Cell Tiss. Res. 150, 463—479 (1974)

The Fine Structure of the Ovarian Follicle of *Alloteuthis subulata* Lam. (Mollusca, Cephalopoda)*

Werner Bottke **

Zoologisches Institut der Universität Münster

Received March 29, 1974

Summary. The structure of the ovarian follicle of *Alloteuthis subulata* Lam. during the euplasmic growth phase and vitellogenesis has been investigated by light and electron microscopy. Oogenesis can be divided into three stages. Oocytes of stage I are not yet surrounded by a follicle cell epithelium. During stage II, infolding of the follicular epithelium into the oocyte and the euplasmic growth phase of the oocyte take place. Follicle cells show all attributes typical for protein synthesizing cells. During stage III, formation of the chorion occurs due to follicle cell activity. In contrast to earlier light microscopical observations, there are no indications of an engagement of the follicle cells in the production of exogenous yolk protein, which could be taken up by the oocyte in pinocytotic vesicles. The observations rather favour the idea of a largely autonomous synthesis of the PAS-positive yolk in the oocyte. The Golgi apparatus seems to be engaged in yolk production. The findings are discussed in comparison with observations on vitellogenesis in other invertebrates and vertebrates.

Key words: Ovarian follicle — Vitellogenesis — Cephalopods — Ultrastructure.

Zusammenfassung. Die Feinstruktur des Ovarfollikels von *Alloteuthis subulata* Lam. während der euplasmatischen Wachstumsphase und der Vitellogenese wurde licht- und elektronenmikroskopisch untersucht. Die Oogenese kann in drei Stadien unterteilt werden. Oocyten des Stadiums I haben noch kein Follikelepithel. Während des Stadiums II faltet sich das Follikelepithel in die Oocyte ein, die ihre euplasmatische Wachstumsphase durchläuft. Die Follikelzellen zeigen typische Merkmale von Zellen mit starker Proteinsynthese. Im Stadium III wird das Chorion von den Follikelzellen gebildet. Im Gegensatz zu älteren lichtmikroskopischen Beobachtungen ergeben sich keine Hinweise, die für eine Beteiligung der Follikelzellen an der Bildung exogenen Proteindotters sprechen. Die eigenen Beobachtungen sprechen vielmehr für eine weitgehend autonome Synthese des PAS-positiven Dotters durch die Oocyte unter Beteiligung des stark ausgebildeten Golgi-Apparates. Die Befunde werden im Vergleich mit Beobachtungen zur Vitellogenese anderer Invertebraten und Vertebraten diskutiert.

Introduction

An unsolved problem of molluscan oogenesis is the origin of yolk. In contrast to the oogenesis of other animal groups, such as insects, crustaceans, amphibians or fishes, where oocytes have been shown to incorporate large amounts of exogenous materials from distant organs (Telfer, 1965; Anderson and Telfer, 1970; Wolin *et al.*, 1973; Jared *et al.* 1973; te Heesen and Engels, 1973), no comparable observations have been made in the case of molluscs. So far, electron microscopical observations of the small oocytes of gastropods and bivalvia have not furnished convincing evidence for pinocytosis, by which exogenous materials could be in-

* This investigation was supported by the Stiftung Volkswagenwerk.

** I am indebted to Prof. Dr. K. Müller for correction of the manuscript. The technical assistance of Miss Ch. Köhn is gratefully acknowledged.

corporated into oocytes (Favard and Carasso, 1958; Recourt, 1960; Beams and Sekhon, 1966; Taylor and Anderson, 1969; Bottke, 1973). The oocytes fulfil however all criteria which are typical for cells with a high synthetic capacity, e.g. large active nucleoli, highly ordered arrays of rough endoplasmic reticulum (ER) and many Golgi fields.

Light microscopical investigations of the large oocytes of cephalopods, which have abundant supplies of yolk, revealed the existence of a large follicular epithelium surrounding the oocyte and presumably providing it with secretory products, which were suggested to build up the yolk during secondary vitellogenesis (Lankester, 1875; Vialleton, 1888; Yung Ko Ching, 1930). In contrast to these findings, early histochemical investigations of Konopacki (1933) prompted Arnold (1971) to suggest, that the mechanism of vitellogenesis in cephalopods might be similar to that of insects.

A recent investigation of oogenesis in *Octopus vulgaris* by O'Dor and Wells (1973) supports strongly the idea of the exogenous nature of the protein yolk in cephalopods. However the authors could not obtain evidence, that the liver, which is the major source of yolk materials in vertebrates, fulfills this role also in *Octopus*. Their findings favour the idea that the synthesis of protein yolk takes place in the follicle cells, as anticipated by the earlier observers. Until now, there exists only one recent observation concerning the alterations of follicle structure during oogenesis (Cowden, 1968). This report deals with light- and electron microscopical observations on follicles of *Alloteuthis subulata* Lam., which provide further information on the oogenesis of cephalopods. As a result, follicle cells have indeed turned out to be well equipped for protein synthesis. Apart from their probable function in producing the chorion of the oocyte, evidences for a transfer of macromolecular products into the oocyte are lacking. It must be suggested that at least a large part of the PAS-positive yolk is synthesized by the oocyte. The Golgi apparatus seems to be engaged in the production of yolk. Further investigations are planned to establish to which extent also exogenous materials are incorporated into the oocyte.

Material and Methods

Adult specimens of *Alloteuthis subulata* Lam. were captured together with masses of spawn during may 1973 between the isles of Langeoog and Spiekeroog (North-Sea). Determination of the species was done after Jaeckel (1958). The follicles were immediately fixed in 2% glutaraldehyde (1 hour), adjusted to pH 7.2 by phosphate-buffer, rinsed for 1 hour in phosphate-buffer and fixed with osmic acid or the fixation medium of Wolfarth-Bottermann (Ruthmann, 1966) for another hour. After rinsing for 30 min with Ringer-solution, the follicles were passed through a graded series of ethanol into propylene-oxide and embedded in Epon. In 70% ethanol, the follicles were stained with 1% uranyl acetate. 1 μm sections were stained for light microscopical examination with alkaline toluidine blue, Azur B at pH 4 (for nucleic acids), or were subjected to the PAS-reaction (Ruthmann, 1966). Ultrathin sections were stained with lead citrate (Reynolds, 1963). Paraplast sections (10 μm) were stained with mercuric bromphenol blue for detection of protein yolk (compare Ubbels, 1968).

Observations

The ovary of *Alloteuthis* contains many oval or pear-shaped follicles of all developmental stages, as was already described by Bergmann (1903) for other

cephalopods. The follicles are connected with the ovarian wall by a narrow, slender stalk of connective tissue, through which the follicles are supplied with blood capillaries. All oocytes, which could be investigated had already passed the pachytene bouquet stage of the chromosomes. Our findings are consistent with the observations of Yung Ko Ching (1930), who could investigate the early meiotic stages only in young animals, since they seem to be extremely rare in adult ones. Also, mitoses could never be observed so that the elucidation of the chromosome number must await further work. Oogenesis of *Alloteuthis* can be divided according to the structure of the follicle into three developmental stages:

Stage I: The oocyte is not yet surrounded by a follicular epithelium but only wrapped into a thin sheath of connective tissue cells (Fig. 1a).

Stage II: At the beginning of this stage, the oocyte becomes totally surrounded by a flat and smooth epithelium of cube-shaped follicle cells (Fig. 1b, c). When the oocyte starts with the growth phase, the follicle cells increase in number and height. Soon a network of infoldings of the follicular epithelium penetrates the oocyte (Fig. 1d, e, f), giving it a rather complex structure when sectioned.

Stage III: During vitellogenesis, the oocyte becomes nearly totally filled with irregular, polygonal yolk platelets and thus shows a clear animal-vegetal polarity. Formation of the chorion starts early in vitellogenesis (Fig. 1g).

Stage I

Stage I oocytes have an average diameter of 100 μm, their nuclei measuring 50–60 μm. The cytoplasm of the oocyte abounds in polysomes and shows many profiles of rough endoplasmic reticulum (ER) and many dictyosomes, located preferentially around the well-rounded nucleus. Extended areas of vesicles (Fig. 5a), containing also coated vesicles which closely resemble Golgi vesicles are also characteristic of this developmental stage.

Mitochondria of the tubulus type are homogeneously distributed over the cytoplasm. Large densely staining clusters of ribosome-like particles, forming complexes of variable structure, are typical for oocytes of this stage as they are for follicle cells of stage II (Fig. 3b, 5d). They are stainable with Azur B and seem to be aggregates of RNP-particles. The oocyte nuclei of stage I contain many spherical inclusions of variable size, which are often vacuolated and consist of a central area of lower density and a fibrillar cortex of higher density (Fig. 6a). They can be stained intensively with Azur B. Later on, one can no longer observe the larger spheres but only loose clusters of many small spheres of identical ultrastructure. They seem to be formed by fragmentation of the large nucleolus-like structures (Fig. 6b). Besides them, the nucleus is filled with a large number of small granules, ordered like beads on a string, which are assumed to be chromomeric granules of lampbrush type chromosomes.

Stage II

a) Follicular Epithelium. Oocytes of the early stage II are surrounded by a simple epithelium of follicle cells with a rather prominent nucleus, showing some heavily stained hook-like or ring-shaped profiles of nucleoli (Fig. 2a). The oval follicle always exhibits a clear polarity, with the oocyte nucleus situated at the

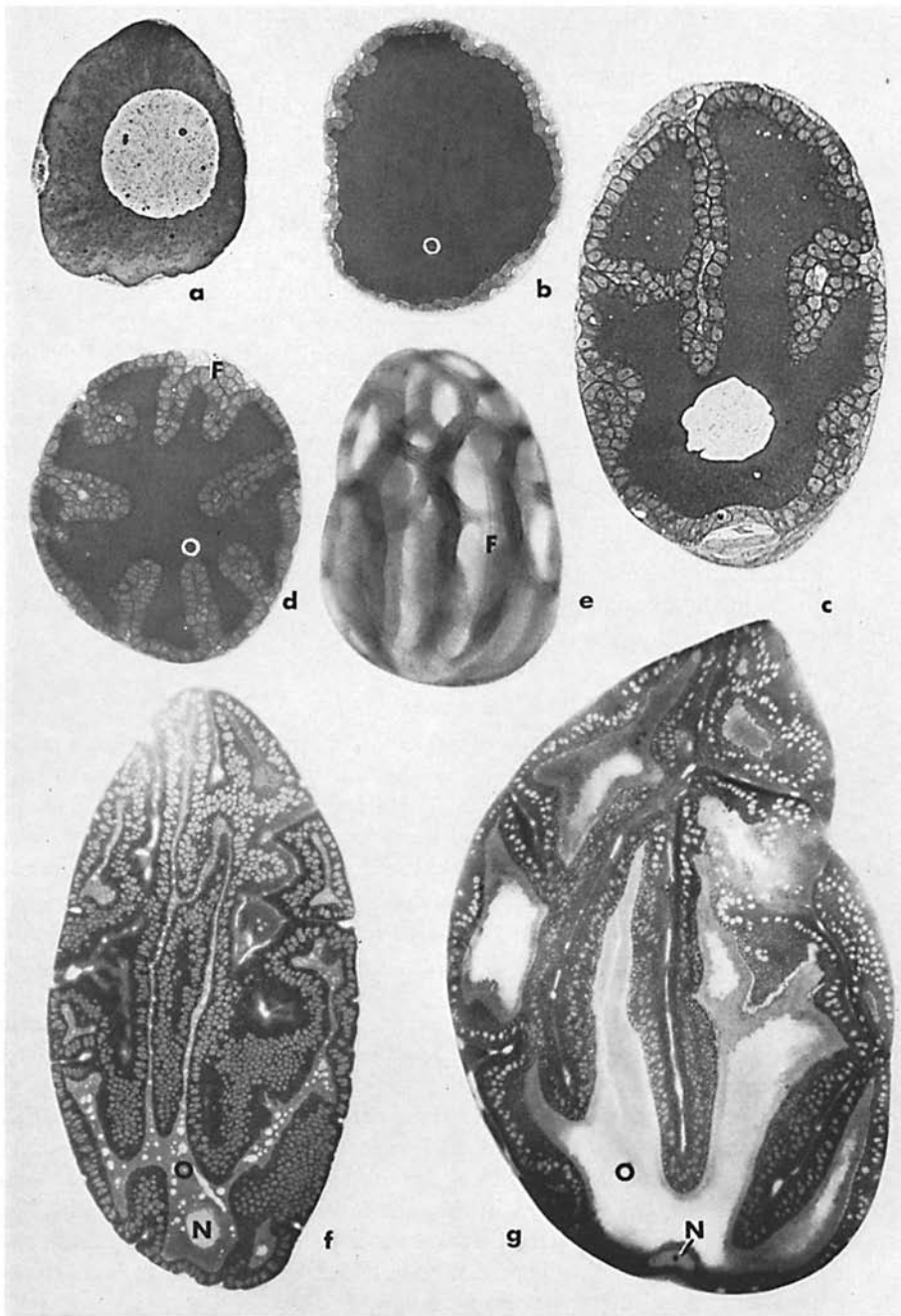

Fig. 1a—g. Developmental stages of ovarian follicles of *Alloteuthis subulata*. a A young oocyte of stage I, not yet surrounded by follicle cells. Magn. ×300. b Cross-section of an early stage II oocyte, which is surrounded by a simple epithelium of follicle cells. Magn. ×120. c Longitudinal section of an early stage II follicle. Magn. ×200 d Cross-section of a medium size follicle (stage II), showing infolding of the follicular epithelium. Magn. ×120. e Total view of a vitellogenic follicle with its network of epithelial infoldings. Magn. ×40. f Longitudinal-section of a late stage II follicle prior to vitellogenesis. Magn. ×80. g Longitudinal-section of a vitellogenic follicle. Magn. ×60. Oocyte (*O*), Follicle cells (*F*), Nucleus (*N*)

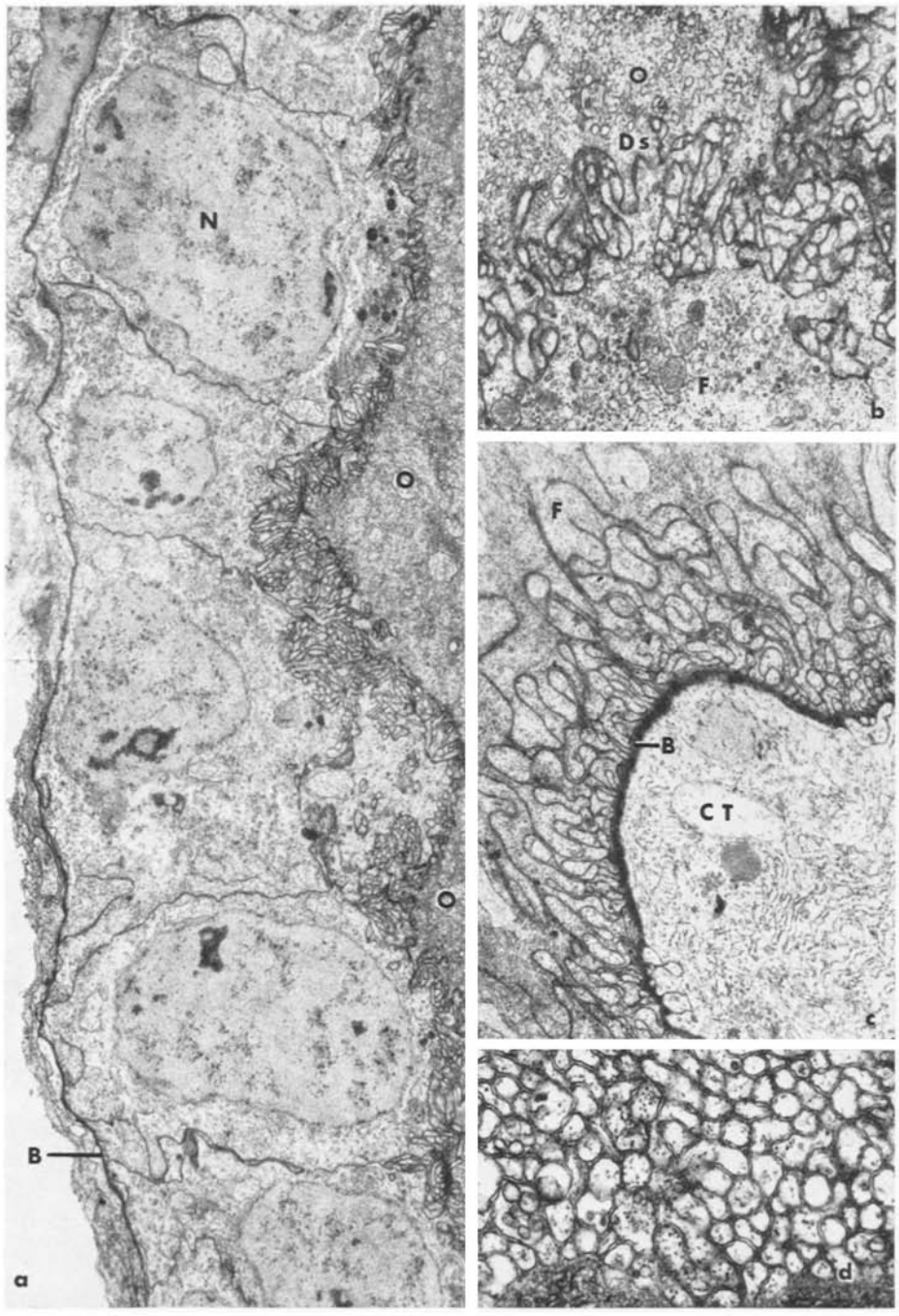

Fig. 2a—d. Ultrastructure of follicle cells during the euplasmic growth phase (stage II). a Total view of the follicle cells of an early stage II follicle. Magn. ×6000. b Zone of contact between the oocyte and a follicle cell. Magn. ×16000. c and d Infolding of the basal plasma membrane of stage II follicle cells. c Longitudinal-section. Magn. ×8000. d Cross-section. Magn. ×24000. Oocyte (*O*), Follicle cell (*F*), Desmosome (*Ds*). Basement membrane (*B*), Connective tissue cell (*CT*)

future animal pole. During the rapid growth phase of the oocyte, which is accompanied by an intensive infolding of the follicle cell epithelium the follicle increases in length from 250 μm to 700 μm (Fig. 1c–f).

The cube-shaped follicle cells of the early stage II have an average height of 8–10 μm. Basally they are situated on a fibrillar basement membrane of an average thickness of 600 Å and do not show infolding of their basal plasma membranes (Fig. 2a). Laterally neighbouring cells are closely connected with each other by desmosomes of the zonula adhaerens type, without forming indentations of their lateral plasma membranes. Apically, however, the cell surface is greatly enlarged by many microvilli-like projections. These projections are tightly connected by zonulae with similar projections of the oocyte, thus forming a complex network of cell projections and giving this zone a honeycomb appearance if sectioned tangentially (Fig. 2a, b). The membranes of this area are often ruptured into vesicles. Although there can be no doubt that rupturing is due to fixation with osmic acid, these observations support the idea of a structural weakness of these membranes due to the physiological state of the follicle cells. The cells contain some microtubules, many tubules of rough ER, large mitochondria and prominent dictyosomes which are accompanied by many vesicles or lysosome-like inclusions, e.g. multivesicular bodies (Fig. 2b).

Infolding of the follicular epithelium is accompanied by a rapid increase in cell number and a considerable growth of the individual cells, which attain a maximum length of 60 μm during vitellogenesis. During the growth phase of the follicle cells an extraordinary increase of the rough ER is seen, whose regularly ordered stacks of lamellae or tubular elements soon fill up the whole cell (Fig. 3b, d), leaving no space for other inclusions. Intercellular spaces between the lateral cell membranes cannot be observed. Many capillaries can be seen invading the infoldings of the follicular epithelium. Follicle cells now start building up an extensive system of infoldings of their basal plasma membrane, the spaces of which are often found to contain osmiophilic material (Fig. 2c, d). Profiles of mitochondria which are easily identifiable by light microscopy on account of their giant dimensions (Fig. 3b) are regularly encountered between the basal infoldings. The occurrence of large dictyosomes is restricted to the apical area of the cells (Fig. 3b, c). They are surrounded by many dense vesicles, vacuoles and tubular structures, which are often in close contact with the apical cell membrane (Fig. 3c). Later stages of follicle development often give the impression that material derived from the Golgi apparatus is transported to the apical cell membrane and released into the intercellular space between the follicle cells and the oocyte. Large membrane-bounded dense granules are also typical for the follicle cells during stage II. In contrast to the strong vesiculation of the follicular epithelium, there are no indications that the oocyte takes up material by pinocytosis, which has been released by the follicle cells.

The nuclei of the follicle cells contain two distinct nucleoli of typical ultrastructure or a network of dense nucleolar strands of ribosome-like particles.

Follicle cells of vitellogenic oocytes (stage III) show no differences in ultrastructure compared with the cells of stage II. During early vitellogenesis, the formation of the chorion starts by action of the follicle cells. The intercellular space between the follicle cells and the oocyte is widened locally and is filled up

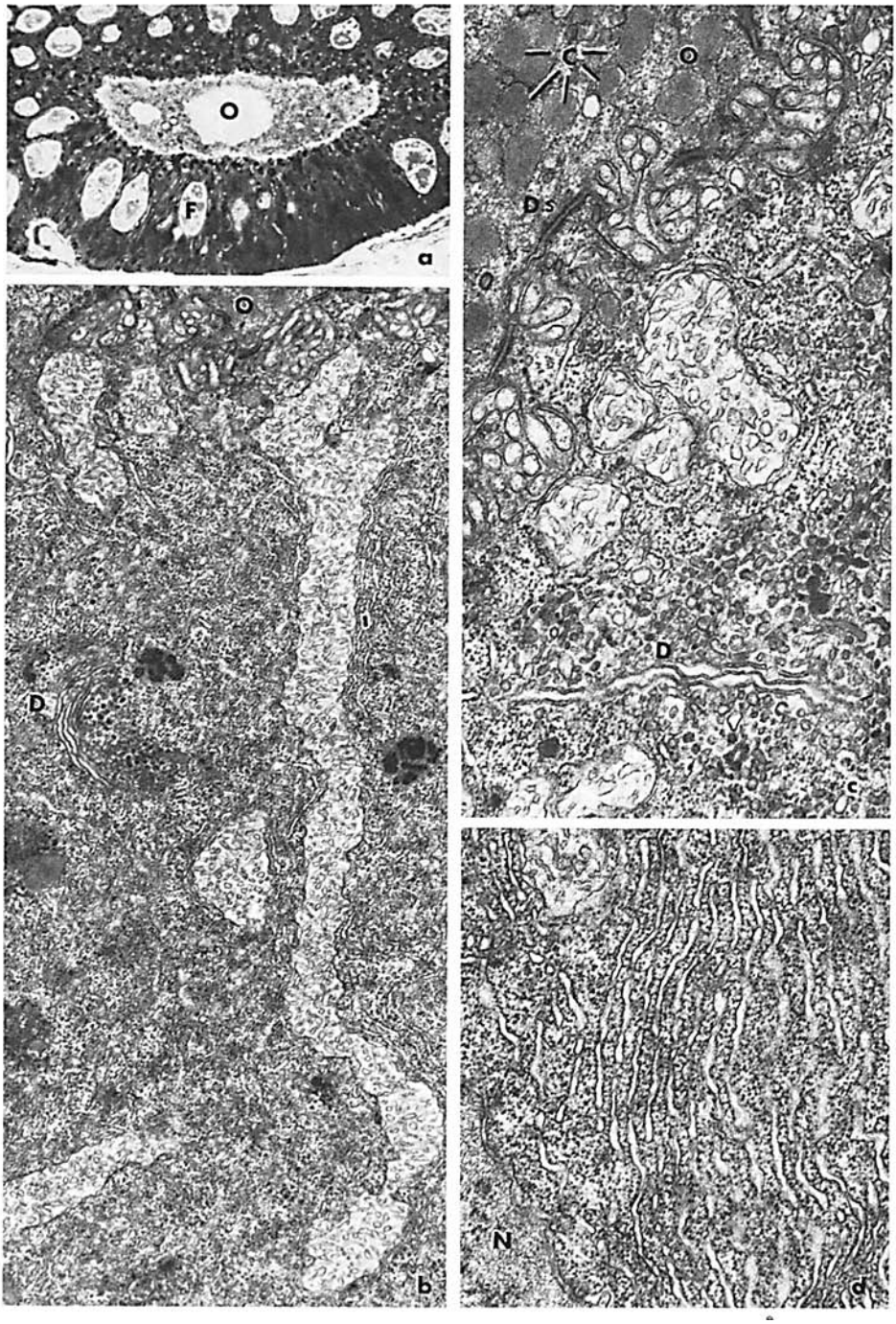

Fig. 3a—d. Ultrastructure of follicle cells during stage II and III. a Light microscopical view of a vitellogenic oocyte with its surrounding epithelium. Magn. ×500. b Giant mitochondrion of a large follicle cell. Magn. ×12000. c Part of the oocyte and the apical region of a stage III follicle cell. Magn. ×24000. d Rough ER of a follicle cell (stage III). Magn. ×24000. Oocyte (*O*), Follicle cell (*F*), Desmosome (*Ds*), Dictyosome (*D*), Crystalline inclusions (*C*)

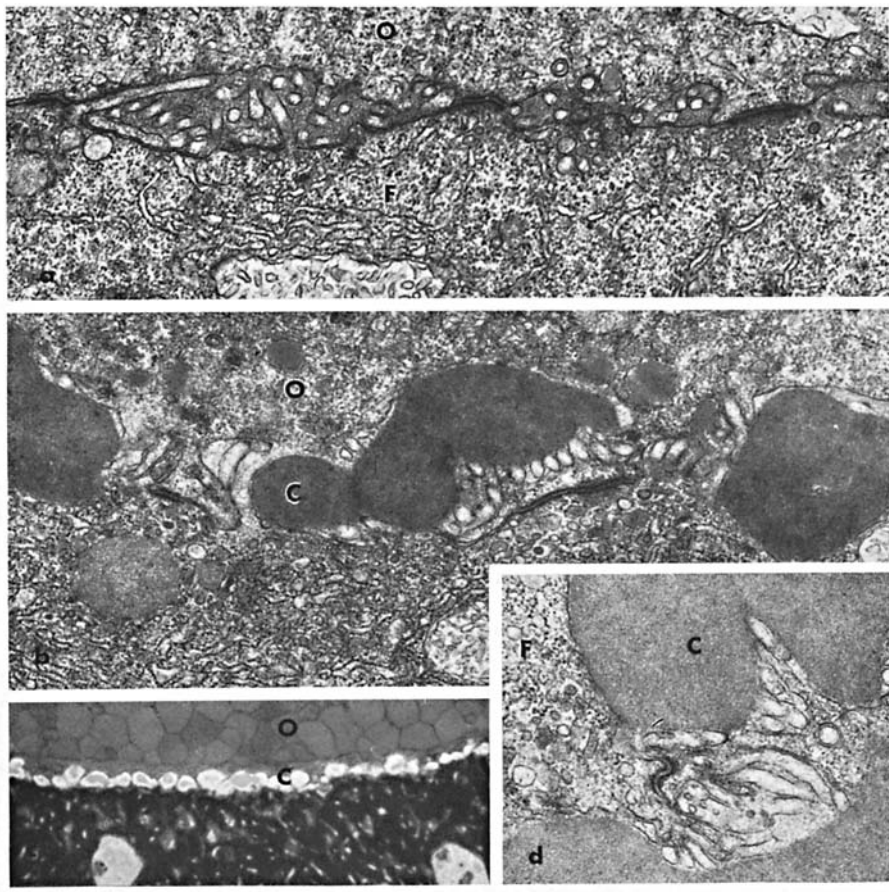

Fig. 4a—d. Development of the chorion. a The intercellular spaces between the oocyte and the follicle cells, filled up with a darker material (early stage III). Magn. ×16000. b Chorion droplets of late stage III follicles. Magn. ×15000. c Light microscopical view of chorion formation. Fusion of the individual droplets occurs late in oogenesis. Magn. ×750. d Oocyte and follicle cells are connected by narrow cytoplasmic channels. Magn. ×20000. Oocyte (*O*), Follicle cells (*F*), Chorion droplet (*C*)

with a darker staining fibrillar material into which the microvilli-like structures of the oocyte and the follicle cells are projecting (Fig. 4a). Obviously this material is not taken up by the oocyte because of the total lack of pinocytotic vesicles. The close connection of the follicle cells and the oocyte is, however, not disturbed by chorion formation, as both stay tightly connected by desmosomes. During the course of vitellogenesis the chorion material can be seen to form irregular droplets, which push apart the membranes of the follicle cells and the oocyte except for those areas, where both are connected by zonulae (Fig. 4b, d). Even during the last stages of follicle development the contact of follicle cells and oocyte remains unaltered, both being connected by narrow cytoplasmic channels between the

chorion droplets. There are no signs of transfer of macromolecular material into the oocyte, which could occur by the cytoplasmic channels during vitellogenesis. The material of the chorion reacts strongly with mercuric bromphenol blue, indicating a considerable protein content. It cannot be stained with the PAS-reaction in contrast to the oocyte plasm (Fig. 7b, c). At the animal pole of the follicle, the epithelium is flattened attaining a minimum height of not more than 1 μm. The individual cells are wedge-shaped but are all connected with the basement membrane. As to their ultrastructure they do not appear to be different from the other follicle cells. Compared with the other regions of the follicle, formation of the chorion seems to be retarded at this area.

b) Oocytes. The nucleus of young stage II oocytes has an average diameter of 80 μm. After staining with Azur B, many chromosomal axes can be seen with lateral projections, suggesting loops of lampbrush chromosomes (Fig. 6c). Some basophilic spherical or drumstick-like structures are often in close connection with the chromosomes. Besides the nucle icontain many little spheres, whose number increases strongly during oogenesis. The cytoplasm of stage II oocytes contains abundantly polysomes. Mitochondria can often be seen wrapped into membranes, which are studded with ribosomes (Fig. 5e, f). Many annulate lamellae (Fig. 5h) are also typical for this stage. The number of dictyosomes is largely increased, compared with stage I. Profiles of dictyosomes, large areas of vesicles or coated vesicles are the most prominent inclusions (Fig. 5b, c). Many of the vesicles contain a crystalline, darkly staining content. The whole plasm of the oocyte becomes gradually filled with poorly contrasted vesicles. It could not be elucidated, whether they are Golgi vesicles or elements of the smooth ER. However, abundance of dictyosomes favours the idea that the greatest part of the vesicles is derived from the Golgi apparatus. Aggregates of presumed Golgi vesicles are often surrounded by lamellae of the rough ER. Large membrane-bounded vacuoles having an average diameter of 3–5 μm and containing aggregates of vesicles and myelin-like membranous structures represent more advanced stages of yolk formation (Fig. 5g), as it is the case in other molluscs. The euplasmic growth phase of the oocyte thus cannot be strictly separated from vitellogenesis. At the end of stage II a clear difference in composition between various parts of the oocyte plasm takes place. The vegetal plasm between the follicular epithelium infoldings contains innumerable vesicles and aggregates of asterisk-like membrane-bounded yolk platelets with low optical density (Fig. 8a). One must assume, that they contain only small amounts of dry matter and are perhaps semifluid, as it is described for *Sepioteuthis* (Arnold, 1971). They are surrounded on all sides by vesicles of similar contrast, which seem to be incorporated into the developing yolk platelets. Yolk platelets often contain vesicles and—due to fixation—a fine fibrillar material. Dictyosomes can often be seen accompanying the yolk platelets, and may be engaged in their production. As vitellogenesis progresses, an increase in contrast of the individual yolk platelet is achieved by condensation and constant incorporation of new materials. The cytoplasm of stage II oocytes is intensively stained by the PAS-reaction, indicating a high carbohydrate yolk content (Fig. 7a).

The animal plasm of the oocyte is densely filled with tubular mitochondria, dictyosomes, many cisterns of the rough ER and many membrane-bounded inclusions, which contain an osmiophilic crystalline material (Fig. 8d). A striking

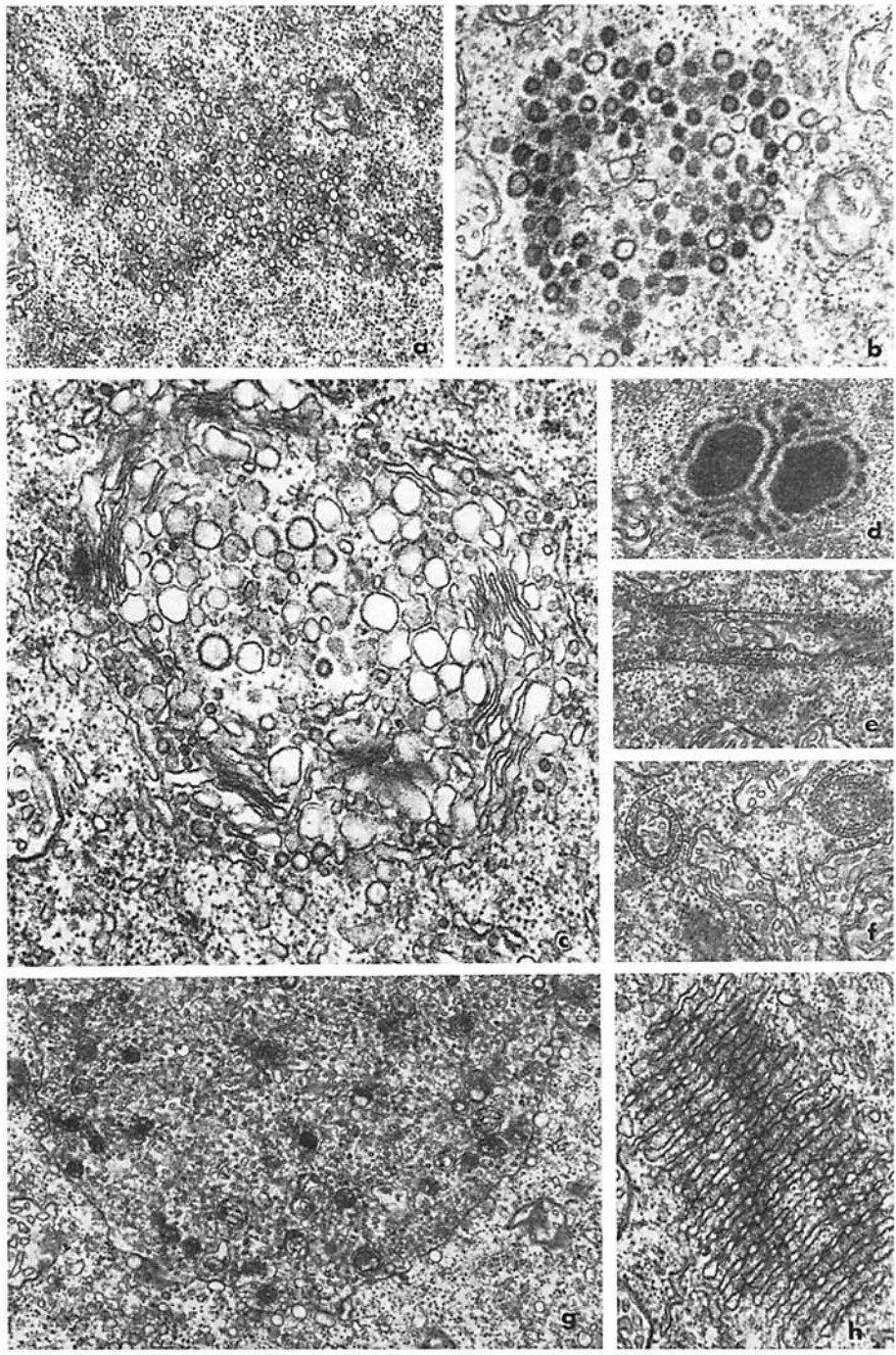

Fig. 5a—g. Oocyte ultrastructure. a Aggregates of Golgi vesicles in an early stage I oocyte. Magn. ×12000. b Aggregates of coated vesicles (stage II). Magn. ×20000. c A typical Golgi apparatus of stage II. Magn. ×32000. d Clusters of ribosome-like particles. Magn. ×20000. e Mitochondrion-membrane-structures; longitudinal-section, f Cross-section. Magn. ×21000. g An early stage of a developing yolk platelet. Magn. 18000×. h Annulate lamellae. Magn. ×24000

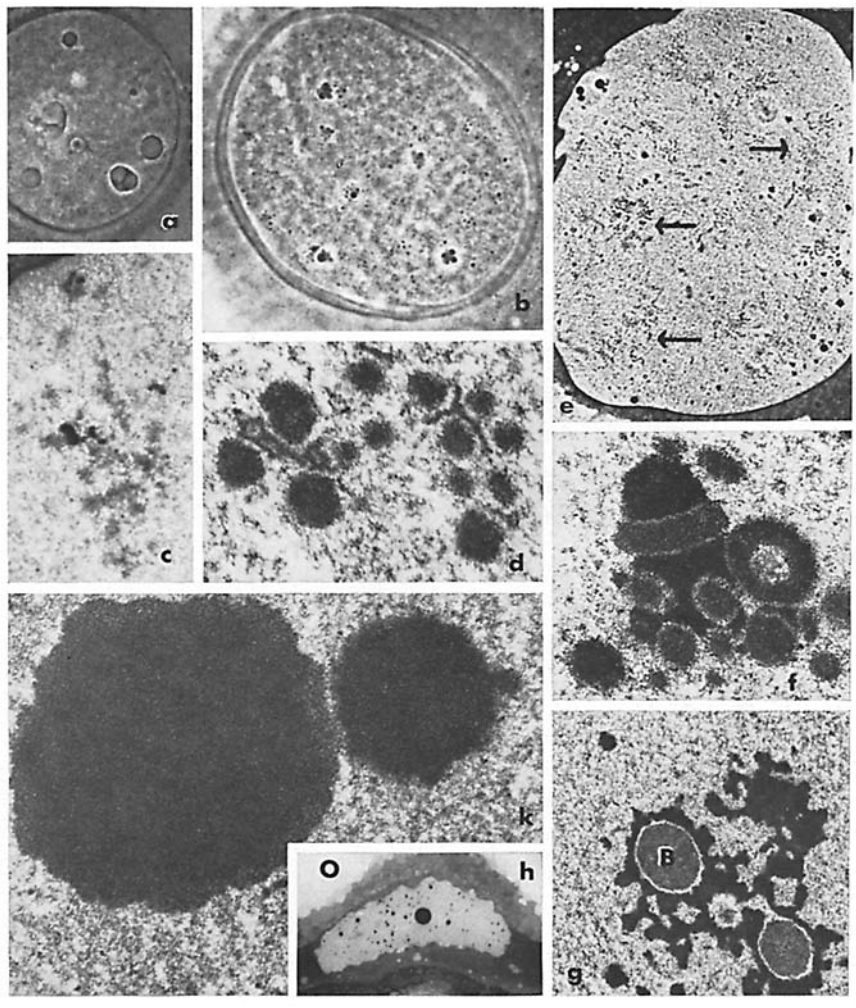

Fig. 6a—k. Structure of the oocyte nucleus. a Stage I nucleus containing nucleolus-like vacuolated inclusions. Magn. ×500. b Inclusions of stage I nuclei give rise to smaller granules by fragmentation. Magn. ×500. c Axial structures, bearing lateral projections, are considered as early lampbrush chromosomes (stage II). Magn. ×750. d Clusters of spheres, connected by narrow stalks, probably differentiations of lampbrush chromosomes. Magn. ×18000. e Section of a large nucleus prior to vitellogenesis with many fibrous structures (↗), which are considered as sections of lampbrush chromosomes. Magn. ×500. f and g Nucleolus-like structures (stage II). Magn. ×15000 and 6000. h Late stage III nucleus with many nucleolus-like particles. Magn. ×300. k Detail from h. Inclusions show typical fibrillar and granular ultrastructure of nucleoli. Magn. ×18000. Oocyte (*O*)

accumulation of this material, the ultrastructure of which reminds strongly of lysosomes (e.g. peroxisomes) occurs along the vitelline membrane of the oocyte (Fig. 3c). Characteristic of the animal plasm are also dense ribbon-like structures, which show a clear periodicity, and which are on higher resolution composed of many ribosome-like particles (Fig. 8c).

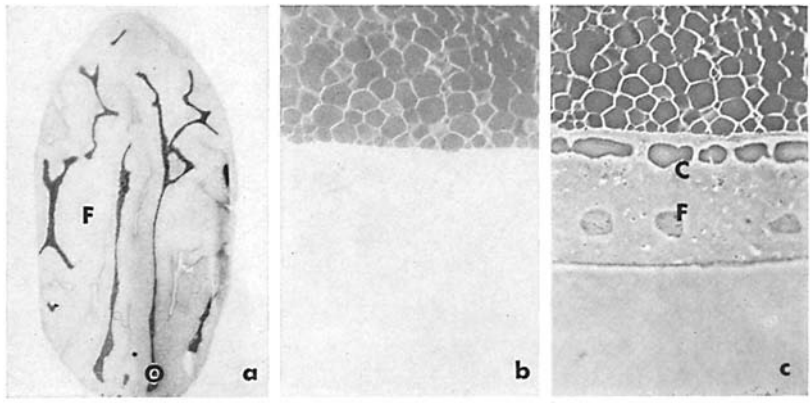

Fig. 7. a Late stage II follicle prior to vitellogenesis, stained by the PAS-reaction. The oocyte reacts strongly positively. Magn. ×60. b Detail from a vitellogenic oocyte and follicular epithelium. The yolk platelets are intensively stained. c Phase-contrast picture of the same region, showing, that the chorion (*C*) is not stained by the PAS-reaction. Magn. ×500

The analysis of serial sections, stained with Azur B, supports the idea, that a well developed lampbrush stage of the chromosomes is also typical for the late stage II oocyte nuclei of *Alloteuthis*. Many loop-like structures can be interpreted as matrix-covered loops of lampbrush chromosomes (Fig. 6e). A major enigma are many Azur-B positive nuclear inclusions, the structure of which is shown in Fig. 6. Dark spheres of all diameters, being often vacuolated (Fig. 6f), are predominant. Clusters of spheres can be seen in connection with small, heavily contrasted and branched stalks (Fig. 6d). Spheres are often surrounded by a more coarse fibrous material of lower density. They resemble strongly the nuclear spheres of gastropod oocytes (Bottke, 1973). Besides them, one can see irregular patches of a more granular material, which often forms branching and anastomosing projections into the nucleus. The granular cortex of these inclusions often surrounds a fibrous central area of decreased stainability with Azur B (Fig. 6g). There can be no doubt that at least a part of these inclusions is part of the nucleolus. Others seem to be specially differentiated loops of lampbrush chromosomes. Less stainable spheres are perhaps identical with the "Proteinkörper" of gastropods (Bottke, 1973) or "Binnenkörper" of insects (Bier *et al.*, 1967).

The largest follicles, which were investigated in our material had a maximum length of 1.3 mm (Fig. 1g). The oocyte is nearly totally filled with polygonal yolk platelets, which are strongly PAS-positive (Fig. 7b, c), and which give only a weak reaction on staining with mercuric bromphenol blue. The euplasm is restricted to a narrow border between the vitelline membrane and the yolk platelets. The late oocyte nuclei contain only well-rounded inclusions with a typical granular structure or fibrillar inclusions with a granular cortex, which look like nucleoli (Fig. 6h and k).

The Sheath of the Ovarian Follicle. Follicles of stage I are only surrounded by a thin sheath of connective tissue (Fig. 1a). When the infolding of the follicular epithelium into the oocyte starts, a new epithelium arises between the connective

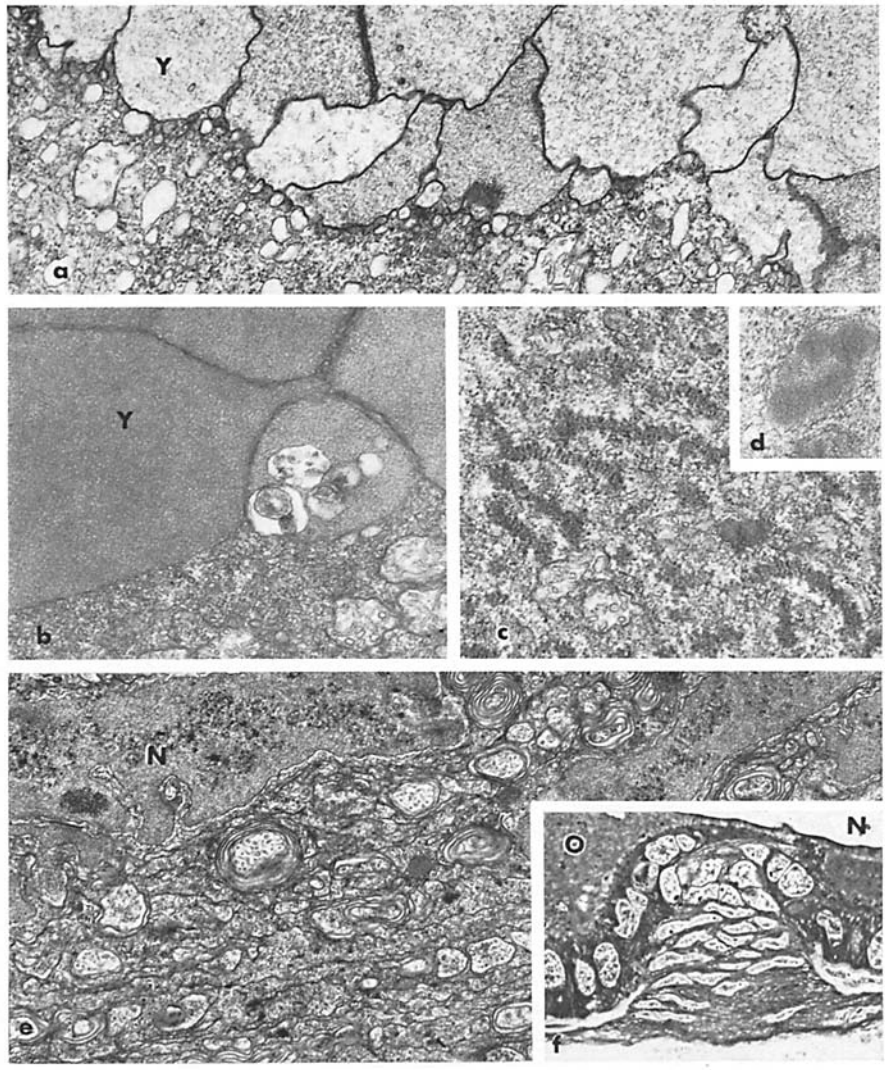

Fig. 8. a Yolk platelets of an early vitellogenic oocyte, surrounded by many vesicles and vacuoles. Magn. ×16000. b Yolk platelets of a late vitellogenic oocyte. Magn. ×16000. c Ribbon-like structures with a clear periodicity, which consist of ribosome-like particles. Magn. ×20000. d A crystalline lysosome-like inclusion of a late oocyte. Magn. ×32000. e Connective tissue cells of the follicle sheath. Magn. ×8000. f The papilla of connective tissue beneath the oocyte nucleus. Magn. ×500. Oocyte (*O*), Nucleus (*N*), Yolk (*Y*)

tissue layer and the follicle cells, which can be distinguished from the follicle cells by its decreased stainability with basophilic dyes. It contains only small amounts of granular ER (Fig. 2c), microtubules, some dictyosomes and lysosome-like inclusions. The nuclei of these elongated cells are preferentially located in the infoldings of the follicular epithelium and in a papilla of connective tissue formed early in stage II follicles (Fig. 8f). Blood capillaries are totally surrounded by these cells. All material, which passes from the blood to the oocyte, has to pass a well-

developed endothelium, the connective tissue cells and the follicle cells. On its outer side the follicle is wrapped into another layer of connective tissue, formed by extraordinary elongated cells, which are connected with each other by desmosomes of the zonula adhaerens type. The branches of these cells contain large mitochondria, which are often enveloped into myelin-like membrane configurations (Fig. 8e) and some lysosome-like inclusions.

Discussion

a) Vitellogenesis. Having investigated yolk protein synthesis in the ovary of *Octopus vulgaris* by biochemical methods, O'Dor and Wells (1973) came to the conclusion that yolk proteins are synthesized by the gonad itself, probably by the follicle cells. These findings appear in a certain contrast to the results obtained in other animal groups, where distant organs, e.g. liver or fat-body have been revealed as the source of yolk precursors (Telfer, 1965; Rudack and Wallace, 1968). They are, however, consistent with observations of earlier investigators who assigned to the follicle cells of other cephalopods the task of producing yolk for the growing oocyte (see also O'Dor and Wells, 1973). Moreover, these results deserve general interest, because comparable observations are lacking for other molluscs. The small oocytes of gastropods and bivalvia are generally assumed to synthesize their yolk autonomously (see also Ubbels, 1968), in many cases by cooperation of the rough ER and the Golgi apparatus. Considering the giant dimensions of the *Alloteuthis* follicles, one is not inclined to think of an autonomous yolk production in this cephalopod.

The architecture of the *Alloteuthis* follicle is in many respects reminiscent of the panoistic insect ovarian follicle. As in the case of insects, the follicle cells of *Alloteuthis* are characterised by their high content of rough ER, by the well developed Golgi system and numerous vesicles and vacuoles (see also Telfer and Smith, 1969). An increase in cell number and a considerable growth process of the cells occurs synchronously with the euplasmic growth phase and vitellogenesis. As it is in insects, also in cephalopods ovarian protein synthesis is under hormonal control (Engelmann, 1970; O'Dor and Wells, 1973). The differences in ultrastructure between insect follicle cells and the cells of *Alloteuthis* are striking, however. Neither does there exist an extended system of intercellular spaces between the follicle cells, by which exogenous material could have access to the oocyte-cortex, nor are there any signs of pinocytotic activity of the oocyte. The lack of typical pinocytotic vesicles, which are always a strong indication for the uptake of heterosynthetic material seems to rule out also the transfer of protein yolk material from the follicle cells into the oocyte. Follicle cells are not subjected to important ultrastructural changes due to the beginning of vitellogenesis. One should expect to encounter secretory granules or a constant influx into the oocyte by pinocytosis. On the other hand, follicle cells of *Alloteuthis* are characterized by many attributes typical for cells, which are engaged in an enhanced transport of materials, e.g. a well developed system of basal plasma membrane infoldings, large longitudinally oriented mitochondria, which are often associated with the basal infoldings and some microtubules. The tendency in cell differentiation is clearly towards enlargement of the contact surface between oocyte and follicle cells, as is indicated by the infoldings of the epithelium into the oocyte and the apical microvilli-like pro-

jections of both cells. It must be assumed from these observations that the follicle cells are engaged in a rapid transport of simple compounds such as sugars or amino acids into the oocyte.

As in other molluscs, e.g. gastropods, vitellogenesis cannot be strictly separated from the euplasmic growth phase. Aggregates of vesicles and vacuoles, which are certainly derivatives of the Golgi apparatus, are already abundant in stage I and II oocytes. Formation of the definitive yolk platelets occurs by intermediate cytosomal precursors, which are at least partially formed by confluence of such vesicles. The plasm of stageII oocytes is PAS-positive, as well as the large polygonal yolk platelets of stage III. In many cases dictyosomes seem to be engaged in the synthesis of carbohydrates (see also Favard, 1969). Formation of the yolk occurs homogeneously. Neither is there any stratification of the oocyte cortex, nor are there different types of yolk platelets of various ultrastructure. We could not detect glycogen by its typical ultrastructure so that the yolk of *Alloteuthis* seems to be composed of carbohydrates other than glycogen. The protein content is rather low, which is also confirmed for other cephalopods (Fujii, 1960). Synthesis of carbohydrate yolk occurs perhaps in a similar way as it is in insects, where rapid glycogen synthesis is catalyzed at the end of oogenesis by enzymes, which have been synthesized early during oogenesis (see also Bier, 1969). This mechanism could perhaps explain the ability of the giant oocyte to build up large amounts of yolk in spite of its low transcription capacity.

To answer this question conclusive information has to be obtained as to the role of the Golgi apparatus. There is also an interesting parallel to vertebrate oogenesis. Whereas the exogenic yolk of the teleost *Brachydanio* consists of a lipoprotein but lacks carbohydrate, the endogenous yolk, which is PAS-positive, seems to be synthesized by the Golgi apparatus of the oocyte (te Heesen and Engels, 1973). The synthesis of chorion material probably takes place by collaboration of the rough ER and the Golgi apparatus of the follicle cells. Golgi vesicles and vacuoles can easily be interpreted as transport vehicles of newly synthesized chorion material, which is finally released into the intercellular gap. It seems reasonable to ascribe the chorion formation to the follicle cells activity alone, since the chorion consists mainly of proteinaceous material and since there is no detectable morphological sign of a contribution of the oocyte.

b) Nuclei. The oocyte nuclei of cephalopods seem to lack typical large molluscan amphinucleoli. It was for the first time described by Brown and Dawid (1968) for the clam *Spisula solidissima*, that amplification of the r-RNA genes takes place also in the oocytes of molluscs. Ribbert and Kunz (1969) reported the occurrence of many nucleolus-like granules in late oocyte nuclei of *Sepia*, which were assumed to be multiple nucleoli. Our own findings are in good agreement with the observations of Bergmann (1903), who described large, vacuolated nucleolus-like structures in young oocyte nuclei of *Sepiola* and *Eledone*, which by fragmentation gave rise to many small granules. In *Alloteuthis* nucleolus-like structures can be observed during all stages of oogenesis. Their number increases considerably during the euplasmic growth phase of the oocyte. As to their ultrastructure, they bear a strong resemblance to typical nucleoli. It shall be elucidated by observation of living oocyte nuclei and autoradiography, whether these structures are nucleoli or specially differentiated loops of lampbrush chromosomes.

References

Anderson, L. M., Telfer, W. H.: Trypan blue inhibition of yolk deposition—a clue to follicle cell function in the cecropia moth. J. Embryol. exp. Morph. **23**, 35–52 (1970)

Arnold, J. M.: Cephalopods. In: Experimental embryology of marine and fresh water invertebrates (ed. G. Reverberi), p. 265–311. Amsterdam: North-Holland Publ. Co. 1971

Beams, H. W., Sekhon, S. S.: Electron microscope studies on the oocyte of the fresh-water mussel (*Anodonta*), with special reference to the stalk and mechanism of yolk deposition. J. Morph. **119**, 477–501 (1966)

Bergmann, W.: Untersuchungen über die Eibildung bei Anneliden und Cephalopoden. Z. wiss. Zool. **73**, 278–301 (1902)

Bier, K. H., Kunz, W., Ribbert, D.: Struktur und Funktion der Oocytenchromosomen und Nukleolen sowie der Extra-DNS während der Oogenese panoistischer und meroistischer Insekten. Chromosoma (Berl.) **23**, 214–254 (1967)

Bier, K. H.: Oogenesetypen bei Insekten und Vertebraten, ihre Bedeutung für die Embryogenese und Phylogenese. Zool. Anz., Suppl.-Bd. 33, Verh. Zool. Ges. 1969, 7–29

Bottke, W.: Zur Ultrastruktur des Ovars von *Viviparus contectus* (Millet, 1813), (Gastropoda, Prosobranchia). II. Die Oocyten. Z. Zellforsch. **138**, 239–259 (1973)

Bottke, W.: Lampenbürstenchromosomen und Amphinukleolen in Oocytenkernen der Schnekke *Bithynia tentaculata* L. Chromosoma (Berl.) **42**, 175–190 (1973)

Brown, D. D., Dawid, I. B.: Specific gene amplification in oocytes. Science **160**, 272–280 (1968)

Cowden, R. R.: Cytological and cytochemical studies of oocyte development and development of follicular epithelium in the squid, *Loligo brevis*. Acta Embryol. Morph. exp. **10**, 160–173 (1968)

Engelmann, F.: The physiology of insect reproduction. Oxford: Pergamon 1970

Favard, P.: The Golgi apparatus. In: Handbook of molecular cytology (ed. A. Lima-De-Faria), p. 1130–1155. Amsterdam: North-Holland Publ. Co. 1969

Favard, P., Carasso, N.: Origin et ultrastructure des plaquettes vitellines de la planorbe. Arch. Anat. micr. Morph. exp. **47**, 211–234 (1958)

Fujii, T.: Comparative biochemical studies on the egg-yolk proteins of various animal species. Acta Embryol. Morph. exp. **3**, 260–285 (1960)

Heesen, D. te, Engels, W.: Elektrophoretische Untersuchungen zur Vitellogenese von *Brachydanio rerio* (Cyprinidae, Teleostei). Wilhelm Roux' Archiv **173**, 46–59 (1973)

Jaeckel, S. G. A.: Cephalopoden. In: Die Tierwelt der Nord- und Ostsee (Hrsg. A. Remane). Leipzig: Akademische Verlagsgesellschaft Geest u. Portig 1958

Jared, W., Dumont, J. N., Wallace, R. A.: Distribution of incorporated and synthesized protein among cell fractions of *Xenopus* oocytes. Develop. Biol. **35**, 19–28 (1973)

Konopacki, M.: Mikrometabolizm podczas owagenezy u. *Loligo vulgaris*. Kosmos (Warsz.) **58**, 133–156 (1933)

Lankester, R. E.: On the developmental history of the mollusca. Phil. Trans. **165**, 1–48 (1875)

O'Dor, R. K., Wells, M. J.: Yolk protein synthesis in the ovary of *Octopus vulgaris* and its control by the optic gland gonadotropin. J. exp. Biol. **59**, 665–674 (1973)

Recourt, A.: Elektronenmicroscopisch onderzoek naar de oogenese bij *Lymnaea stagnalis* L. Thesis : Utrecht 1961

Reynolds, E. S.: The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol **17**, 208–213 (1963)

Ribbert, D., Kunz, W.: Lampenbürstenchromosomen in den Oocytenkernen von *Sepia officinalis*. Chromosoma (Berl.) **28**, 93–106 (1969)

Rudack, D., Wallace, R. A.: On the site of phosvitin synthesis in *Xenopus laevis*. Biochim. biophys. Acta (Amst.) **155**, 299–301 (1968)

Ruthmann, A.: Methoden der Zellforschung. Kosmos. Stuttgart, 1966

Taylor, G. T., Anderson, E.: Cytochemical and fine structural analysis of oogenesis in the gastropod, *Ilyanassa obsoleta*. J. Morph. **129**, 211–247 (1969)

Telfer, W. H.: The mechanism and control of yolk formation. Ann. Rev. Entomol. **10**, 161–184 (1965)

Telfer, W. H., Smith, D. S.: Aspects of egg formation. In: Insect ultrastructure (ed. A. C. Neville). Oxford and Edinburgh: Blackwell Scientific Publ. 1970

Ubbels, G. A.: A cytochemical study of oogenesis in the pond snail *Lymnaea stagnalis*. Thesis, Rotterdam 1968

Vialleton, L.: Recherches sur les premières phases du développement de la seiche (*Sepia officinalis*). Ann. Sci. Nat. Zool. **6**, 165–280 (1888)

Wolin, E. M., Laufer, H., Albertini, D. F.: Uptake of the yolk protein, lipovitellin, by developing crustacean oocytes. Develop. Biol. **35**, 160–170 (1973)

Yung Ko Ching, M.: Contribution à l'étude cytologique de l'ovogénèse, du développement et de quelques organes chez les céphalopodes. Thèses, Paris 1930

Dr. Werner Bottke
Zoologisches Institut
der Universität
44 Münster
Badestraße 9
Federal Republic of Germany