Recent Advances in Insect Embryology in Japan and Poland
Edited by H. Ando and Cz. Jura
Arthropod. EmbryoL. Soc. Jpn.
(ISEBU Co. Ltd., Tsukuba) 1987

Inductive Effect of Oocyte Nucleus on Ovarian Follicle Morphogenesis in Water Bugs (Heteroptera)

Antoni OGORZAŁEK

Synopsis

Using electron (TEM, SEM), polarization and fluorescence microscopies oogenesis in following water bugs was studied: *Notonecta glauca*, *Ilyocoris cimicoides*, *Ranatra linearis*, *Nepa cinerea* and *Corixa punctata*. The aim of the study was to analyse factors causing a diversification of the uniform prefollicular cell population into subpopulations of different morphology and function. The results indicate that the inductive effect of the oocyte nucleus is one of such factors determining the morphogenesis and biology of ovarian follicles in the studied species. The "compartmentation" of the previtellogenic ovarian follicles causes a temporal and spatial differences in the exposition of the follicular cells to this factor. This brings about a functional discoordination of the epithelium which subsequently determines the deutoplasm distribution and the egg capsule formation.

Introduction

Egg capsule (chorion) of bugs are often provided with adaptive structures which serve to fasten the eggs to or inside plants and enable gas exchange between embryo and environment (Hinton, 1981). These structures are formed by modified follicular cells. Observations on the interdependence between the oocyte nucleus position, the range of the trophic cord penetration in ovarian follicles in previtellogenesis and the epithelium deversification during vitellogenesis and choriogenesis, in eight bug species (*Notonecta glauca*, *Ilyocoris cimicoides*, *Ranatra linearis*, *Nepa cinerea*, *Corixa punctata*, *Gerris lacustris*, *Pyrrhocoris apterus* and *Lygaeus equestris*) suggest that the oocyte nucleus is an inductor of the follicular cell differentiation (Ogorzałek, 1969, 1974, 1975a, b, 1984, in press). The results of new studies on this interdependences are presented in this paper.

Material and Methods

Females of *Notonecta glauca*, *Ilyocoris cimicoides*, *Nepa cinerea*, *Ranatra linearis* and *Corixa punctata* (imagines and last instar larvae) were collected in the spring and summer 1983, 1984 and 1985 in the clay pits near Wrocław (Lower Silesia). Ovaries were dissected in Ringer solution for *Drosophila* and fixed in Carnoy's (6: 3: 1) fixative, 5% formaldehyde in 0.1 M phosphate buffer, pH 7.4 and in 5% glutaraldehyde in the same buffer. Sections were stained using the panoptic method according to Pappenheim and with methylene blue and borax, ultrathin sections were stained with uranyl acetate and lead citrate. Observations were made in fluorescence microscope Ljuman L-1, polarization-interference microscope MPI-3 (PZO Warsaw), TEM Tesla BS-613 and SEM Tesla BS-300. The material for SEM studies, prior to fixation, was placed in distilled water. Following the osmotic disintegration of the epithelia, their remnants were removed and the egg capsules at various chorionogenesis stages were repeatedly rinsed in distilled water. After the fixation, postfixation and dehydration in alcohol series and acetone the material was dried and coated with carbon and gold.

Results

I. *Ovary structure*

The general ovary structure in the studied species resembles that of the other Heteroptera (for review see King and Büning, 1985). The oocyte growth in the ovarioles is asynchronous while in all the species except *C. punctata* there is a horizontal interovariole synchronization. The analysis of various portions of ovarioles indicates interspecific differences in the process of the ovarian follicle formation, growth and differentiation: the differences consist in unequal time of differentiation of various parts of the epithelium as well as in the range of the diversification.

A. Primary diversification of the follicular epithelium

The diversification of the originally homogeneous group of prefollicular (stem) cells into two subpopulations takes place at the moment of the ovarian follicle formation in the meristematic portion of the ovariole, in all species according to a similar scheme. A part of the cells assume a lateral localization relative to the oocyte and contact with the ovariole wall, others are localized between the oocytes in the paraaxial ovariole portion with no direct contact with haemolymph. The former group, in the further course of the oogenesis, forms a high prismatic epithelium built of binucleated cells, and surrounding cylindrically the proximal ovariole portions. The latter group, as a rule mononuclear, form interfollicular septa, still without a contact with the epithelium basal membrane (Fig. 16). Consecutive endomitoses that increase the follicular cell ploidy (in *I. cimicoides* to 32n, Ogorzałek, 1978) initially occur in the prismatic portion of the epithelium which leads to a quick diversity in size of both cell types. The cytoplasm of the interoocyte septum cells shows a higher affinity to basic stains which

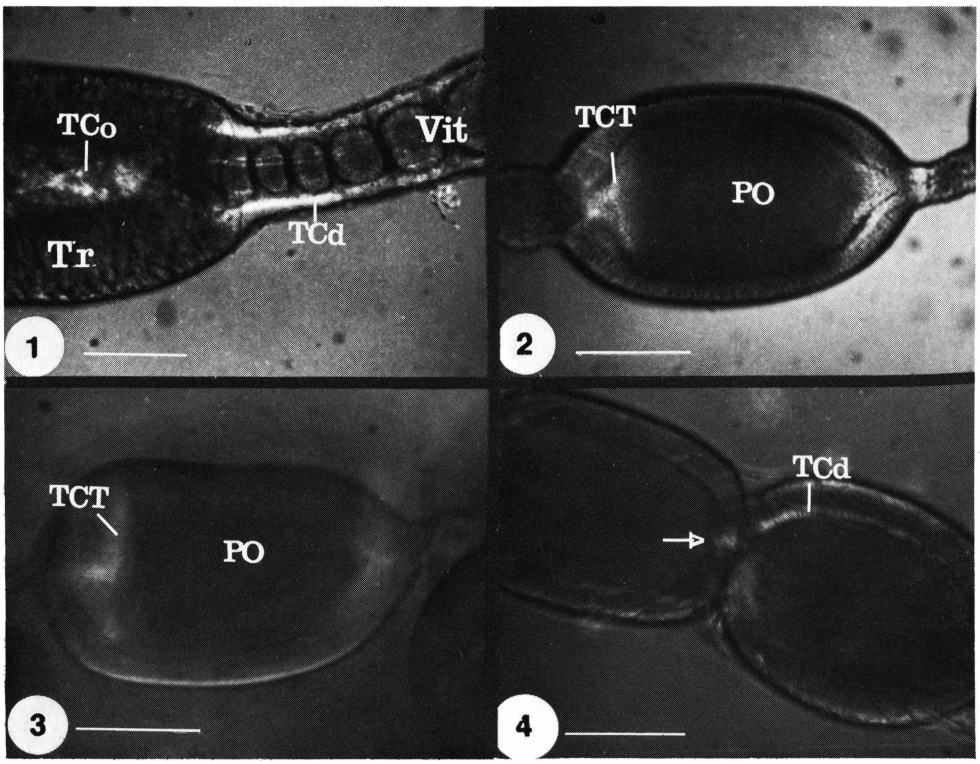


Fig. 1. *Ilyocoris cimicoides*. Tropharium (Tr) and proximal part of vitellarium (Vit) viewed in polarization microscope. Trophic core (TCo) and trophic cord (TCd) lighten. Scale: *ca.* 150 μm.

Fig. 2. *Ilyocoris cimicoides*. Previtellogenic ovarian follicle. Structures causing a rotation of polarization plane penetrate proximal part of follicle (TCT). PO, proper ooplasm. Scale: *ca.* 150 μm.

Fig. 3. *Ilyocoris cimicoides*. Early vitellogenic ovarian follicle. Lighter territory in proximal part of follicle (TCT) asymmetrical. PO, proper ooplasm. Scale: *ca.* 170 μm.

Fig. 4. *Notonecta glauca*. Previtellogenic ovarian follicle. Trophic cord (TCo) only slightly penetrates into the ovarian follicle (arrow). Scale: *ca.* 150 μm.

allows a good distinction between them and the remaining epithelium cells. At the stage of budding of elliptical ovarian follicles off the end of the cylindrical ovariole portion, the septum cells undergo a reorganization and form a "casing" of the trophic cord outlets to ovarian follicles, and interfollicular stalks (Figs. 7, 18, s-cells). This process is accompanied by mitotic divisions which by that time have already stopped in the remaining part of the epithelium. This arrangement persists until the end of vitellogenesis and in the chorionogenesis the s-cells build the micropylar apparatus.

B. "Compartmentation" of the ovarian follicle content

At the end of the previtellogenesis in the studied species (except *N. cinerea* and *R.*

linearis) a division of the ovarian follicle content into two territories ("compartments") takes place. Their apical portions are penetrated by the trophic cords and thus the trophic cord area can be distinguished, as well as the area of the "proper" ooplasm containing the oocyte nucleus.

1. Studies in polarization microscope

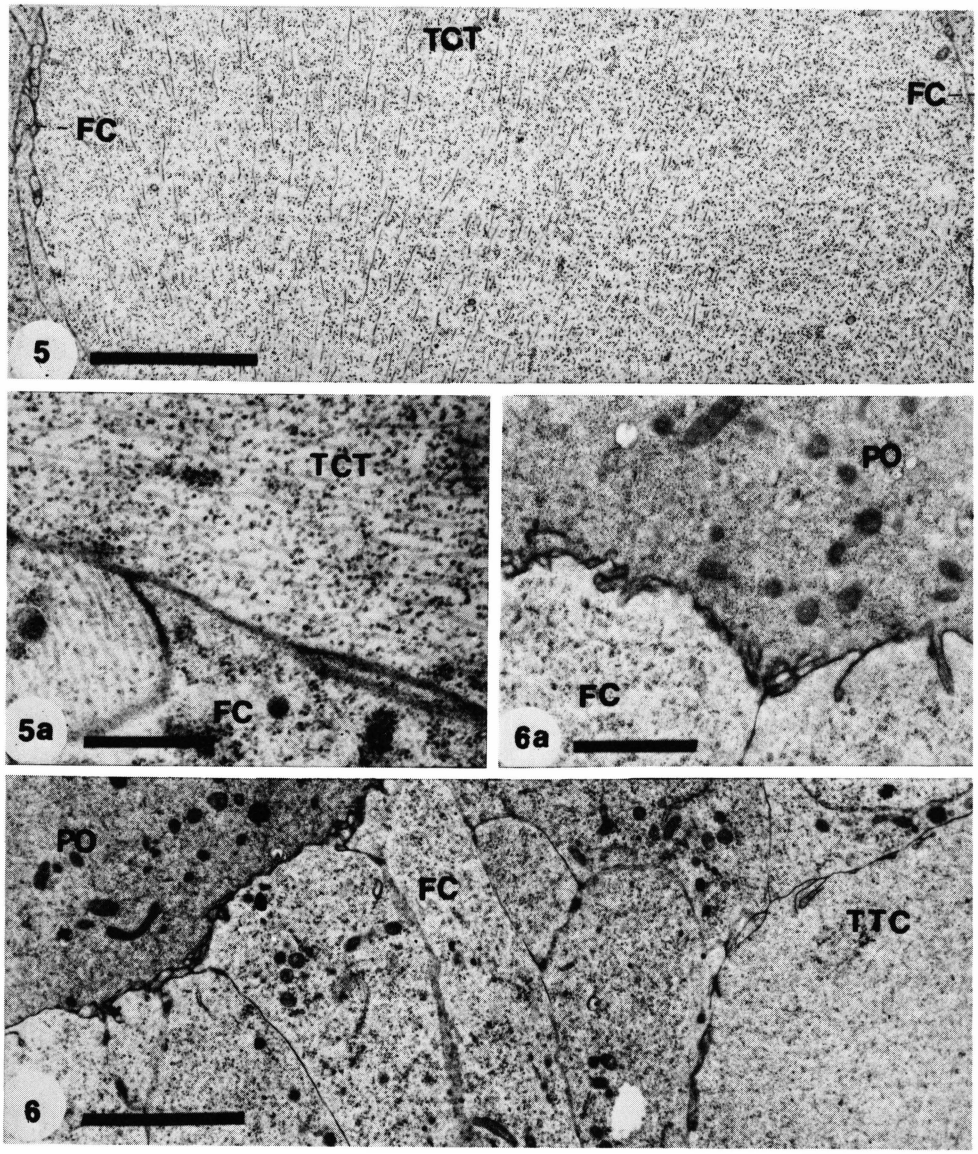
A great number of parallelly arranged microtubules results in the trophic core area and the trophic cord area in bug ovarioles causing a rotation of the polarization plane and both these structures are easily revealed in polarization microscope (Stebbings, 1971). This allows to determine the penetration range of the trophic cords inside the ovarian follicles (Figs. 1 — 4). The range of the trophic cord viewed in polarization microscope in greatest in *I. cimicoides*. The rotation of the polarization plane in this species comprises apical portions of the previtellogenetic and early vitellogenetic ovarian follicles, the outline of the trophic cord area being already asymmetrical (Fig. 3). In *R. linearis* and *N. cinerea* the area of the arranged microtubules reaches the level of apical portions of the cell adjoining the trophic cord outlet or in *Not. glauca* (Fig. 4) slightly exceeds this zone. In *C. punctata* (Fig. 21) an intermediate condition is observed namely the arranged microtubules penetrate the follicle but the area occupied by them and their density are smaller than in *I. cimicoides*.

2. TEM studies of *Ilyocoris cimicoides*

In *I. cimicoides* the "transit" trophic cords running inside the follicular epithelium of the previtellogenic and early vitellogenic ovarian follicles have a round smooth outline in transverse section, and there is a close contact between the trophic cord membrane and the follicular cell membrane. Only sporadically on small areas systems of short, flatly arranged and overlapping microvilli are observed which on transverse section in electron microscope appear as rows of round or oval, adjoining outlines.

Microtubules, arranged parallelly to long axes of the trophic cords and ribosomes, are rather evenly distributed in the trophic cords. Mitochondria, fine and filled with electron dense matrix, lie peripherally. In the proximal portion of the trophic cord territory, already inside the follicle, the microtubules are still arranged parallelly and rather regularly (Figs. 5, 5 a). In the distal portion the paracentral bundles of microtubules take a wave-like course of increasing amplitude, while the direction of the peripheral microtubules changes from the parallel to the epithelium surface to the centripetal, also wavy. Beside the fine mitochondria already mentioned, in the distal portion of the trophic cord territory, much larger ones, also peripherally situated are observed. They are of discoidal shape and lie parallelly to the epithelium surface.

In spite of the lack of any border structure, the border between the trophic cord territory and the proper ooplasm is relatively distinct. No syntopy of the ooplasm rich in microtubule bundles and ribosomes, and the deutoplasmic elements was observed. Also the large number of mitochondria in the proper ooplasm, and their almost total absence in the intrafollicular area of the trophic cord are good criteria distinguishing



Figs. 5, 5 a. *Ilyocoris cimicoides*. Proximal part of early previtellogenic ovarian follicle. TEM. Follicular cells (FC), trophic cord territory (TCT) with microtubules and ribosomes. Border TCT—follicular cells smooth. Scales: *ca.* 15 μm (Fig. 5), *ca.* 5 μm (Fig. 5 a).

Figs. 6, 6 a. *Ilyocoris cimicoides*. Distal part of ovarian follicle, stage as on Fig. 5. TEM. Proper ooplasm (PO) rich in ribosomes and mitochondria. On border PO—follicular cells (FC) microvilli form. TTC, transverse section of "transit" trophic cord. Scales: *ca.* 15 μm (Fig. 6), *ca.* 6 μm (Fig. 6 a).

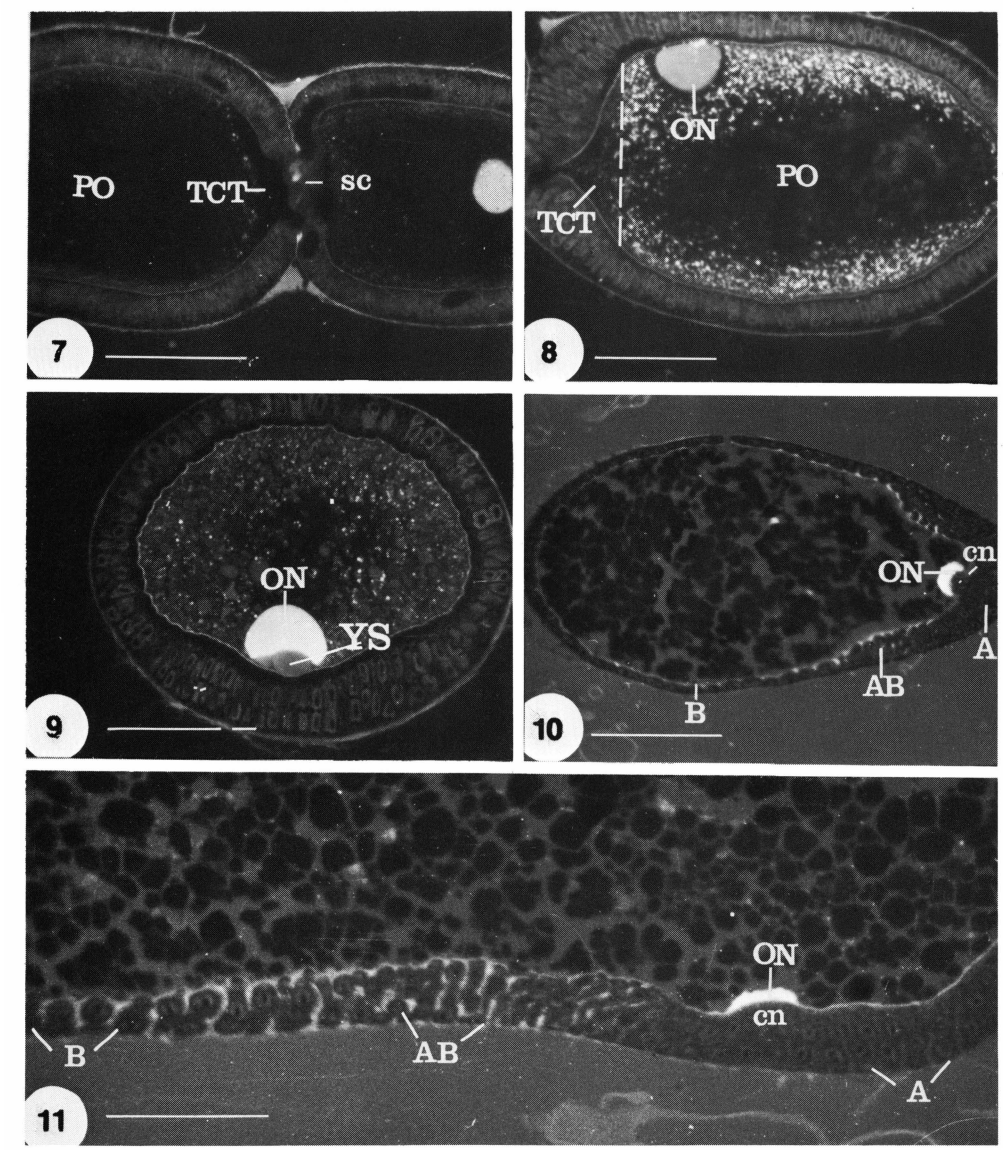


Fig. 7. *Ilyocoris cimicoides*. "Closing" of columnar epithelium part in early previtellogenic ovarian follicle. Follicle content divided into trophic cord territory (TCT) and proper ooplasm (PO). In cells originating from septum (sc) mitoses visible. Scale: *ca.* 70 μm.

Fig. 8. *Ilyocoris cimicoides*. Early vitellogenic ovarian follicle. Follicle content divided into trophic cord territory (TCT) and proper ooplasm (PO) (broken line). Epithelium diversified into prismatic and low, deutoplasm gradient decreasing with the distance from the oocyte nucleus (ON). Scale: *ca.* 80 μm.

Fig. 9. *Ilyocoris cimicoides*. Early vitellogenic ovarian follicle (later than of Fig. 8). Transverse section. Cells adjoining the oocyte nucleus (ON) decreased in height, nucleus surface facing the epithelium concave. YS, yolk spheres. Scale: *ca.* 80 μm.

both territories (Figs. 6, 6 a).

C. Secondary diversification of follicular epithelium — retardation

The division of the ovarian follicle content into the trophic cord territory and the proper ooplasm takes place when the elliptical ovarian follicles are covered with a uniform high prismatic epithelium (Fig. 7). In the further oogenesis, the cells adjoining the trophic cord area retain this original morphology and arrangement. Their nuclei are situated above one another, their apical portions are flat and until the advanced vitellogenesis they retain a rather close contact with the oolemma. Until the end of the chorionogenesis these cells adjoin each other (A-cells, Figs. 8, 10, 11).

The cells contacting with the proper ooplasm, beginning with the late previtellogenesis undergo a sequence of morphological changes typical of the bug vitellogenic epithelium. They decrease in height and the epithelium becomes low- instead of high-prismatic, and then iso-prismatic. The shape of the cells, initially polygonal, grows more or less oval. At the end of this sequence of the differentiation changes the cells move apart, their only contact being by cytoplasmic arms, and their apical portions form bulb-like protrusions in the ooplasm. As the cells decrease in height and move apart, their nuclei, initially situated above one another, arrange on a level. Their flattened surfaces facing each other at the end of the vitellogenesis undergo invagination resulting in a cylindrical space of an internuclear cytoplasm, perpendicular to the ooplasm surface (B-cells).

The space between the follicular cells that have move apart is limited by the epthelium basal membrane at one side, and by the oolemma at the other. It is filled with a material showing an affinity to eosin in panoptic staining according to Pappenheim. This makes it possible to use a patency index (Davey and Huebner, 1974) in fluorescence microscope (see Fig. 11). The index shows that the final distance between B-cells varies between species and at the end of vitellogenesis it is in *I. cimicoides* more than 5, in *R. linearis*, *N. cinerea* and *Not. glauca ca.* 3 and in *C. punctata ca.* 2.

With the advance of the vitellogenesis the trophic cord territory is gradually "pushed out" from the ovarian follicle. As a result, a part of the cells originally adjoining the trophic cord area enters (somewhat later than the other cell) in contact with the proper ooplasm. During the advanced vitellogenesis they show a mixture of characters namely they are high prismatic, their nuclei lie above one another, the cells themselves being moved apart and connected by cytoplasmic arms and their apical portions from bulb-like swellings (AB-cells).

Fig. 10. *Ilyocoris cimicoides*. Vitellogenic ovarian follicle. Transverse section. Oocyte nucleus (ON) situated on cumulus nucleophorus (cn). A-, AB- and B-cells visible. Scale: *ca.* 150 μm.

Fig. 11. *Ilyocoris cimicoides*. Vitellogenic ovarian follicle. Longitudinal section. Epithelium diversified into A-, AB- and B-cells. Fluorescence substance filling intercellular spaces allows a determination of the epithelium patency. Scale: *ca.* 90 μm.

(Figs. 7 — 11; formaldehyde, paraffin sections, Pappenheim panoptic method, fluorescence microscope.)

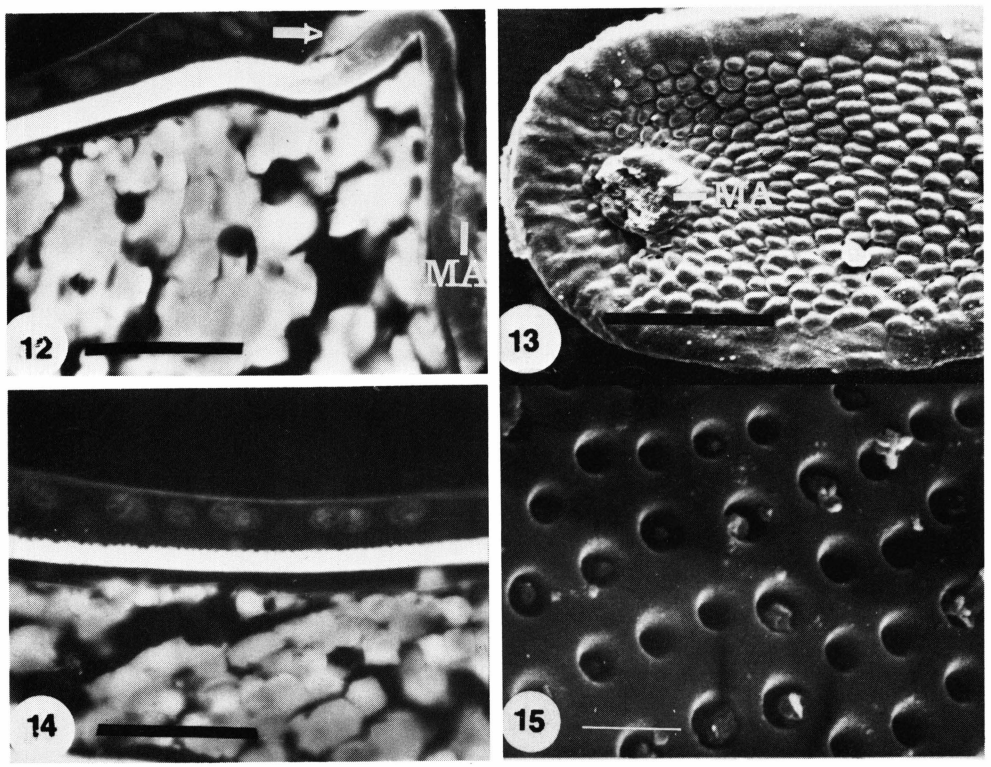


Fig. 12. *Ilyocoris cimicoides*. Apical part of ovarian follicle — chorion formation. Chorion plate formed by A-cells is thicker, deeper sculptured and less eosinophilous than the other parts of egg capsule. MA, micropylar apparatus. Scale: *ca.* 40 μm.

Fig. 13. *Ilyocoris cimicoides*. Chorion plate in apical part of egg capsule. SEM. Deep prints of A-cells apical portions, micropylar apparatus (MA), chorion collar, covering the junction between the plate and the rest of egg capsule, formed by AB-cells. Collar visible also on Fig. 12 (arrow). Scale: *ca.* 65 μm.

Fig. 14. *Ilyocoris cimicoides*. Subapical part of ovarian follicle — chorion formation. B-type follicular cells compact, chorion strongly eosinophilous, in outer capsule part — pits. Scale: *ca.* 40 μm.

Fig. 15. *Ilyocoris cimicoides*. Chorion in subapical part of egg capsule. SEM. Outer layer of egg capsule perforated. Scale: *ca.* 12 μm.

(Figs. 12, 14; Carnoy, paraffin sections, Pappenheim panoptic method, fluorescence microscope.)

D. Lateralization of the oocyte nucleus

During the previtellogenesis in all the studied species a migration of the nucleus takes place from the paracentral portion of the oocyte to its periphery. The time of the migration and the trajectory of the nucleus as well as its ultimate localization in the paraepithelial ooplasm are species-specific (see Fig. 27).

In *Not. glauca* and *I. cimicoides* the migration takes place at the time of closing of the cylindrical part of the epithelium in the process of budding off of the ovarian folli-

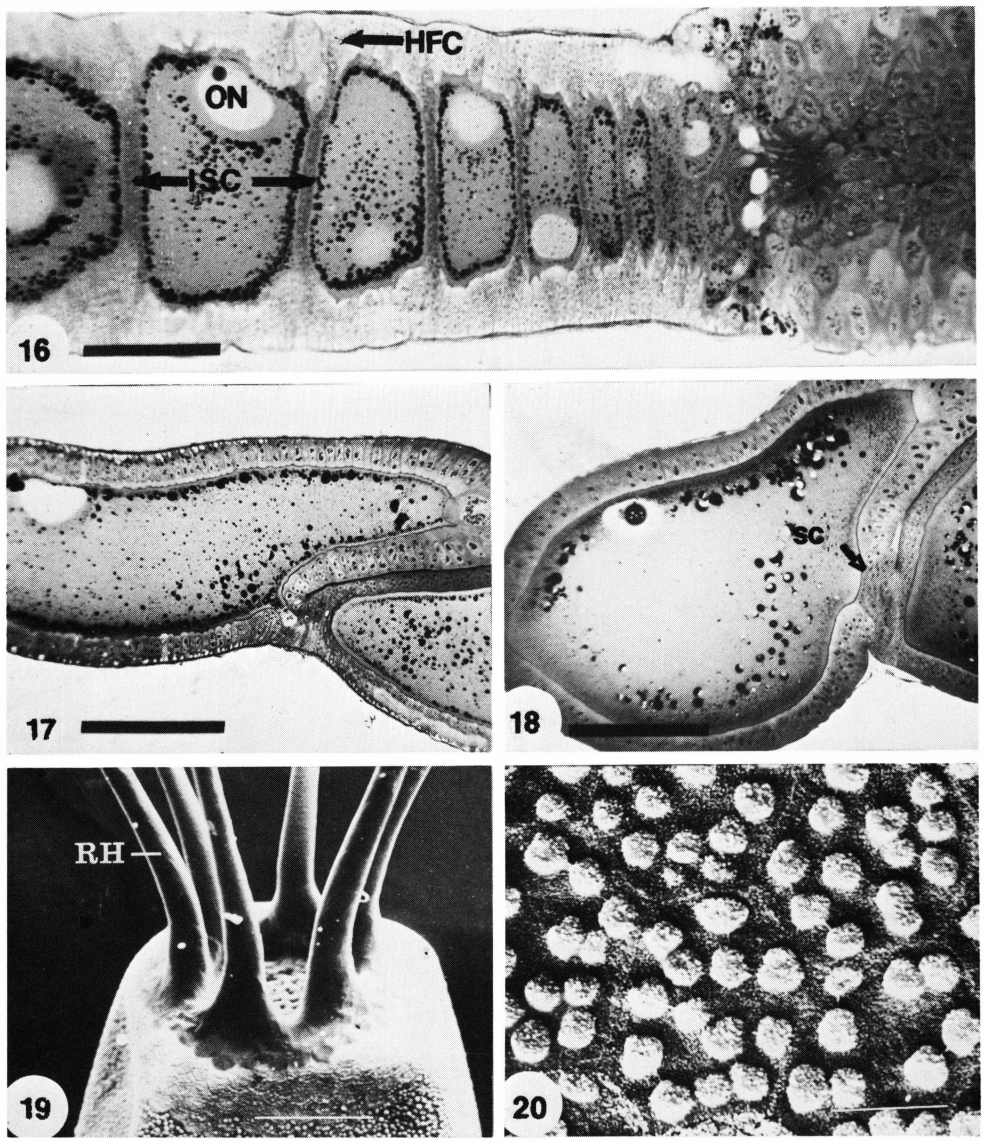


Fig. 16. *Ranatra linearis*. Fragment of germarium and proximal part of vitellarium. Oocyte nuclei (ON) in consecutive follicles alternately arranged. Cells on contact epithelium — ON — hypertrophied (HFC). Epithelium primary diversified into high prismatic and innerfollicular septum cells (ISC). Scale: *ca.* 60 μm.

Figs. 17, 18. Late previtellogenic ovarian follicles in *Ranatra linearis* (Fig. 17) and *Nepa cinerea* (Fig. 18). Epithelium diversified into super-B-, B- and (around trophic cord outlet) s-cells (sc). Scales: *ca.* 120 μm.

Fig. 19. *Nepa cinerea*. Proximal part of egg capsule. SEM. Respiratory horns formed by super-B-cells surround the "honeycomb" chorion plate. Scale: *ca.* 110 μm.

Fig. 20. *Nepa cinerea*. Subapical part of egg capsule. SEM. Prints of follicular cells and filler material accumulations visible. Scale: *ca.* 15 μm.

(Figs. 16—18; semithin sections, metylene blue and borax.)

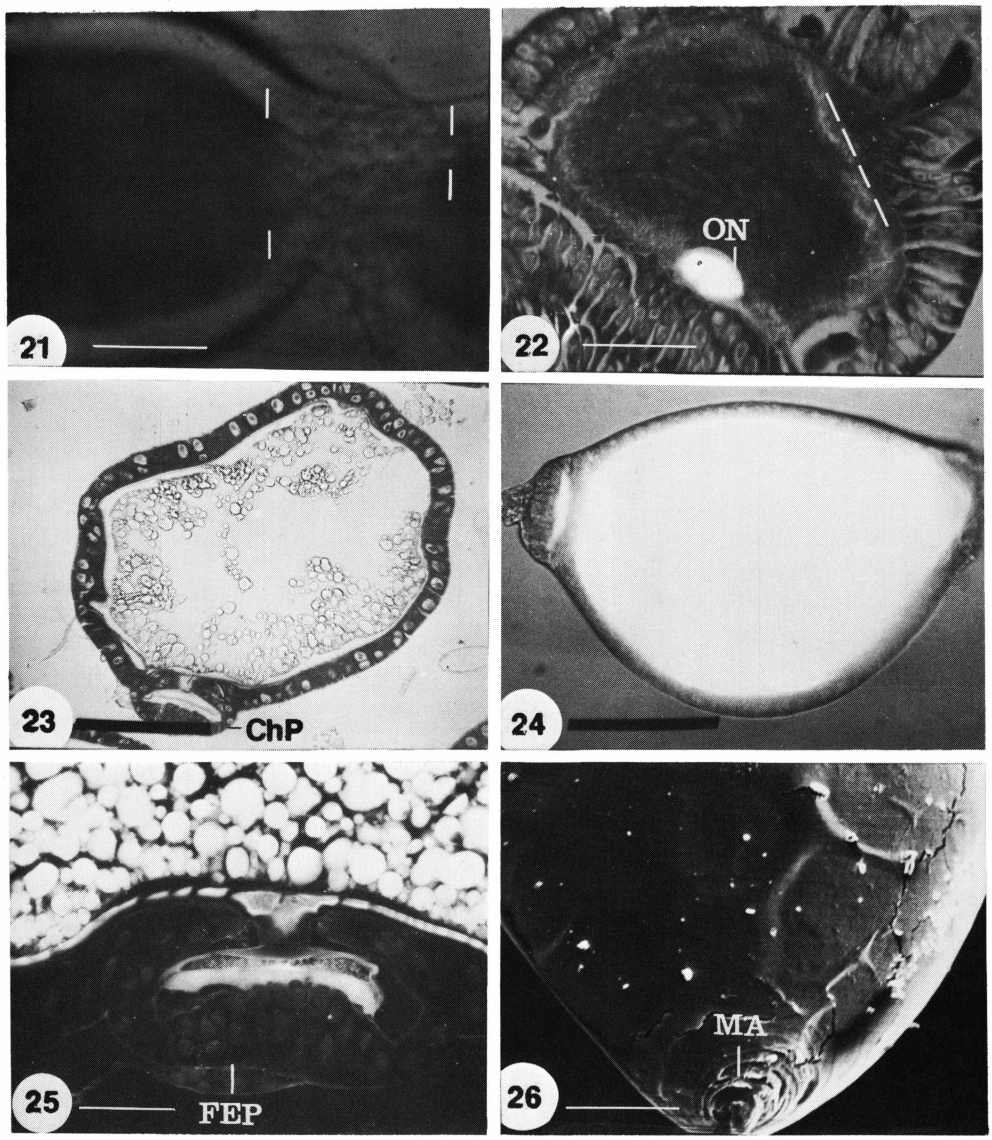


Fig. 21. *Corixa punctata*. Previtellogenic ovarian follicle. Polarization microscope. Polarization-active structure penetrates the follicle (arrows). Scale: *ca.* 65 μm.

Fig. 22. *Corixa punctata*. Late previtellogenic ovarian follicle. Oocyte nucleus (ON) moved to distal part of the follicle. Follicle content divided into trophic cord territory and proper ooplasm (broken line). Scale: *ca.* 40 μm.

Fig. 23. *Corixa punctata*. Early chorionogenic ovarian follicle. In epithelial pocket forming chorion process (ChP) visible. Panoptic Pappenheim method, light micrtoscope. Scale: *ca.* 140 μm.

Fig. 24. *Corixa punctata*. Grown ovarian follicle. Polarization microscope. On proximal pole micropylar apparatus forms, on distal pole, in epithelial pocket adaptive chorion structure visible. Scale: *ca.* 150 μm.

cles. The nucleus reaches the periphery in equatorial portion of the oocyte. In *Not. glauca* it remains in this position till the end of vitellogenesis, in *I. cimicoides* it migrates farther, already in contact with the epithelium, to the subapical portion of the follicle, its ultimate position being near the trophic cord outlet (Fig. 8). In *C. punctata* the migrating nucleus is situated first in the distal oocyte pole (Fig. 22). Then (at the end of the previtellogenesis) the nucleus migrates ultimately to the posterio-lateral part of the oocyte. In *R. linearis* and *N. cinerea* the nucleus peripherization occurs in V larval stage ovaries. The nucleus migration is preceded by a folding of the nuclear envelope on the side turned in the direction of the migration. In imaginal ovarioles, in the differentiated ovarian follicles, the nucleus assumes an extremely eccentrical position, at the opposite side relative to the trophic cord outlet (Fig. 16).

E. Secondary diversification of the follicular epithelium—development acceleration

The process of the secondary diversification of the columnar epithelium part in *R. linearis* and *N. cinerea* is opposite (negative) as compared with the retardation diversification described above. A group of cells (in *R. linearis*, 2; in *N. cinerea*, 6—7) which already in the larval ovary has come to lie in the immediate vicinity of the oocyte nucleus starts growing faster than the other cells and differentiate into B-cells (Figs. 16—18). Their shape becomes oval, the nuclei localize on one level and the apical parts protrude to the ooplasm. Grains of the prechorional material appear in their cytoplasm earlier than in the remaining epithelium cells (super-B-cells).

II. *Vitellogenesis*

In all species studied fluorescent, eosinophilous granules are first observed in the space between the peripherally situated oocyte nucleus and the follicular epithelium. The only exception is the vitellogenesis in *R. linearis* where first yolk spheres appear in the ooplasm pocket adjoining the hypertrophic follicular cells, while at the same time the remaining ooplasm is still of previtellogenic character. However, in advanced vitellogenesis, in both *R. linearis* and *N. cinerea* yolk spheres are observed in the space between the oocyte nucleus and the epithelium, the two territories of increased vitellogenic activity being separated.

In *Not. glauca* and *I. cimicoides* the oocyte nucleus surface facing the epithelium invaginates at the beginning of the vitellogenesis. The resulting space between the nucleus and the epithelium has, in *Not. glauca* a lenticular shape, while in *I. cimicoides* it is gutter-like, parallel to the follicle long axis, and widens proximally. In both the early

Fig. 25. *Corixa punctata*. Adaptive chorion structure on distal pole of egg capsule. Thick, deeply sculptured chorion plate formed by prismatic cells of epithelial pocket (FEP) visible. Scale: *ca.* 40 μm.

Fig. 26. *Corixa punctata*. Proximal part of the egg capsule. SEM. Homogeneous chorion surface formed by B-cells and micropylar apparatus (MA) visible. Scale: *ca.* 90 μm.

(Figs. 22, 25; Carnoy, paraffin sections, Pappenheim panoptic method, fluorescence microscope.)

and the advanced vitellogenesis the yolk spheres in this space (and in *I. cimicoides* on its prolongation as well) are many times larger than in the remaining ooplasm (Fig. 9). In the advanced vitellogenesis, in all the species, a gradient of vitellogenic activity is observed, the activity decreasing gradually with the increasing distance from the oocyte nucleus. This results in an early asymmetry of the oocytes because of the deutoplasm distribution.

III. *Morphological modifications of the epithelium related to the neighbourhood of the oocyte nucleus*

In *Not. glauca* and *I. cimicoides* follicular cells situated in the nearest vicinity of the oocyte nucleus, decrease in height forming in vetellogenesis a pit, bordered by a group of cells higher than in the remaining part of the epithelium. In *I. cimicoides* the phenomenon if accompanied by a nuclearward arrangement of the apical parts of the cells, which on tangential sections gives local radial patterns. In this species, at the end of the vitellogenesis the oocyte nucleus, with its surface facing the epithelium strongly folded because of the pressure exerted by yolk spheres, migrates and adjoins closely the epithelium. At the contact the cells grow higher than the surrounding ones and a "cumulus nucleophorus" arises on which the nucleus forms a characteristic cap (Figs. 10, 11).

A different reaction of the epithelium to the neighbourhood of the oocyte nucleus takes place in *C. punctata* where on the distal oocyte pole, at the first contact epithelium-nucleus, a pit forms and then an epithelial pocket (Figs. 23, 24). Morphological changes in the cells surrounding this separated ooplasm fragment in the pre- and the early vitellogenesis are synchronous with the changes in the remaining epithelium part, though the space bordered by them is devoid of yolk. At the end of the vitellogenesis the pocket undergoes flattening and the cells that form its distal part become high prismatic. They enter the chorionogenesis earlier than the remining part of the epithelium (Figs. 23, 25).

IV. *Chorionogenesis*

The egg capsule formation is preceded by a reorganization of the follicular epithelium. The B-type epithelium gets flat and its patency disappears. The nuclei, situated at one level, often change their position and arrange above one another or in such a way that the axis connecting their centres is oblique relative to the oocyte surface. In *I. cimicoides* the process of "closing" of intercellular spaces begins in the vicinity of the cumulus nucleophorus and encompasses first AB-cells, and then B-cells.

The chorion produced by the high prismatic cells (plate on the proximal pole of the egg capsule in *I. cimicoides* — Figs. 12, 13; chorion plate between the respiratory horns in *N. cinerea* — Fig. 19; distal part of the discoidal chorion process in *C. punctata* — Fig. 25) is always thick, deeply sculptured and azurophilous, which results in its fluorescence on sections stained with Pappenheim's method being lower than the remaining part of the egg capsule. The chorion formed by the B-cells is thinner and on its surface prints of apical portions of the follicular cells are visible. It can be covered

with pits (*I. cimicoides* — Figs. 14, 15), accumulations of filler material (*N. cinerea* — Fig. 20) or uniformly smooth (*C. punctata* — Fig. 26). The super-B-cells form respiratory horns in the proximal portion of the egg capsule (*N. cinerea* — Fig. 19).

Discussion

I. *Diversification of the follicular epithelium in bugs*

A. Morphology

The polymorphism of the follicular cells within the ovarian follicle in bugs connected with the formation of egg capsule respiratory horns ("Atmungsschlauche") was described in *N. cinerea* and *R. linearis* by Korschelt (1884, 1885, 1887, 1891) and Korschelt and Heider (1902). Gross (1901) described the presence of various morphological types of follicular cells in bugs and the part of the modified cells play in formation of various chorion appendages. Huebner and Anderson (1972) when studying the fine structure of the follicular cells in *Rhodnius prolixus*, found that the epithelium on the proximal pole of the follicles is compact and high prismatic. Further results (Anderson, 1974) showed that the prismatic epithelial cells (A-cells) produce the deeply sculptured cover of the egg capsule, similar in structure to the honeycomb. Studies of Huebner (1981) and Huebner and Injeyan (1981) show that in the ovarian follicles in *Rho. prolixus* gap junctions are present both between the follicular cells themselves, and between the follicular cells and the oocyte. Studies of Woodruff and Anderson (1984) on small milkweed bug *Oncopeltus fasciatus* indicate a spatial differentiation of the distribution of fluorochromes injected into the ooplasm in various epithelium parts. This evidences the heterogeneous pattern of distribution of the above mentioned gap junctions in the pre- and early vitellogenic ovarian follicles.

B. Mechanism

Experimental studies showed that the juvenile hormone (JH) is responsible for the regulation of the morphogenesis of the follicular cells in *Rho. prolixus* (Pratt and Davey, 1972). The increasing distance between the epithelium cells which can be expressed as patency index, is *in vitro* a function of the JH concentration in the medium (Davey and Huebner, 1974). The reaction of the follicular cells to the effective titer of JH is changes in cytoskeleton (Abu-Hakima and Davey, 1977) and shrinking of the cells caused by loss of water (Abu-Hakima and Davey, 1979). As a result of these changes the shape of the follicular cells changes from prismatic, determined by mechanical effect of neighbouring cells into oval, with additional protrusions directed to the ooplasm. The cells move apart contacting by cytoplasmic arms. On place of their contact septate and gap junctions are observed (Huebner, 1981, Huebner and Injeyan, 1981).

The sequence of morphological changes of the follicular cells, identical in all the species described in the present paper, allows a supposition that the mechanism of the follicular epithelium differentiation is similar in most bugs. The transformation of the

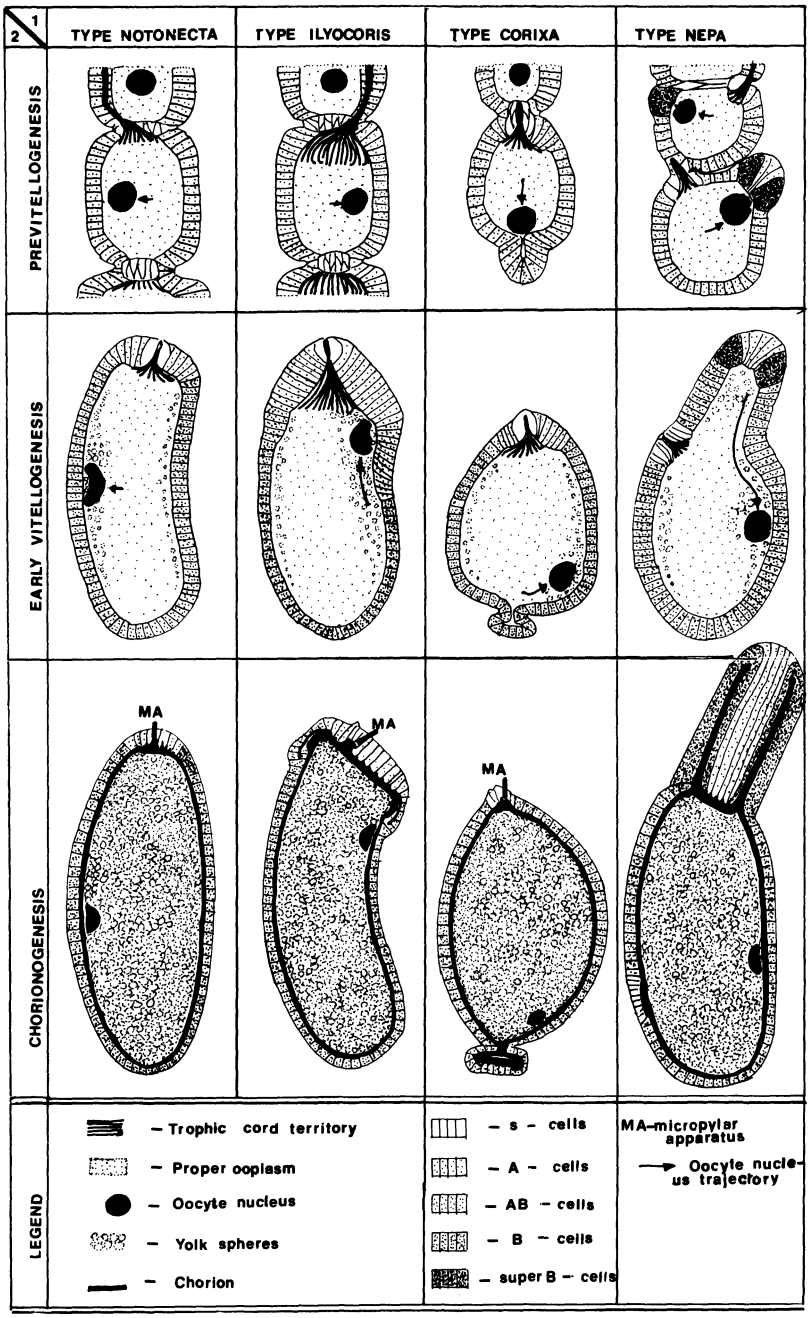


Fig. 27. Diagram showing four basic morphogenesis types in ovaries of water bugs — comparison. 1, morphogenesis type; 2, stage of oocyte growth.

chaotic, blastic mass of the prefollicular cells into the epithelium also requires (in *Pyrrhocoris apterus*) the presence of JH in haemolymph (Masner, 1968).

II. *Asynchrony of follicular cells differentiation*

When analyzing consecutive stages of the oocyte growth in the ovaries of each species, it can be stated that both the interovariole asynchrony (within an ovariole previtellogenic, vitellogenic and chorionogenic follicles may occur simultaneously) and the developement epithelium discoordination within a follicle occurs. The original cause of the discoordination phenomena (A-cells retardation, super-B-cells acceleration and hypertrophy) lies in temporal differences in the differentiation processes in various epithelium parts. Assuming that all the follicles are equally exposed to the factors containing in haemolymph (see Riddiford, 1985, Epilogue) it can be supposed that the ovarian follicles in the species studied show a considerable autonomy. The autonomy of the ovarian follicles (egg chambers) in *Bombyx mori* was postulated by Legay (1979), in bugs by Ogorzałek (1975b). The results of the present study shows, though indirectly, that in the studied species the asynchrony and the discoordination can be reduced to the statement that the trigerring and the possible interruption of the above differentiation mechanism in follicular cells are generated in the follicles themselves.

III. *Bug ovarian follicle as an inductive system*

The morphological analysis of the interdependence between the compartmentaion of the ovarian follicle content in consecutive oogenesis stages, the oocyte nucleus translocalization and the morpho-functional diversification of the follicular epithelium makes it possible to regard the ovarian follicle as an inductive system. According to the handbook definition such a system consists of two components: an active one (inductor) and a reactive one. The retardation diversification of the epithelium in which the cell differentiation depends on their contacts with the proper ooplasm (cells with no such contact do not differentiate and retain ancestral characters) roughly point out to the oocyte as an active component and to the follicular epithelium as a reactive one. The acceleration differentiation, as well as the later, local modification of the epithelium connected with the neighbourhood of the oocyte nucleus indicate that the oocyte nucleus is the actual inductor in the ovarian follicle morphogenesis in the studied species. Purely morphological character of the present results and the indirect reasoning do not allow for a definition of the mechanism of the described interaction. It enables only a construction of a simple model of the ovarian follicle morphogenesis in bugs.

IV. *The morphogenesis model*

The proposed model is based on a hypothetical assumption that the oocyte nucleus emits a factor (evocator ?, morphogene ?) which initiates the follicular cell differentiation. The chemical nature of this factor is unknown. If the gap junctions are the

only physical canal through which it could penetrate to the follicular cells, the upper limit of its molecular weight will be below 1,500. In the proposed model the factor is called FCDF (follicle cell differentiating factor) according to its postulated function and it is assumed that the time of the emission depends on the oogenesis stage.

The sequence of events described above, which leads to the increase in epithelium patency is a reaction of the follicular cells on FCDF (requiring an effective titer of JH in haemolymph). When the oocyte reaches the threshold size, the differentiation program is stopped in all the cells at the same time, irrespective of which part of it has been effected, and the cells synchronously start building the chorion.

The compartmentation of the ovarian follicle content results, in turn, in the asynchronous (and probably uneven) exposition of the cells to JH (primary epithelium diversification depending on the contact *vs.* lack of contact with heaemolymph) and to FCDF (secondary differentiation of the epithelium, both retardation and acceleration). The discoordination "relief" diversification of the epithelium determines the rough morphology of the egg. The high prismatic nature of the juvenile cells (A-cells) and the decrease in height of the cells in the remaining parts of the epithelium cause the modified inner outline of the epithelium on the proximal egg pole, modelling the oocyte shape, while the outer outline of the follicle remains unchanged.

If the postulated hypothesis on the inductive, morphogenetic role of the oocyte nucleus in functioning and formation of bug ovarian follicle is accepted, the haemolymph with its chemical milieu can be regarded as an environment interfering with the processes that take place in the growing oocyte, albeit with no effect on the spatial diversification of eggs and their capsules. In this connection follicular cells would be a "mediator" between the genetic and epigenetic system of the primary oocytes on one hand, and the insect internal environment on the other. Their behaviour can be regarded as a resultant of the reaction to the environment factors (hormonal signals, yolk precursors or nutritive materials) and the signals coming from the oocyte itself (most probably its nucleus). Due to the latter signals in the spatially homogeneous internal environment of the insects, morphogenetic processes in the ovarian follicles take place, resulting in the species-specific egg morphology in bugs.

References

Abu-Hakima, R. and K. G. Davey, 1977. The action of juvenile hormone on the follicle cells of *Rhodnius prolixus*. The effect of colchicine and cytochalasin B. *Gen. Comp. Endocrinol.* 32: 360-370.

————, and ————, 1979. A possible relationship between ouabain-sensitive ($Na^+ - K^+$) dependent ATPase and the effect of juvenile hormone on the follicle cells of *Rhodnius prolixus*. *Insect Biochem.* 9: 195-198.

Anderson, E., 1974. Comparative aspects of the ultrastructure of the female gamete. *Int. Rev. Cytol., Suppl.* 4, 1-70.

Davey, K. G. and E. Huebner, 1974. The response of the follicle cells of *Rhodnius prolixus* to juvenile hormone and antigonadotropin *in vitro*. *Can. J. Zool.* 52: 1407-1412.

Gross, J., 1901. Untersuchungen über das Ovarium der Hemipteren, zugleich ein Beitrag zur Amitosenfrage. *Z. Wiss. Zool.* 69: 139-201.

Hinton, H. E., 1981. Biology of Insect Egg. Vols. 1-3. Pergamon Press, Oxford, New York.

Huebner, E., 1981. Oocyte-follicle cells interaction during normal oogenesis and atresia in an insect. *J. Ultrastruct. Res.* 74: 95-104.

———, and E. Anderson, 1972. A cytological study of the ovary of *Rhodnius prolixus*. I. The ontogeny of the follicular epithelium. *J. Morphol.* 136: 459-493.

———, and H. Injeyan, 1981. Follicular modulation during oocyte development in an insect: Formation and modification of septate and gap junctions. *Devl. Biol.* 83: 101-113.

King, R. C. and J. Büning, 1985. The origin and functioning of insect oocytes and nurse cells. *In* G. A. Kerkut and L. I. Gilbert (eds.), Comprehensive Insect Physiology, Biochemistry and Pharmacology, Vol. 1, Embryogenesis and Reproduction, 37-82. Pergamon Press, Oxford, New York.

Korschelt, E., 1884. Die Bildung des Chorions bei einigen Wasserwanzen. *Zool. Anz.* 7: 500-504.

———, 1885. Zur Frage nach dem Ursprung der verschidenen Zellelemente der Insektenovarien. *Zool. Anz.* 8: 581-599.

———, 1887. Uber einige interessante Vorgänge bei der Bildung der Insekteneier. *Z. Wiss. Zool.* 45: 327-379.

———, 1891. Beiträge zur Morphologie und Physiologie des Zellkerns. *Zool. Jb. Anat. Ontog.* 4: 1-154.

———, and W. Heider, 1902. Lehrbuch der vergleichenden Entwicklungsgeschichte der Wirbellosen Tiere. Allgemeine Teil. Vol. 1. Gustav Fischer, Jena.

Legay, J. -M., 1979. Oocyte growth. *Biochemie* 61: 131-145.

Masner, P., 1968. The inductors of differentiation of prefollicular tissue and the follicular epithelium in ovarioles of *Pyrrhocoris apterus* (Heteroptera). *J. Embryol. Exp. Morphol.* 20: 1-13.

Ogorzałek, A., 1969. Zachowanie się komórek folikularnych w czasie oogenezy u *Ranatra linearis* i *Nepa cinerea* (Insecta, Heteroptera). *Mat. IX Zjazdu P. T. Zool. Lublin.* 96.

———, 1974. Oogenesis in the water bugs. I. Cytochemical investigations of the follicular cells in *Nepa cinerea* L. and *Ranatra linearis* L. *Zool. Pol.* 24: 25-39.

———, 1975a. Oogenesis in the water bugs. II. The interaction of the germinal vesicle and the follicular epithelium in *Naucoris cimicoides*. *Zool. Pol.* 25: 197-209.

———, 1975b. Wpływ jądra oocytu na różnicowanie się i funkcjonowanie komórek folikularnych podczas oogenezy u pluskwiaków różnoskrzydłych. *Acta Univ. Wrat.* 301: 41-55.

———, 1978. Oogenesis in the water bugs. III. The behaviour of chromatin in the oogenesis of *Naucoris cimicoides*. *Zool. Pol.* 27: 81-90.

———, 1984. SEM, TEM and fluorescence microscope observations on egg chambers morphogenesis in water bugs. *III Int. Congr. Cell. Biol., Tokyo*, 5683.

———, Ovarian follicle morphogenesis and egg capsule formation in *Nepa cinerea* L. (Heteroptera, Nepidae). *Acta Univ. Wrat.* (in press).

Pratt, G. E. and K. G. Davey, 1972. The corpus allatum and oogenesis in *Rhodnius prolixus* (Stal). I. The effects of allatectomy. *J. Exp. Biol.* 56: 201-214.

Riddiford, L. M., 1985. Hormone action at the cellular level. *In* G. A. Kerkut and L. I. Gilbert (eds.), Comprehensive Insect Physiology, Biochemistry and Pharmacology, Vol. 8, Endocrinology II, 37-84. Pergamon Press, Oxford, New York.

Stebbings, H., 1971. Influence of vinblastine sulphate on the development of microtubules and ribosomes in telotrophic ovarioles. *J. Cell Sci.* 8: 111-125.

Woodruff, R. J. and K. L. Anderson, 1984. Nutritive cord connection and dye-coupling of the follicular epithelium to the growing oocytes in the telotrophic ovarioles in *Oncopeltus fasciatus*, the milkweed bug. *Roux's Arch. Devl. Biol.* 193: 158-163.

Author's address: Dr. A. Ogorzałek
Department of General Zoology, Institute of Zoology, Wrocław University, ul. Sienkiewicza 21, 50-335 Wrocław, Poland