the angle of incidence of the light of the microscope, as is normally achieved by turning the micrometer screw, the garnet red colour tends towards a yellow tone. Under the microscope with polarizing filters set at 90°, the nucleolini appear divided into 4 very bright quadrants by a black interference cross. In the present state of our studies, it appears that the nucleolini possess no staining affinity and that even less do they have positive cytochemical reactions. There is not even a Feulgen-positive reaction. One can be deceived into thinking that a reaction of this kind exists by the characteristic garnet red colour of the nucleolini; however, it is easy to check this by varying the angle of incidence of the light of the microscope. Under the polarizing microscope, in the preparations obtained by the ordinary techniques, the nucleolini still show the characteristic black interference cross.

4. Nucleolini may coexist, in one and the same nucleolus, with one or more vacuoles. There is a very sharp difference between them, since the former show high refraction, which is not revealed by the content of the latter.

5. Nucleolini are especially abundant in the nucleoli of vitellogenetic oocytes and developed neoplastic cells, and this is found in concomitance with the following conditions presented by these nucleoli: a more or less marked affinity for the aniline blue of Mallory's method, little or no affinity for the pyronine of the Unna-Pappenheim method and the toluidine blue of the Dominici method. A check with ribonuclease shows that the affinity for pyronine and toluidine blue is due to RNA. Thus it is that the nucleolini are found in greater numbers in those nucleoli in which there is a condition of lower density of their constituents[2], as also of a smaller quantity – or possibly the absence – of ribonucleoproteins.

6. In the cells observed in vivo, the nucleolini are evident, especially in nucleoli of oocytes and neoplastic cells. They appear of a faint red colour, which turns to a more yellow tone as the angle of incidence of the light of the microscope is varied; these colours stand out against the background of the nucleoli, which is faintly green. Under the phase-contrast microscope, as also under the Nomarski's interference phase-contrast microscope (Figure 1 a and b), the nucleolini appear more evident than under the ordinary microscope. Under the microscope with polarizing filters set at 90°, it is not possible to identify the black interference cross.

Regarding the earlier statement that the greater the volume of the nucleoli the more numerous were the nucleolini, previous studies[17] had been carried out on the oocytes of *Bufo vulgaris*, which are polynucleolate. Also RAMON CAJAL[18] had pointed out, in nerve cells, a relationship between the number of the nucleolar granules and the dimensions of the nucleoli. We have now turned our attention to the oocytes of the Echinoid *Echinus melo*; during their whole growth these oocytes have only one nucleolus. For the species under examination, the criteria adopted for elaborating the statistics were the same as those employed for the oocytes of *Bufo*; the measurements were taken in 115 nucleoli of different oocytes.

The smallest nucleolus diameter was 1.5 μm, the largest 10.5 μm; the maximum number of nucleolini counted was 61, a number that may be considered as the experimental limit of the count. Up to a nucleolus diameter of 2.7 μm no granules were found. The mean values obtained were: diameter of nucleoli: 5.8 μm; number of nucleolini: 13.7. With the values found, the dispersion diagram (Figure 2) was drawn up relative to nucleoli diameter and number of nucleolini, as well as the correlation Table. From these were obtained a coefficient of regression (by/x) equal to 0.15 and a coefficient of correlation (r) equal to 0.82. From the diagram it is easily deduced that the number of nucleolini increases in relation to the increase in the nucleolar diameters. However, the distribution of the points does not appear uniform along the line of regression, since there are maximum values of one dimension that do not correspond to the maximum values of the other. Since the coefficient of correlation is equal to 0.82, we can equally assert that between the two magnitudes considered in the oocytes of *Echinus melo* there is a significant correlation on the statistical level.

Riassunto. I risultati delle ricerche condotte al microscopio fotonico sul nucleolo di cellule vegetali e animali, somatiche e germinali, normali e tumorali hanno permesso di affermare ancora una volta che le sole strutture presenti in esso sono i nucleolini. L'esistenza dei nucleolini è stata comprovata in vivo, come pure con varie tecniche in seguito ad osservazioni compiute con diversi tipi di microscopio. L'analisi statistica effettuata su ovociti di *Echinus melo* ha permesso di confermare che i nucleolini, che sono assenti nei nucleoli più piccoli, sono poi tanto più numerosi per quanto più elevato è il volume dei nucleoli più grandi.

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Morphological Basis of Follicle Cells - Oocyte Interaction in Normal Pupae and Isolated Pupal Abdomina of *Galleria mellonella* L.

The complex structure of follicular cell/oocyte interface in vitellogenetic insect oocyte was observed by several authors. However, the observations on the changes of its pattern in the course of later development of the egg vesicle in normal and hormonally disturbed conditions have not been signalized. The present study gives some informations on this subject.

Material and methods. The follicular cell/oocyte interface was studied in the egg vesicles of *Galleria mellonella* L. bred on bee's wax under laboratory conditions. The

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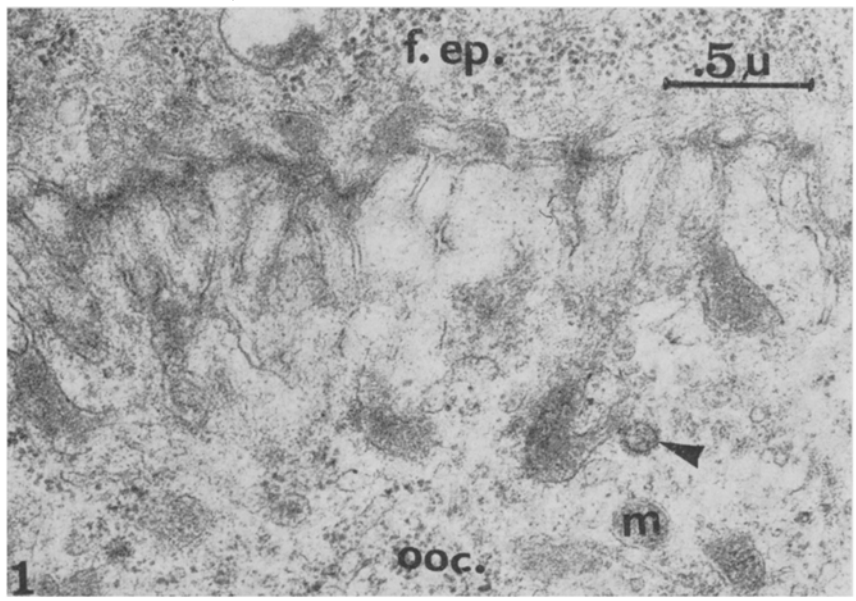
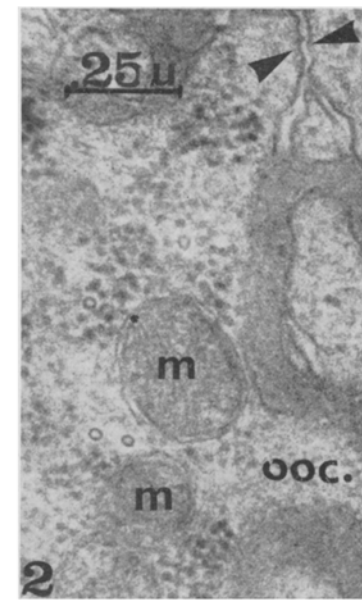
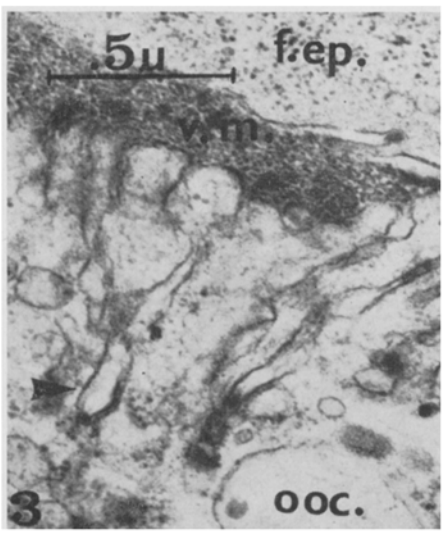
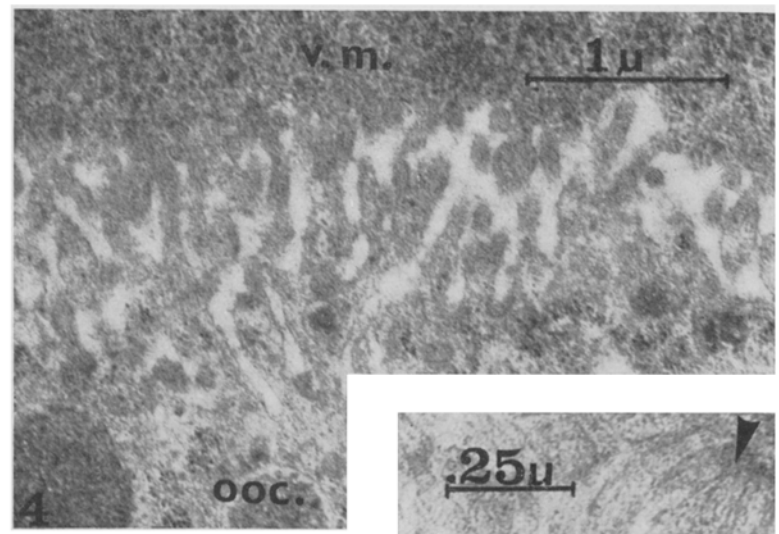
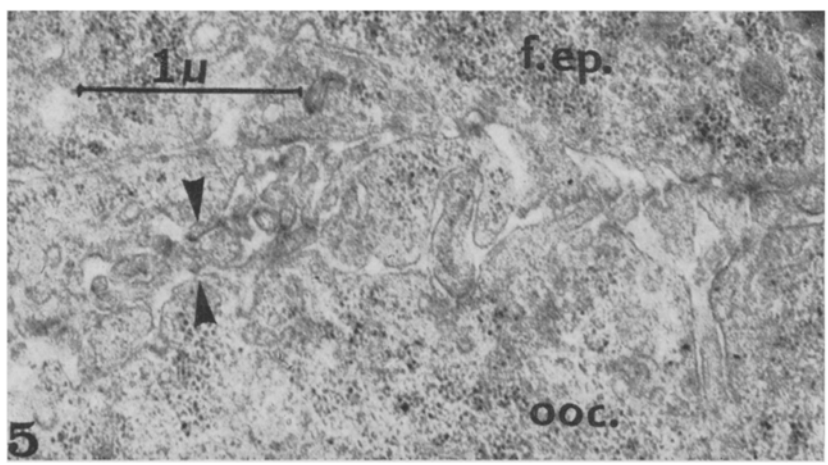
Fig. 1. Oocyte (ooc.)/follicular epithelium (f. ep.) interface in terminal egg vesicle of 6th-day pupa. Numerous pinocytotic canaliculi and vesiculi are seen. m, mitochondrium. Fig. 2. Fragment of a similar region as in Figure 1. The oocyte and follicular epithelium plasmalemma are marked. Fig. 3. Oocyte/follicular epithelium interface in egg vesicle at early vitellogenesis. The agglomeration of electron dense material filling the interspace of these cells is visible. Pinocytotic vesicles marked by arrow. vm., vitelline membrane. Fig. 4. Oocyte/follicular epithelium

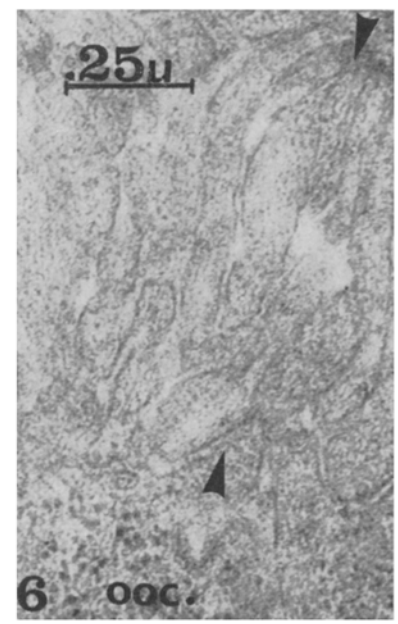












interface of an egg vesicle at advanced vitellogenesis. Fragment of a non-homogeneous vitelline membrane is seen. Fig. 5 and 6. Fragments of oocyte/follicular epithelium interface in egg vesicles developing in isolated abdomina of pupae. The isolation was performed at the 2nd day after pupation; dissection of ovarioles – 5th day after operation. No close contact between plasmalemma of oocyte and of the follicle cell is seen. Numerous intercalating protrusions formed by both plasmalemmas are seen, which do not, however, form pinocytotic vesicles.

vesicles were produced either from pupae, or from newly hatched imagoes. For studying the effect of hormonal deficiency on the development of the oocyte, 2nd-day pupae were deprived of brain, of corpora cardiaca, corpora allata and prothoracic glands by cutting off the anterior part of the body after the technique of WILLIAMS[1]. The ovarioles were dissected from the isolated abdomina obtained by this technique on the 5th day after the operation, that is on the day which for normally developing pupae is the second last day of pupal life. Normally, at that time the terminal egg vesicles of the ovarioles contain fully developed oocytes[2].

The tissue was dissected without narcosis, fixed in glutaraldehyde in cacodylate buffer at pH 7.3 and embedded in Epon 812[3]. The material was sectioned on a Reichert ultramicrotome and subsequently contrasted with uranyl acetate followed by lead citrate[4,5] and examined in the JEM 7A electron microscope.

Results and discussion. In the terminal egg vesicles found in pupae of *G. mellonella* at the 6th day after pupation, that is at the onset of vitellogenesis[2], numerous protrusions of the plasma membrane extending to the cortical zone of the oocyte are conspicuous. Similar protrusions were observed in oocytes of several other insects[6–8]. However, in contrast to what was found in the egg vesicles of *Hyalophora*, no special microvilli in the apical part of the follicle cells and no brush border on the oocyte surface were observed. In *Galleria*, the pattern of both the follicular cell and the oocyte plasmalemma may be easily followed (Figure 1). These two membranes seem to be in close contact with one another, the distance between them measuring about 150 Å, when not filled with an amorphous adielectronic material. The agglomeration of this material is accompanied by local extension of the intercellular space. Numerous slender plasma membrane invaginations appear, which are partially filled with a substance of similar electron density as that found in the intercellular space. The invaginations seem to be formed by the oocyte plasmalemma only, indicating high pinocytotic activity of this membrane (Figure 2) similarly as was found in *Colorado beetle* egg vesicles[9].

In the course of vitellogenesis, the follicular/oocyte interspace becomes gradually more extended and nearly uniformly filled with electron dense material.

The developing oocyte in the course of vitellogenesis is provided with proteins[10,11] and probably lipids[12] produced by the fat body and transported by the haemolymph. However, the follicle cells themselves also provide the developing oocyte with newly synthetized lipids[13,14], proteins and glycoproteins[15]. Some of these components are most probably used for the formation of the vitelline membrane, which, as has been found in the oocytes of *Drosophila*, contains lipids, proteins and polysaccharides[16]. This chemical heterogeneity of the vitelline membrane is reflected in the observed non-homogeneity of its ultrastructural pattern (Figures 3 and 4). The material accumulating at the oocyte border and forming the membrane is not of equal electron density, which is especially distinct at the first stages of the membrane formation. It is worth noting that, even in very advanced stages of vitellogenesis, the vitelline membrane retains its spongy ultrastructure, which probably enables it to continue the pinocytotic activity. Even when is it about 1 μm thick, the delicate invaginations of the oocyte plasmalemma ending with pinocytotic vesicles are seen.

It is known that cutting off the normal hormone inflow disturbs the normal development of the egg vesicles of *G. mellonella*. Light microscopic investigations showed that oocytes growing in isolated abdomina do not initiate vitellogenesis and reach not more advanced stages than those found in 4-day-old normal pupae[17].

The EM study shows that development stops even at an earlier stage, since, contrary to the terminal oocytes of 4th-day pupae, the plasmalemma of terminal oocytes of experimental individuals fails to show the pinocytotic activity. The external epithelial sheath of these egg vesicles is distinctly enlarged, as well as the follicular epithelium which even becomes multilayered. However, the contact of the apical part of the follicle cells with the oocyte seems to be rather loose and no accumulation of material in the interfollicular cell spaces and at the oocyte/follicle interface can be observed (Figures 5 and 6).

It seems probable that the lack of the pinocytotic activity of oocyte plasmalemma in ovarioles developing in hormonal deficiency is caused by the lack of nutritive materials provided to the oocytes. This may be the consequence as well of the diminution of synthetic activity in follicle cells, as of inhibition of production or release of nutritive material from the fat body involved by the experiment[18].

Résumé. Le plasmalemme des oocytes se caractérise par une grande activité pinocytosique au cours de la vitellogenèse. Celle-ci semble être contrôlée par des facteurs hormonaux, car l'ablation des glandes endocrines bloque complètement cette activité.

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Reduced Foetal Calcium Without Skeletal Malformations in Rats Following High Maternal Doses of a Strontium Salt

Skeletal malformations have been reported following high maternal doses of 'bone-seeking' metal ions such as lead[1,2], cadmium[2–4], zinc[3] and lithium[5–7] to laboratory mammals. The teratogenic activity of strontium, a metal ion thought to be essential in trace amounts for initial osteogenesis[8], has not been studied. In mature animals,