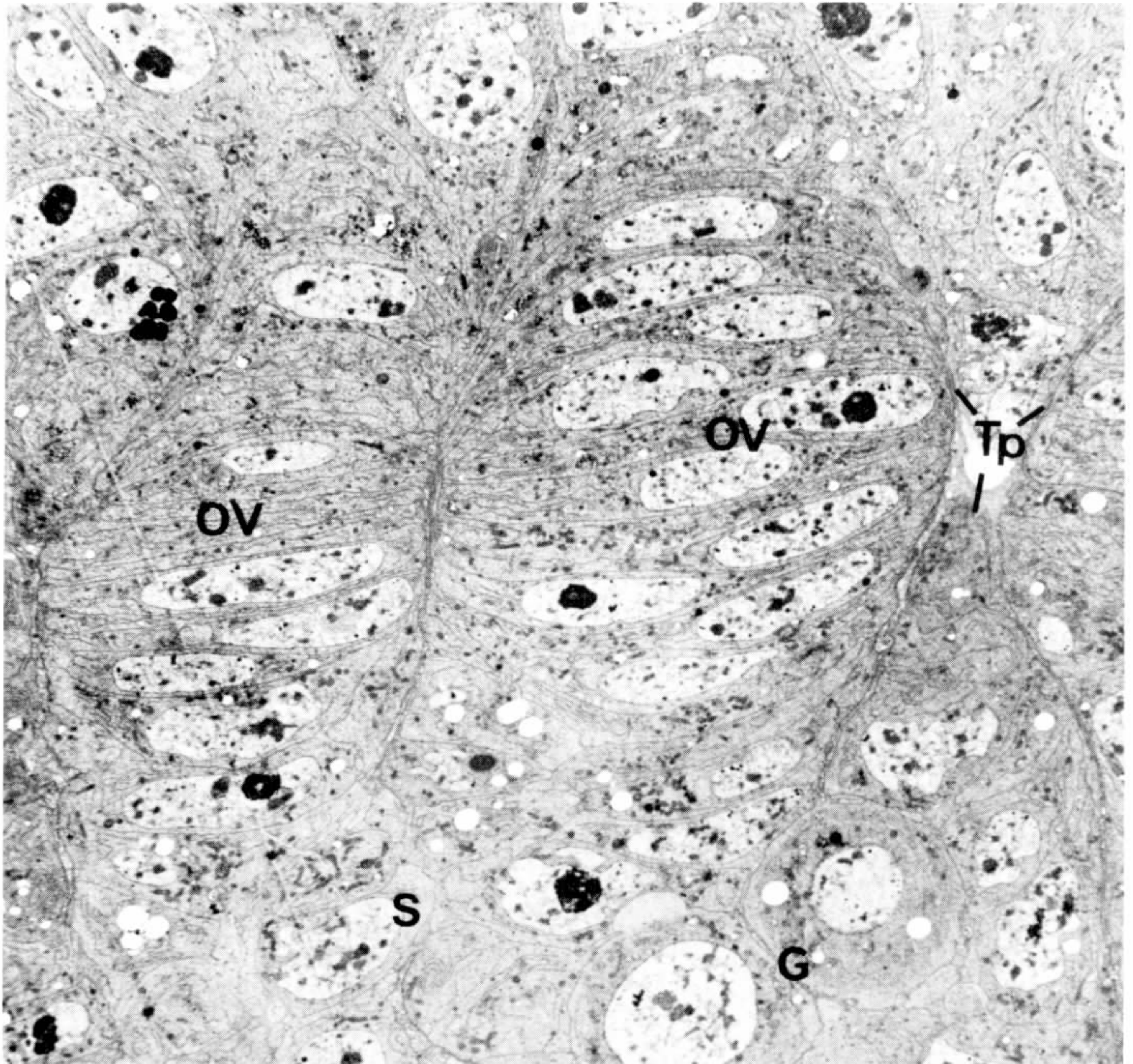


Fig. 2: median region of the ovary in 4-day old pre-pupa. Terminal filament of the ovariole (OV); interstitial somatic cell (S); gonial cell (G); tunica propria (Tp). X 3500.

In 5 to 7-day old pupae the tunica propria is formed by several layers of acellular material (Fig. 4A). In the interstitial somatic cells from the apical region of the ovary, adjacent to the oblong somatic cells, there is an increase of the rough endoplasmic reticulum that may be dilated containing a delicate filamentous material (Fig. 4B). Similar material is also observed in the interstitial space, among these cells (Fig. 4C) and between them and the tunica propria (Fig. 4D). In older pupae this filamentous material was not seen in the cytoplasm of the interstitial somatic cells nor in the intercellular spaces.

DISCUSSION

The formation of ovarioles' tunica propria in insects is not well known; it appears to be related to the formation of the terminal filament that is the first structure to be morphologically recognized.

According to King et al. (1968) and King (1970), in *D. melanogaster*, the formation of the tunica propria starts after completion of the terminal filament. They suggest that the apical cells of the ovary recognize the cells of the terminal filament as non self and secrete

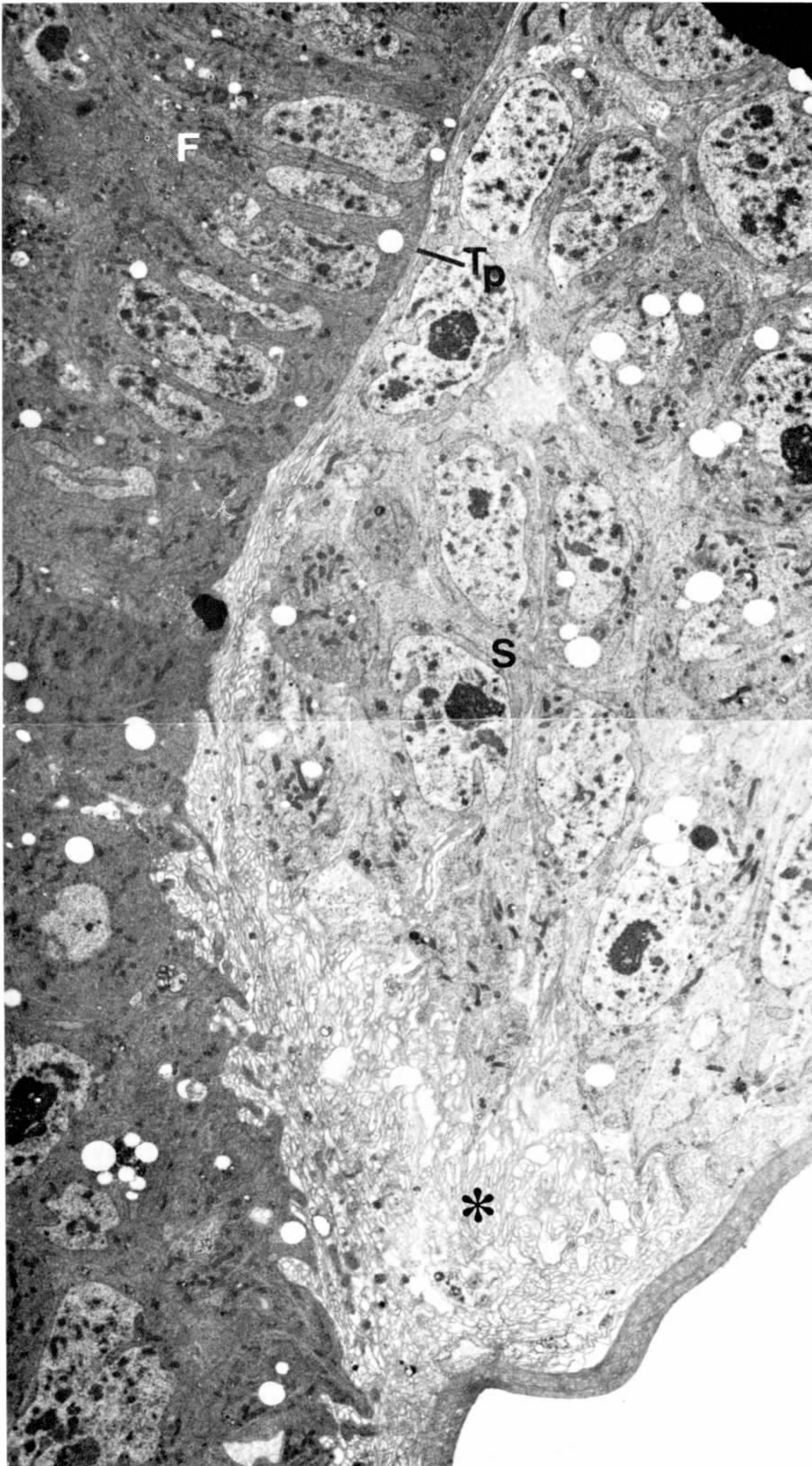


Fig. 3: ovary in 4-day old pre-pupa. Groove and apical regions. Note the continuity between the fibrillar portion of the external tunica (*) and the tunica propria (Tp) of the ovariole. Terminal filament (F); interstitial somatic cells (S). X 5000.

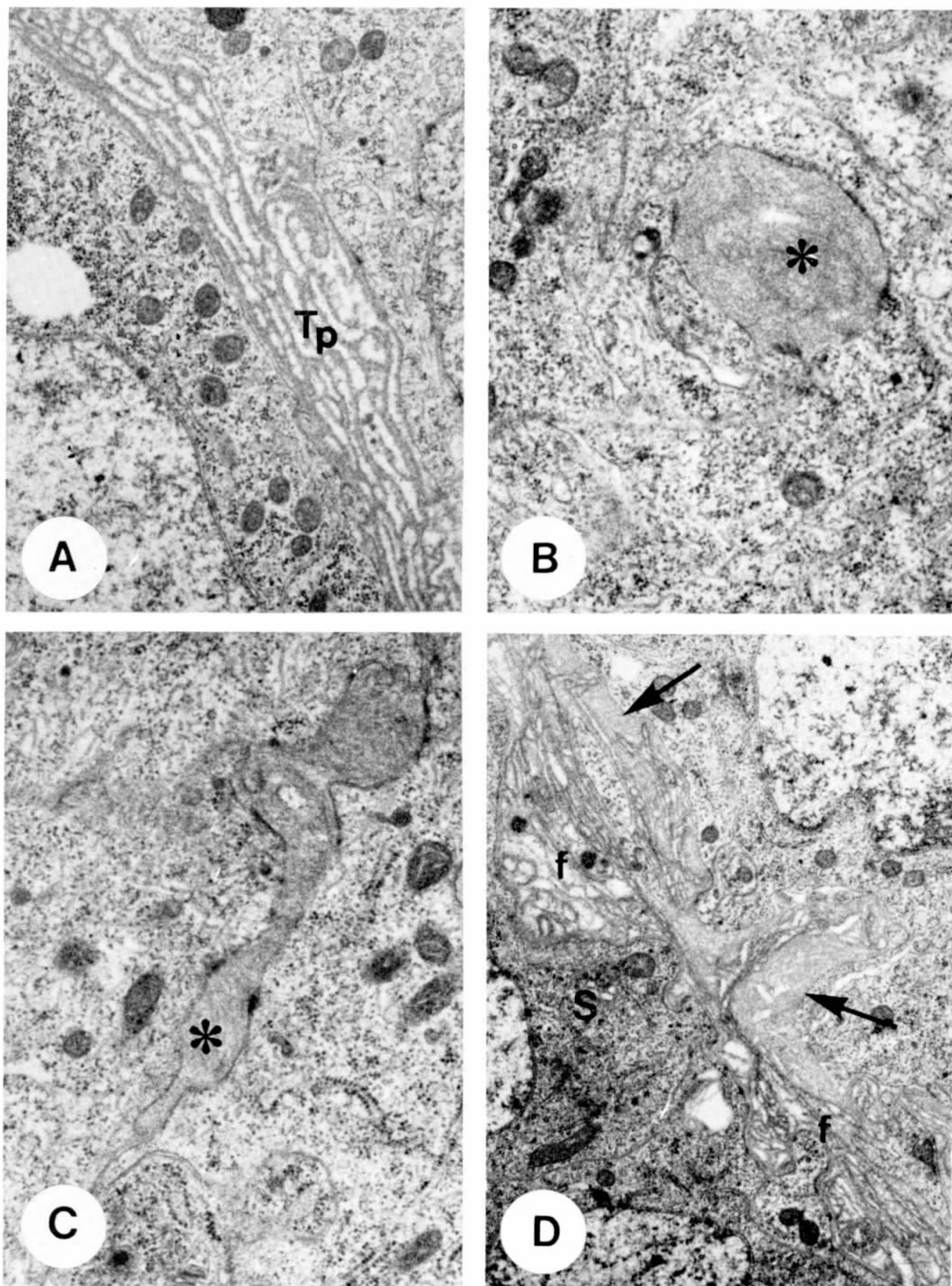


Fig. 4: ovary in 7-day old pupa. A: detail of the tunica propria (Tp) arrangement in layers. X 16900. B: detail of an interstitial somatic cell. Note the dilated rough endoplasmic reticulum full of filamentous material (*). X 27500. C: filamentous material (*) accumulated among interstitial somatic cells close to the ovarioles. X 27500. D: filamentous material (arrows) being incorporated to the fibrillar material (f) of the tunica propria: ovariole somatic cells (S). X 14400.

and deposit the material on the surface of these "foreign" cells. On the other hand, Boulard (1967) in *Hipoderma*, describes that even

before the morphological definition of the ovarioles the apical cells of the ovary secrete a membrane that divides it in two distinct

zones. Later this acellular membrane forms plicas into which gonial and somatic cells from the central region penetrate and where the somatic cells from the apical region pile up to form the terminal filament.

Lello (1979) and Lello et al. (1985) described in *D. hominis* that the terminal filament and the tunica propria appear at the same time in 7 to 8-day old pupae. They did not define which cells formed the tunica propria suggesting that it originates from the amorphous membrane surrounding the ovary. They also suggested that the oblong somatic cells move from the periphery to the interior of the gonad dragging with them part of the external amorphous membrane.

Our observations in the same material using electron microscopy also show the appearance of the terminal filament and tunica propria at the same time, but earlier than observed at optical level. In 3-day old pre-pupae we noticed that interstitial somatic cells from the median region of the ovary start to stack having on their proximity fibrillar material similar both, to the future tunica propria and to the external tunica of the ovary. In 4-day old pre-pupae the continuity of the tunica propria with the internal fibrillar portion of the tunica externa of the ovary is evident. In ovaries of *Sialis flavilata*, Buning (1979) called the attention to the similarity between the tunica propria of the ovarioles and the external acellular layer, not only of the ovary but of all the gonial tube. All these data agree with the assumption of Lello (1979) and Lello et al. (1985).

The participation of apical cells in the formation of the tunica propria in *D. hominis* can not be discarded. We observed immediately after the appearance of the ovarioles, the

presence of a delicate filamentous material, among the interstitial somatic cells as well as inside of their rough endoplasmic reticulum. This material was also observed being incorporated to the material that penetrates from the tunica externa into the interior of the gonad. These observations suggest that somatic apical cells do not begin the formation of the tunica propria of the ovarioles, but they contribute to its final composition.

ACKNOWLEDGEMENTS

To Mrs Maria E. L. Peres and Miss Maria H. Moreno for their skillful services.

REFERENCES

- BOULARD, C. H., 1967. *Étude du développement post-embryonnaire des gonades d'Hipoderma (Diptera: Oestridae)*. Thèse du Doctorat 3^e cycle, l'Université de Paris, 61 p.
- BUNING, J., 1979. The telotrophic-merostic ovary of Megaloptera. I. The ontogenetic development. *J. Morph.*, 162: 37-66.
- GREGÓRIO, E. A.; SECCO, V. N. D. P.; TOLEDO, L. A. & LELLO, E. Ultrastructure of the ovary of *Dermatobia hominis* (Diptera: Cuterebridae). I. Development during the 3rd larval instar. *Mem. Inst. Oswaldo Cruz*, 85:
- KING, R. C., 1970. *Ovarian development in Drosophila melanogaster*. Academic Press, New York and London, p. 227.
- KING, R. C.; AGGARWAL, S. K. & AGGARWAL, V., 1968. The development of the female *Drosophila* reproductive system. *J. Morph.*, 124: 143-166.
- LELLO, E., 1979. *Desenvolvimento pós-embriônico das gônadas de Dermatobia hominis (Linnaeus Jr. 1781) (Diptera: Cuterebridae)*. Tese de Livre Docência, IBBMA/UNESP, 73p.
- LELLO, E.; GREGÓRIO, E. A. & TOLEDO, L. A., 1985. Desenvolvimento das gônadas de *Dermatobia hominis* (Diptera: Cuterebridae). *Mem. Inst. Oswaldo Cruz*, 80: 159-170.
- LELLO, E.; TOLEDO, L. A. & GREGÓRIO, E. A., 1984. Diferenciação morfológica das gônadas em larvas de *Dermatobia hominis* (Diptera: Cuterebridae). *Mem. Inst. Oswaldo Cruz*, 79: 211-219.