Studies on the Origin and Composition of Yolk in Oxya velox (Orthoptera)

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A cursory survey of the pertinent literature brings to focus the widely divergent views on the origin, development and composition of yolk in the eggs of insects, even within members of the same taxonomic group. However, all the investigators, who have studied the yolk formation per se, conform to the fact that the Golgi bodies are directly transformed into fatty yolk, or, at any rate, they are intimately implicated in its formation. And yet authors like Payne (1932) and Gresson (1930) deny any participation whatsoever by the Golgi bodies in the formation of fatty yolk. Likewise, opinion on the formation of compound yolk is divided; namely: 1) it is derived from extraovarian source (Anderson 1964, Roth and Porter 1964, Nath et al. 1958 b), 2) its precursors migrate from the follicular epithelium to the oocyte and contribute to its origin (Nath et al. 1958 a and 1959 b), 3) it originates as carbohydrate granules from the circumnuclear zone of oocytes (Bonhag 1956, Gupta 1968).

In an attempt to assess the relative merits of these views the present work was undertaken and thus deals with a cytochemical analysis of yolk formation during oogenesis of the grasshopper Oxya velox.

Material and methods

Female specimens of Oxya velox were collected locally and reared in the laboratory. The ovaries were dissected out and specifically processed for Golgi bodies, lipids, proteins and carbohydrates. Such standard techniques as Kolatchev, Aoyama, Hermann’s postosmification, 2% osmic acid etc. were employed for the study of Golgi bodies. Cytochemical techniques, as detailed in Pearse (1968), were adopted for the detection of chemical constituents of the oocytes.

Observations

Golgi bodies and lipids: The young oocytes of Oxya velox display their granular Golgi bodies in the juxtanuclear zone (Fig. 1). On further growth of oocytes they become perinuclear (Fig. 2) and later on dispersed in the ooplasm (Fig. 3). However, during previtellogenesis the Golgi bodies assume a duplex appearance near the cortical ooplasm (Fig. 4) and from where, finally, they move to the interior of the oocyte in course of vitellogenesis (Fig. 5).

Acid-haematin stains the granular Golgi bodies deeply and uniformly blue, indicating their phospholipid content. On treatment of fresh tissue with 2%
osmic acid, these Golgi bodies readily turn dark as they seem to have highly unsaturated lipids. Also in the Nile blue sulphate preparation their acidic lipid (phospholipid) content can be detected. The phospholipid component of Golgi bodies in the younger oocytes is referred to as L₁ bodies. During previtellogenesis the Golgi bodies seem to undergo a chemical change and assume a duplex appearance

Figs. 1 to 5. Camera lucida diagrams of oocytes. Aoyama's silver method. ×430. 1, showing juxtanuclear Golgi bodies (GB). 2, showing perinuclear Golgi bodies. 3, showing dispersed Golgi bodies. 4, showing duplex appearance of Golgi bodies. 5, camera lucida diagram of a part of section of oocyte (Sudan III and IV). Showing L₂ and L₃ bodies and also sudanophobic compound yolk (CY). ×430.
(Fig. 6). This is evident in a 2% osmic acid preparation in which the 'externum' of duplex Golgi bodies becomes blackened in a short time, say within 15', but not the 'internum' even after similar treatment for six hours. Plausibly this implies the development of saturated lipids in the zone of internum. Or we can say that the Golgi bodies are partly masked with saturated lipids, thus giving a duplex appearance. This is confirmed as they give a uniformly black colouration without duplex appearance when treated with 1% phenol (Gupta 1958) for 48 hours prior to staining with Sudan black B. This may mean the presence of triglycerides in the zone of internum. These Golgi bodies therefore contain another variety of lipids which is referred to as L₂ bodies. The duplex structure of L₂ bodies is clearly marked in the acid-haematin preparation since the externum is stained blue-black and the internum light gray. When exposed to Nile blue sulphate the externum is stained blue and the internum pink. Some of these observations reasonably establish that the externum contains phospholipids while the internum a mixture of phospholipids and triglycerides. During vitellogenesis most of the phospholipids in the L₂ bodies are transformed into triglycerides as seen by their very weak reaction to acid-haematin, by their uniformly pink colouration in Nile blue sulphate...
and by their orange colouration in a mixture of Sudan III and IV. Thus, by further alteration in the chemical make-up of L₂ bodies, their duplex appearance is lost, giving rise to a homogeneous structure (Figs. 5 and 7). The latter are referred to as L₃ bodies. None of these bodies (L₁, L₂ and L₃) however, contain protein and carbohydrate as reflected in their negative response to mercuric bromophenol blue, coupled tetrazonium, ninhydrin-Schiff and PAS tests.

**Compound yolk**: Minute yolk spheres appear even during previtellogenesis, exuding from the follicular epithelium (Fig. 8). They accumulate near the cortical ooplasm and from here they migrate to the central ooplasm. Larger globules of yolk are however extruded from the follicular epithelium (Fig. 9) during vitellogenesis. The yolk globules fuse to form still larger globules which gradually occupy the whole of the oocyte (Figs. 10 and 11). Being completely sudanophobic, the yolk globules appear as empty spaces in Sudan black B and Sudan III and IV preparations (Figs. 5 and 7). They give intense blue-black colouration in the acid-haematin preparation (Fig. 12) and which persists even after extraction with pyridine, thus pointing to the absence of phospholipids in these bodies. The persistence of colouration is probably due to the presence of some other chemical components. This is confirmed when these yolk globules are seen to react very strongly to the specific tests for carbohydrate and protein (Figs. 10 and 11). The occurrence of carbohydrate (1:2 glycol group) is further reinforced by acetylation and KOH reversal controls of PAS test. A completely negative reaction with PAS, following treatment with saliva, indicates the presence of glycogen in the yolk globules. This is confirmed by their intense colouration in Best's carmine preparations. However, the yolk globules respond negatively to alcian blue and toluidene blue meaning thereby a lack of acid mucopolysaccharides. Mercuric bromophenol blue, ninhydrin-Schiff and coupled tetrazonium stain the yolk globules positively indicating the presence of different groups of protein.

**Golgi bodies and lipids**: The formation and chemical composition of fatty yolk do admit of sufficient controversy even within a few species of orthopterous insects that have been chosen to settle this point. For example, in case of Chrotogonus (Nath et al. 1959b), the lipid bodies (L₁) acquire a uniform distribution in the ooplasm of younger oocytes and are substantially composed of lipoprotein. Some of the L₁ bodies, which are at the periphery of the cell, grow and change chemically to give a duplex appearance (L₂) in Sudan black and Sudan III and IV preparations. By employing specific histochemical tests these authors find the L₁
bodies to consist of a highly unsaturated phospholipid sheath and an inner core of a highly saturated masked lipids. The lipids in the core are believed to be largely triglycerides with traces of phospholipids. In mature oocytes the L₂ bodies lose their duplex appearance during vitellogenesis and stain homogeneously with Sudan black B. These are the so-called triglyceride-rich L₃ bodies which gradually increase in number and eventually collect between the compound yolk globules, presenting a honey-combed pattern. The same workers, however, find variations in distribution, behaviour and chemical contents of lipid bodies in the grasshopper Gryllodes. Here, no sooner than the lipid bodies (L₁) acquire a uniform distribution in the ooplasm, some of them, which remain on the periphery, become associated in groups of two, three and even more. In later oocytes all these lipid bodies (L₃) collect at the periphery of the cell. The L₁ bodies appear to be chemically similar to those in Chrotogonus. The L₂ bodies, unlike in Chrotogonus, colour homogeneously with Sudan colourants and thus no longer show the duplex structure. Nevertheless they possess an unmasked triglyceride core with traces of phospholipids and are encased in a phospholipid sheath. They now begin to grow in size and move to the centre of the oocyte. Growth and movement completed, they are now called L₃ bodies. The latter, however, possess only saturated triglycerides with no traces of phospholipids. It has been observed in Locusta (Gupta 1968) that the L₁ bodies remain uniformly distributed and consist of only phospholipids but in due course they are transformed into duplex L₂ bodies with their cortex composed of phospholipids and their sphere of triglycerides. With further growth of oocytes the phospholipids in L₃ bodies are completely transformed into triglycerides, thus producing homogeneous L₃ bodies. The distribution and behaviour of lipid bodies (L₁, L₂ and L₃) of Oxya velox, included in the present study, parallel those of Chrotogonus. The L₁ bodies of Oxya contain phospholipids and the L₂ bodies have phospholipids in their externum and a mixture of phospholipids and triglycerides in their internum. The L₃ bodies, on other hand, are mostly of triglycerides with traces of phospholipids. Evidently, the chemical framework of the lipid bodies (L₁, L₂ and L₃) in different grasshoppers is not the same. Thus, while L₁ bodies of Oxya are similar to those of Locusta, the duplex L₂ and homogeneous L₃ bodies simulate those of Chrotogonus in their chemical make-up. It is, however, quite clear that the L₁, L₂ and L₃ bodies form a graded series in all the grasshoppers and since so, the L₃ bodies may have been derived from L₁ and L₂ from the L₃. In short, our observations in Oxya velox confirm and extend those of Nath and his associates (1958a–e, 1959a–d) that the L₁ and L₃ bodies appear to be homologous with the Golgi vesicles and the L₂ bodies with the fatty yolk; or, more pointedly, the L₃ bodies are deduced directly from the L₂ and the L₂ from L₁ bodies.

**Compound yolk:** Variations exist with respect to the origin and chemical composition of compound yolk in different species having a panoistic ovary. While Nath et al. (1959b) find the yolk globules in the oocytes of Chrotogonus and Gryllodes composed of protein and carbohydrate (including glycogen as one of the constituents), Gupta (1968) fails to find glycogen in Locusta even though proteins and carbohydrates (1:2 glycol group) do occur in them. Thus, as stated earlier, the yolk globules in the oocytes of Oxya velox are broadly similar to those
of Chrotogonus and Gryllodes in chemical composition. As to the origin of the yolk globules, Nath et al. have stated from their studies on Chrotogonus and Gryllodes that the yolk spheres make their first appearance at the periphery of the oocyte, close to the follicular epithelium; these gradually grow in size and invade the more internal regions so that the cytoplasm becomes loaded with the yolk bodies as to be obliterated by them during vitellogenesis. In Locusta the compound yolk first appears as carbohydrate granules in the circumnuclear zone of the oocyte; then they become scattered in the entire ooplasm but soon after they concentrate in the cortical ooplasm where they acquire proteinous substances to form the compound yolk. In Oxya velox, however, the situation is entirely different. Here the yolk globules first appear as minute PAS-positive and mercuric bromophenol blue-positive granules in the follicular epithelium. From here they move to the periphery of the oocyte and then suddenly grow and invade the entire ooplasm. A similar origin of compound yolk has been reported by Nath et al. (1958c) for Periplaneta. In cockroach Anderson (1964) has derived the protein content of yolk from RNA-rich particles which invade the ooplasm and the polysaccharide content from the follicular epithelium. Gupta has also observed a similar type of origin of the protein content of yolk but he differs from Anderson in the origin of its carbohydrate content. The granules of carbohydrates, as observed by Gupta, first appear near the circumnuclear region and from where they disperse in the cytoplasm, forming the yolk globules. In our studies there is, however, little evidence to support that the protein content of yolk globules is contributed by the extruded nuclear RNA since the minute granules of yolk from the follicular epithelium offer positive response to both protein and polysaccharide tests. These clearly show a variation in the origin and chemical composition of the compound yolk in insects, which may even belong to the same group.

Summary

The cytochemical studies of vitellogenesis in Oxya velox establish the following facts:

1. The Golgi bodies are clearly involved in the formation of fatty yolk which, however, consists predominantly of triglycerides with traces of phospholipids.

2. The compound yolk originates from the follicular epithelium and is composed of carbohydrates (1:2 glycol group and glycogen) and different groups of protein.

Literature cited


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