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Oögenesis in a Braconid, *Apanteles glomeratus* (L.) Possessing an Hydropic Type of Egg

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Summary. 1. Oögenesis in a braconid parasitoid *Apanteles glomeratus* (L.) is described and compared with that in other hymenoptera.

2. The processes are similar, except that there is no uptake of protein for yolk formation with the associated configurations in the follicle cells and the periphery of the oöcyte.

Key-Words: Oögenesis — Hymenopteran — Hydropic egg.

Introduction

Apanteles glomeratus (L.) is a parasitoid (Doutt, 1959) which oviposits in the first instar larva of the lepidopteran, *Pieris brassica* (L.) and, developing within the host eventually emerges from its last larval instar and thus kills it.

Insect oögenesis has been reviewed by Bonhag (1958), Raven (1961), Telfer (1965), Nørrevang (1968) and Engelmann (1970). During their development, insect eggs pass through a germarial phase followed by growth, vitellogenic and chorion-forming phases. In most species the yolk, which is deposited during vitellogenesis within the ovariole, consists of protein, lipoid and sometimes glycogen moieties, though the composition varies quantitatively and qualitatively between species and sometimes with the time of year within a single species. Flanders (1942), described two types of insect eggs, the "anhydropic" and "hydropic" types. The former has sufficient yolk for the subsequent formation and development of the embryo to eclosion. This is the type present in most hymenopterans, examples being the non-parasitoid *Bombus terrestris* (L.) which has abundant yolk (Hopkins and King, 1966) and the parasitoid *Nasonia vitripennis* (Walker) which has a full complement of yolk qualitatively but less than in *Bombus terrestris* quantitatively (Hopkins and King, 1966; King and Richards, 1969). The structure and formation of chorions of anhydropic eggs have been described by Richards (1969) and King, Richards and Copland (1968). The hydropic chorions surround eggs, which have little yolk and obtain their nourishment from the body fluids of the host. *A. glomeratus* has an "hydropic" type of chorion (King, Ratcliffe and Copland, 1969) and a reduced amount of yolk both quantitatively and qualitatively, protein yolk being absent from the unlaid egg (King and Ratcliffe, 1968) and only the lipoid moiety present.

Since the formation and vitellogenesis in particular, of this type of egg, has not been previously examined, it was considered to be of interest. The process involved, which in other eggs is rather obscured by vitellogenesis and especially by the production of protein can be examined in these eggs.

Materials and Methods

A. glomeratus was cultured in the laboratory on the larvae of *Pieris brassicae*.

Histological observations were made on material fixed in Carnoy's 6:3:1 or formol-calcium-cadmium and embedded in Ester wax, sectioned and stained with Mallory's triple stain.

For electron microscopy, whole ovaries were dissected in saline and fixed immediately in Palade's (1952) 1% osmium tetroxide at 0–4° C for 1 hour then rapidly dehydrated in graded cold ethanols and embedded in Epon. Other ovaries were fixed in glutaraldehyde and postosmicated. Sections were cut on a Huxley ultra-microtome, stained with uranyl acetate (60 mins) followed by lead citrate (1 min) and viewed in an Akashi tronscope 50.

Sections of fixed abdomens, embedded in paraplast were subjected to one of the following histochemical procedures:

Carbohydrates. The periodic-acid Schiff technique (Hotchkiss, 1948) was used after fixation in Carnoy's 6:3:1 solution. Sections were oxidised for 9 mins in either 5% periodic acid or 0.1 N HNl. Control sections were left unoxidised or extracted with 1% diastase for 30 mins. The alcian blue (Steedman, 1950) technique was used to test for mucopolysaccharides. Lead tetra-acetate/Schiff technique and Best's carmine methods were used as described by Pearse (1960). Unless enzyme extraction techniques were employed, sections were protected with celloidin. The controls were treated with 1% diastase for 30 mins.

Proteins. Carnoy-fixed material was employed in all the protein tests. The mercury-bromophenol-blue method was used without modification (Pearse, 1960), the ninhydrin-Schiff test for NH_2 (Yasuma and Itchikawa, 1953) and the chloramine-T/Schiff method (Pearse, 1960) were employed. The Sakaguchi reaction for arginine 1 was used (Baker, 1947) and the Morel-Sisley protein diazotization procedure for protein bound tyrosine (Pearse, 1960).

Lipids. Whole ovaries, fixed in cold formalin were stained with sudan Black B.

Nucleic acids. The pyronin/methyl green technique (Brachet, 1953) was used as a general test for nucleic acids. R.N.A. was selectively extracted with 1% R.N.A-ase. The Feulgen "nucleal" technique was used according to Pearse (1960). To identify D.N.A., control sections were left unhydrolysed.

Observations and Discussion

Each ovary of *A. glomeratus* consists of two polytrophic ovarioles. In each ovariole there is a swollen germarium which is relatively wide compared with that of other insects. This leads into the rest of the ovariole, where growth and development of the oöcytes occurs and this then opens into a large reservoir.

The oögonia in the germarium divide continuously. After a series of divisions, which are of an incomplete type, similar to that described in *Drosophila melanogaster* (King, 1963), *Apis mellifera* (Hegner, 1915), *Bombus terrestris* (Hopkins, 1964) and *N. vitripennis* (King and Richards, 1969) a cluster of cells is formed consisting of the oöcyte and a number of trophocytes. These cells are connected to each other by a simple opening in their membranes and not by a complex "ring canal" structure of the type described in the germarium of *B. terrestris* by Hopkins (1964).

In addition to the oögonia, the germarium contains a mass of prefollicular tissue which is not of germ cell origin (Bonhag, 1958). The dividing oögonia are surrounded by these prefollicular cells (Fig. 1). In the germarium region chromosomes are visible in many sections suggesting that there is a very rapid rate of cell division. Passing down the ovariole, cell division ceases, and the growth phase begins. The rate of growth of the oöcyte follicle initially equals that of the trophocyte follicle.

Oöcyte. The nucleus is at first relatively large but, as growth continues, the oöplasm increases more rapidly in volume than the nucleus. The nucleoplasm is finely particulate with occasional larger adielectronic particles, and several aggregations of particles which presumably constitute the nucleolus (Fig. 4). Initially the oöcyte membrane becomes folded then gradually short microvilli form on it and a "space" appears between it and the surrounding follicle cells (Figs. 2, 3). Early in development the peripheral oöplasm contains numbers of small mitochondria, each of which has one or two longitudinal cristae and, as development proceeds, the number of mitochondria increases (Figs. 1, 4, 5). These are produced, in part at least, by budding from existing mitochondria. In addition to these organelles the peripheral oöplasm contains occasional golgi complexes and short strands of endoplasmic reticulum. Some of these strands are closely associated with the mitochondria at the time when these are increasing in numbers but the significance of this association was not determined (Fig. 5). The oöplasm also contains many free ribosomes (Figs. 2, 4). When the oöcyte follicle has grown until it equals the size of the trophocyte follicle and both are about to enter the reservoir, the nuclear envelope of the oöcyte disappears and the less adielectronic nucleoplasm, still with patches of adielectronic material scattered in it, becomes confluent with the oöplasm (Figs. 6, 7). Around the zone of nucleoplasm appear stacks of endoplasmic reticulum, annulate lamellae and golgi complexes (Fig. 8). It has been shown that the accessory nuclei arise in this zone by King and Fordy (1970). Structures in this zone, with double membranes around them become filled with lipoid (Figs. 7, 8). Initially the concentration of these lipoid inclusions is greater in the region around the nucleoplasm than elsewhere in the oöplasm and this provides the main source of lipoid in the yolk (Fig. 9). Tawfik (1957) recorded alkaline phosphatase activity in this region of the eggs of *A. glomeratus* and this may be related to the membrane activity. Eventually a pedicel forms at the end of the egg and this consists mainly of vitelline membrane and chorion. It is probably concerned chiefly with attachment of the egg to suitable internal organs of the host after laying. In this condition the egg awaits oviposition within the storage reservoir of the ovary.

Trophocytes. Soon after formation, each trophocyte contains a number of small, sometimes elongate and budding, peripherally situated mitochondria (Figs. 10, 11). Each of these has only one or two longitudinally orientated cristae and they are similar to those described in the oöcyte (Fig. 11). The trophocytes are connected with each other and with the oöcyte by simple openings in their adjoining membranes (Fig. 12).

At the next stage growth occurs which involves the utilization of precursors of small molecular size by the numerous free ribosomes since no pinocytosis was observed (Fig. 10). There is no endoplasmic reticulum present in the cytoplasm at this stage. Subsequently the nuclear envelope becomes folded and, closely associated with its pores are numerous RNA-positive emissions. These emissions form a cloud round the nucleus and from the outer membrane of the nuclear envelope, strands of perforated reticulum which may be annulate lamellae, are produced (Fig. 11). Some of these strands arise by a process of delamination and they extend and fuse with each other to enclose a part of the emission cloud. Eventually these structures move away from the nucleus and frequently they

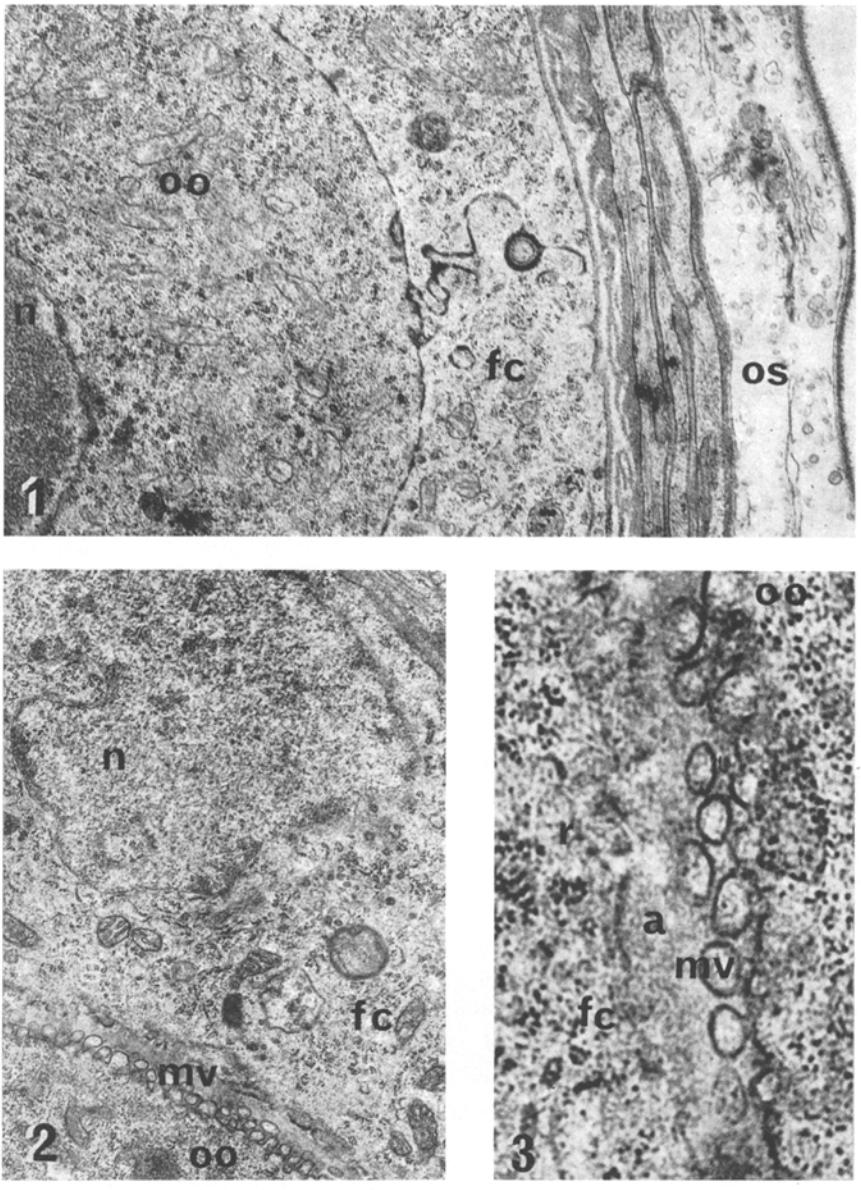
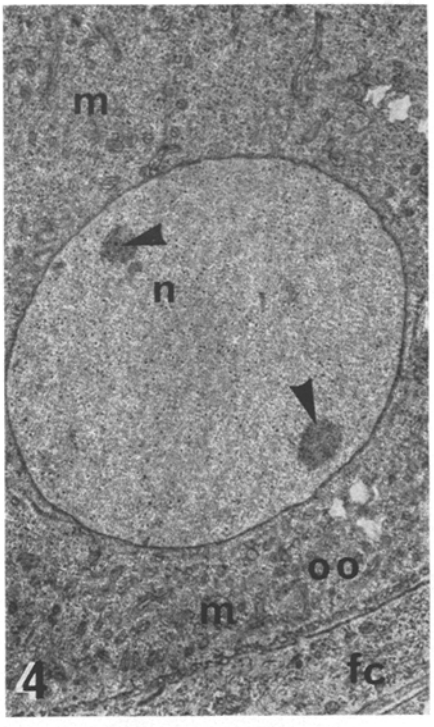
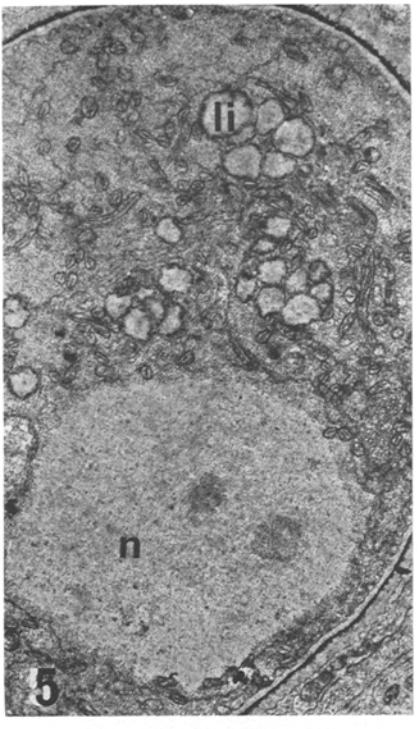


Fig. 1. Periphery of oöcyte follicle cells (*fc*) and ovariole sheath (*os*) in the region of the germarium showing oöcyte nucleus (*n*) oöplasm (*oo*) with numerous small mitochondria and free ribosomes. × 13200. Glutaraldehyde/osmic fixation

Fig. 2. Follicle cells (*fc*) and nucleus (*n*) and the periphery of an oöcyte (*oo*) showing the microvilli on the membrane of the oöcyte during its growth phase. × 12250. Glutaraldehyde/osmic fixation

Fig. 3. The periphery of a follicle cell (*fc*) and an oöcyte (*oo*) showing the microvilli on the oöcyte (*mv*), the adielectronic material in the lumen between the follicle cell and the oöcyte(*o*) and the free ribosomes (*r*). × 41000. Glutaraldehyde/osmic fixation





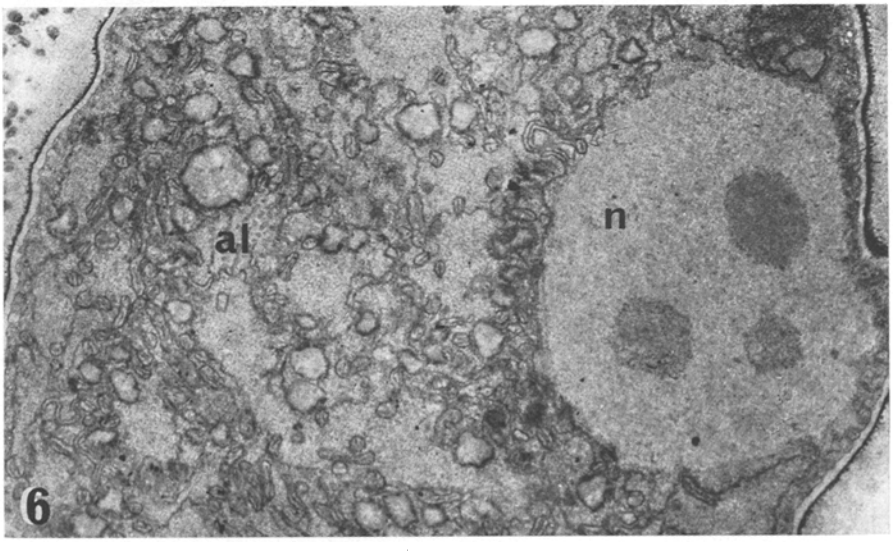


Fig. 4. Follicle cell (*fc*) and oöcyte (*oo*) during the growth phase of the latter showing the numerous small mitochondria in the oöplasm (*m*) and the nucleus (*n*) containing several adielectronic inclusions (arrows). × 7000. Glutaraldehyde/osmic fixation

Fig. 5. Later stage in the development of the oöcyte showing an increase in the number of mitochondria, lipoid-filled inclusions (*li*) and a nucleus (*n*) which has just lost its envelope. × 8500. Glutaraldehyde/osmic fixation

Fig. 6. Later stage in the development of the oöcyte showing the nucleus (*n*) with several adielectronic inclusions but no envelope, but a cloud of mitochondria and membrane structures around it. The oöplasm contains more mitochondria and lipoid-filled inclusions together with a number of annulate lamellae (*al*). × 10500. Glutaraldehyde/osmic fixation

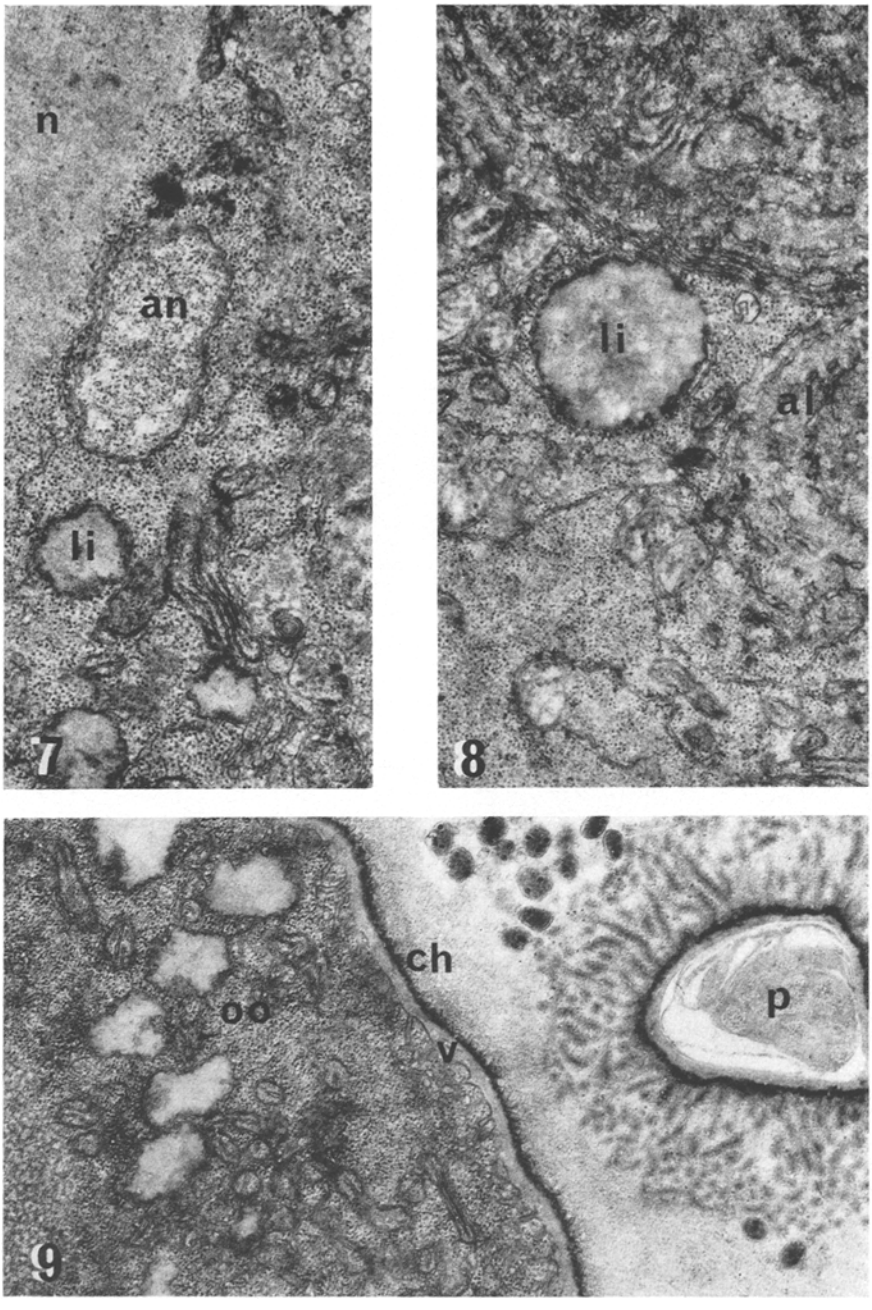


Fig. 7. Periphery of the oöcyte nucleus (*n*) showing the breakdown of the envelope and the formation of accessory nuclei (*an*) which later contain lipoid (*li*). × 25000. Glutaraldehyde/osmic fixation

Fig. 8. Region of the oöplasm near to the nucleus containing many membrane configurations including annulate lamellae (*al*). Some lipoid-filled inclusions (*li*) are also present in this zone. × 19250. Glutaraldehyde/osmic fixation

retain the remains of their nuclear connection in the form of a tail. Within these structures there is a gradual deposition of lipoid until the whole area within the membrane is filled with it and in some examples the inner membrane disappears. This is probably similar to the process described in the oöcyte. The structures are not as numerous in the trophocytes as the oöcytes, however (Fig. 10).

The mitochondria continue to increase in number by budding but there is no detectable increase in the basophilia of the trophocyte cytoplasm. During this time, the nucleus is sub-spherical in shape but there are few RNA-inclusions within it or emissions from it. This stage coincides with the release of the follicles into the reservoir of the ovary. In the reservoir the nuclear-cytoplasmic ratio increases until the nucleus occupies almost the whole cell and is no longer spherical.

During the entry of the oöcyte and trophocyte follicles into the reservoir the trophocytes and follicle cells break down. In the final stages of this process the basement membrane is thickened and folded into the cells (Fig. 13).

In most insects there are three types of yolk body in a developing oöcytes; (1) the large yolk spheres, which form the major part, by volume, of the deutoplasm and have been shown to consist of a protein/carbohydrate complex (Bonhag, 1955, 1959; Aggarwal, 1962; Krainska, 1961; Roth and Porter, 1964; Hopkins and King, 1966; King and Richards, 1969); (2) the lipid yolk which Nath (1960) showed could be separated on histochemical grounds into three forms of body and (3) glycogen yolk, which has not been demonstrated in all species (Bonhag, 1959; Aggarwal, 1962; Lusis, 1963) is present during yolk synthesis in most polytrophic ovarioles. In *A. glomeratus* the only yolk present is lipoid.

Attempts have been made to determine the origin of yolk. The trophocytes have been shown to pass formed yolk materials to the oöcyte in *Bombus* (Palm, 1948; Hopkins and King, 1966), *Drosophila melanogaster* (Hsu, 1952, 1953; King and Mills, 1962), *Apis mellifera* (L.), *Vespa crabro* (L.) *V. germanica* (F.) and *Formica rufa pratensis* (L.) (Bier, 1954), *Oncopeltus fasciatus* (Dallas), *Anisolabis maritima* (Gene) (Bonhag, 1958), *Acanthoscelides obtectus* (Say) (Mulnard, 1954), *Cynips quercus foli* (L.) (Krainska, 1961), *Bombyx mori* (L.) (Aggarwal, 1962), *Calliphora erythrocephala* (Meigen) (Bier, 1962) and *Nasonia vitripennis* (Walker) (King and Richards, 1969). No histochemically detectable material is transferred, however, in *Culex fatigans* (Nath, 1925; Nath, Gupta and Bains, 1959). In *A. glomeratus* the presence of ribosomes and occasional mitochondria in the cytoplasm, within the nutritive pore, suggests that these may be passed from the trophocytes to the oöcyte, although no lipoid was detected within the nutritive pore, as described in *N. vitripennis* and the amounts of the materials within the oöcyte were far less than in those of that insect or of *B. terrestris*. Although yolk-rich oöcytes can be produced by insects with panoistic ovarioles in which no trophocytes are present, trophocytes probably aid rapid egg production by supplying the

Fig. 9. Periphery of an egg ready for laying showing the oöplasm with many small mitochondria, lipoid inclusions and densely packed ribosomes. The periphery is surrounded by a vitelline membrane (*v*) and a rudimentary chorion (*ch*). The pedicel (*p*) of another egg is shown the peripheral extensions which presumably help its attachment to the organs of the host. × 20000. Glutaraldehyde/osmic fixation

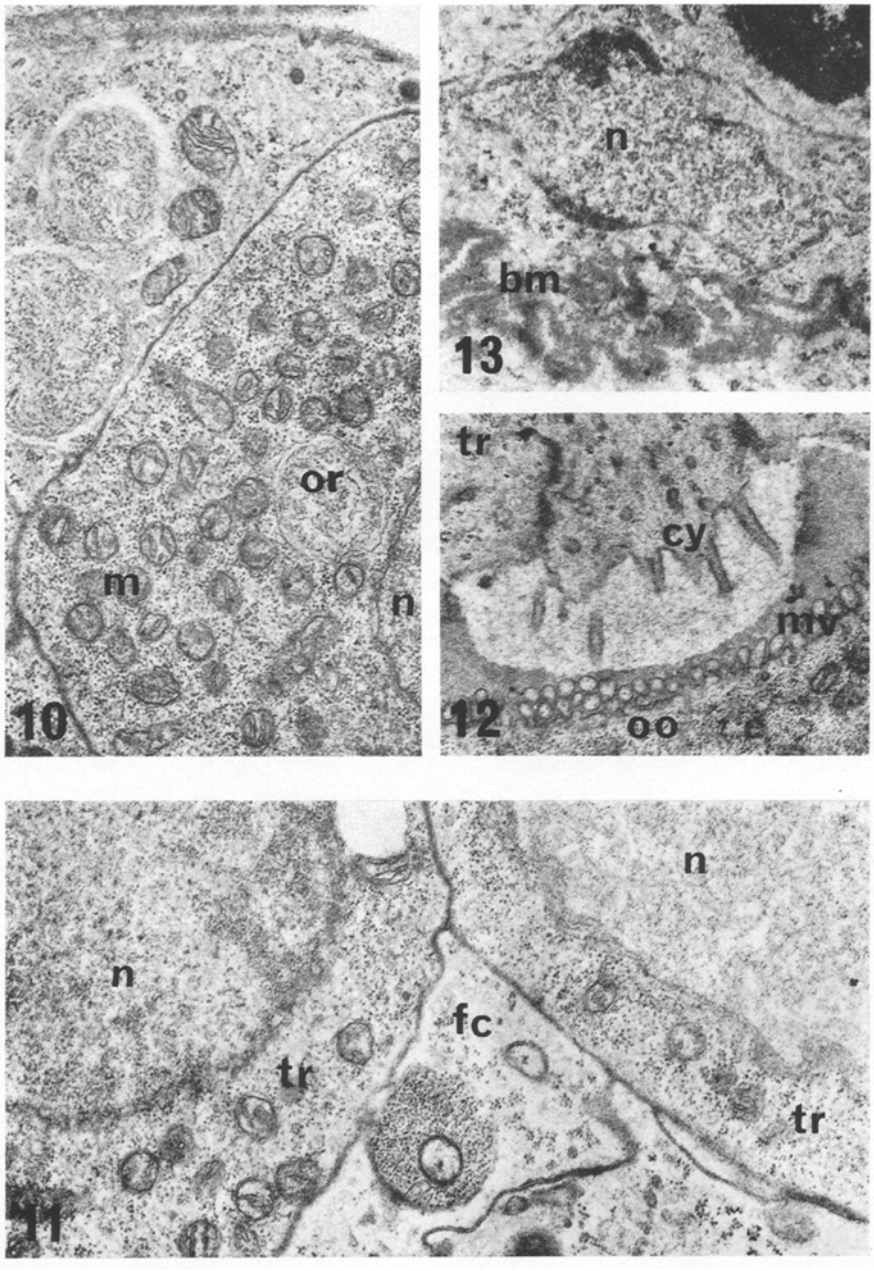


Fig. 10. Trophocyte at the beginning of the growth phase showing the nucleus (*n*), numerous mitochondria (*m*) and organelles bounded by two membranes (*or*). × 16000. Glutaraldehyde osmic fixation

Fig. 11. Follicle cell (*fc*) of the trophocyte follicle and two trophocytes (*tr*) showing the relatively large nuclei (*n*) present during the growth phase. × 19200. Glutaraldehyde/osmic fixation

mechanism for growth rather than the raw materials. It is possible that since the type of oögenesis in *A. glomeratus* has evolved from a more normal type, the trophocytes are no longer essential or they may contribute at the growth phase of egg development, although there is no evidence that they play any appreciable role at vitellogenesis.

The trophocytes arise during the germarial phase of oöcyte development by division of the primary oögonium. Dederer (1915) first described the formation of cytoplasmic bridges connecting the trophocytes with the oöcyte. These bridges are the result of incomplete cell division. In *B. terrestris*, which produces an egg containing abundant yolk, the connections have complex ring canals (Hopkins, 1964). Similar structures have been described in *Drosophila melanogaster* (Koch and King, 1969) and in *Habrobracon juglandis* (Cassidy and King, 1969). In *A. glomeratus* which has less yolk than even *N. vitripennis* the connections are also simple and the cytoplasm within the pore is less basophilic than the rest of the cytoplasm so that perhaps even ribosomes are not passed. There is no evidence that the pore is ever occluded by a plug as described in *D. melanogaster* (Meyer, (1961). When the follicle, during its movement away from the germarium reaches the reservoir, a process of resorption occurs within the trophocytes of *A. glomeratus*. Oösorption has been recorded in many insect species including the hymenopterans *B. terrestris* (Palm, 1948), *Formica rufa*, *Camponotus ligniperda* (Weyer, 1928), *Diadromus varicolor* (Labeyrie, 1959), *Brachymeria euploea*, *Euchalcidia caryobori* (Schneider, 1941) and *N. vitripennis* (Edwards, 1954). The histochemistry (Hopkins and King, 1964) and the ultrastructure of oösorption (King and Richards, 1968a, b) were studied in *N. vitripennis* and trophocyte breakdown outlined in the same species (King and Richards, 1969). The process of trophocyte degeneration in *A. glomeratus* resembles oösorption in *N. vitripennis*. After degenerative changes in the organelles the basement membrane folds into the cytoplasm and presumably helps in the passage outwards of the product of degeneration. The ultimate destination of these products was not ascertained and presents a problem since the trophocytes are actually inside the reservoir of the ovary at this time.

Ries (1932), Palm (1948), Bonhag (1955) and Anderson and Telfer (1969, 1970) have suggested that in polytrophic ovarioles the follicular epithelium synthesises and contributes formed yolk directly to the oöplasm. There is no indication that this occurs in *A. glomeratus* and the only function so far attributed to the follicular epithelium in this insect is the formation of at least part of the reduced vitelline membrane and chorion.

Wigglesworth (1943) suggested that protein yolk is taken up by the oöcyte from the haemolymph in *Rhodnius prolixus* Stål and *Pediculus humanus* L. This

Fig. 12. Oöcyte (*oo*) and trophocyte (*tr*) showing a section through the cytoplasmic bridge (*cy*) which unites their cytoplasm during development. × 15000. Glutaraldehyde/osmic fixation

Fig. 13. Resorbing trophocyte follicle after release of the oöcyte showing the nucleus (*n*) and the infolded and thickened basement membrane (*bm*) × 15750. Glutaraldehyde/osmic fixation

has been supported by investigations using electrophoresis (Laufer, 1960; Telfer, 1954), autoradiography (Bier, 1962, 1964; Anderson and Telfer, 1969, 1970) and immunology (Telfer, 1954, 1960). Electron microscopy shows that proteins pass through the ovariole sheath into spaces between the follicle cells occurring at this stage of development in *Hyalophora* (Telfer, 1961), *Calliphora* and *Musca* (Bier, 1964), *Panorpa* (Bier, 1964), *Aedes* (Roth and Porter, 1964), *Bombus terrestris* (Hopkins and King, 1966) and *N. vitripennis* (King and Richards, 1969). The protein is then taken up at the periphery of the oöcyte by pinocytosis. This occurs in all insects examined (Roth and Porter, 1962, 1964; Anderson, 1964; Kessel and Beams, 1963; Bier and Ramamurty, 1964; Stay, 1965; Hopkins and King, 1966; King and Richards, 1969) except *A. glomeratus*. This insect, having no protein moiety in its yolk, lacks all these processes and the follicle cells remain closely adpressed to each other throughout development until they degenerate at the time the follicle reaches the reservoir (Fig. 14). In many other insect species examined, the follicle cells remain around the egg even after the formation of the vitelline membrane and chorion, and are lost during oviposition. No oösorption was observed in *A. glomeratus* and this may be correlated with the absence of follicle cells around the egg, awaiting laying, since these cells were shown to play in important part in the oösorptive process (King and Richards, 1968a).

Since McCullock (1952) first observed annulate lamellae in *Arbacia* eggs, this little understood cytomembrane system has been given a variety of names. They have been recorded most abundantly in developing stages of both male and female germ cells, in embryonic cells and in neoplastic cells, though they do occur in some normal somatic cells. In oöcytes they have been recorded in mammals, reptiles, amphibians, fish, a variety of invertebrates and arthropods (Kessel, 1968). The present study supports the view that annulate lamellae are formed from the nuclear envelope and contribute to the formation of the accessory nuclei in this species (King and Fordy, 1970). They may also contribute to the formation of the agranular endoplasmic reticulum (Kessel, 1968; King and Richards, 1968b). In the oöcytes of *Acmaea* (Kessel, 1968) and *N. vitripennis* (King and Richards, 1968b) there is some relationship between the annulate lamellae and the lipoid bodies. Light microscopists attributed the origin of lipoid yolk to the golgi complex (Nath and Mohan, 1929; Gresson, 1931; Nath and Mehta, 1929), the golgi complex transferred from the trophocytes freely in the cytoplasm or in the "yolk nucleus" (Raven, 1961). In several cell types an intimate association between mitochondria and lipoid has been described Bak and Elliot (1962), and Ratcliffe and King (1969). Nath (1960) described several histochemically distinct lipoid moieties in the yolk of many insect eggs. In *A. glomeratus* some lipoid appears early in development near the oöcyte periphery and later spherical, double membraned, annulated organelles, originating in the membrane complex round the germinal vesicle slowly convert their contents to lipoid. Whether this represents the end products of an energy producing system, as proposed in mitochondrial change (Ratcliffe and King, 1969) is uncertain. Structures with a double membrane and annuli have been called "accessory nuclei" in some insect oöcytes (Bonhag, 1958). They have been claimed as yolk precursors (Loyez, 1908; Gresson, 1930; Palm, 1948), contributors without becoming themselves converted (Hopkins, 1967b), producers of the vitelline membrane (Cruikshank, 1964;

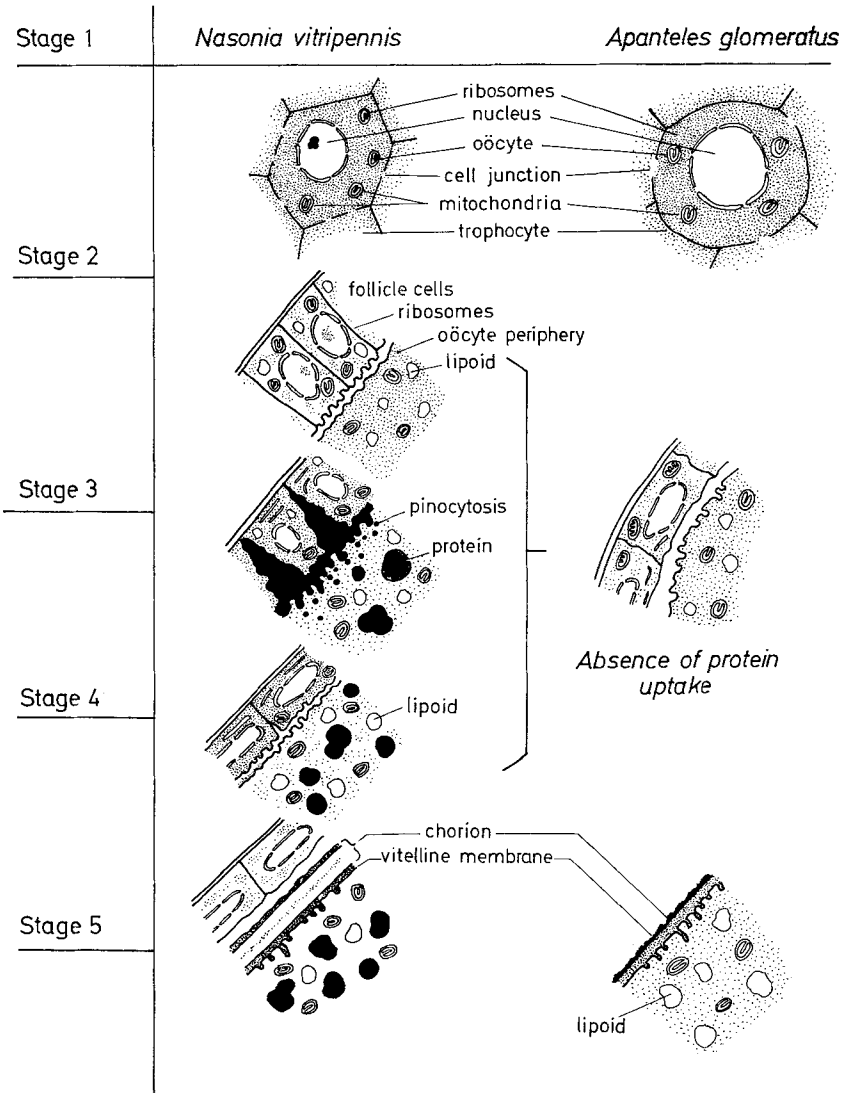


Fig. 14. Diagram comparing the processes occurring in the ovariole during growth and vitellogenesis in *Apanteles glomeratus* and a chalcid *Nasonia vitripennis* which, although it has a reduced amount of yolk resembles the stages in other insects with yolk-rich eggs

Hopkins, 1964b). In *N. vitripennis* they are closely associated with annulate lamellae (King and Richards, 1968b). Considering their origin and membrane structure the organelles in oöcytes of *A. glomeratus* are probably equivalent to "accessory nuclei" of some other insect oöcytes. The region round the oöcyte nucleus at the time when the annulate lamellae and golgi complexes appear resembles the "yolk nuclei" or Balbiani bodies described in a wide variety of

animal oöcytes (Nørrevang, 1968). These zones contain varying combinations of mitochondria, whorls of endoplasmic reticulum, golgi complex, ribosomes and annulate lamellae depending upon the species.

From this discussion it can be appreciated that oögenesis in *A. glomeratus* differs markedly in a number of points from the process described in other insects. This may be attributed to modified mode of development of the insect in which the laid egg, which has only thin surrounding membranes, is bathed in nutrient rich host blood. Eggs of *A. glomeratus* dissected from first instars larvae of *Pieris brassicae* 24 and 48 hours after deposition showed that the laid eggs of this species, in common with those of *A. thompsoni*, increase in size markedly during incubation, presumably as a result of the entry of materials from the host haemolymph. This suggests that during its growth the egg produces the mechanisms for nutriment utilization rather than the materials. The role of the lipoid has not been determined. It may be equivalent to some of the lipoid present in other insect eggs, in which case the eggs of *A. glomeratus* present strong evidence for the origin of this material. Dumont and Anderson (1967) pointed out that the contribution to yolk provided through pinocytosis in oöcytes falls into a sequence ranging from almost total, in such forms as the cockroach (Anderson, 1964), *Cecropia* (Stay, 1965) and cricket (Favard-Séréno, 1964), through the guppy (Droller and Roth, 1966) and amphibia (Wartenberg, 1964), with a moderate contribution to an apparently small contribution in the crayfish (Beams and Kessel, 1963), fresh water mussel (Beams and Sekhon, 1966) and tunicates (Kessel, 1966). In the forms in which micropinocytosis makes a major contribution, there is a complete or almost complete absence of golgi complexes and endoplasmic reticulum but in those oöcytes in which a minimal contribution to yolk formation is made by micropinocytosis there is a well developed endoplasmic reticulum and numerous golgi complexes. *A. glomeratus* lacks pinocytosis but has lost most of the golgi complexes and endoplasmic reticulum and shows the situation in eggs which have pinocytosis, though in them usually obscured by the yolk.

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