Origin and Cytochemical Analysis of Yolk in Oogenesis of *Locusta migratoria*

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Introduction

A considerable amount of work has been carried out in recent years on the origin and cytochemistry of yolk in insect oogenesis (Hsu 1953, Colombo 1955, Bonhag 1955a, b, 1956, 1958 and 1959, Nath *et al.* 1958a, b, 1959a, b, c, King 1960, Krainska 1961, Aggarwal 1962 and 1964, Roth and Porter 1964, Sharma 1966 and Gupta 1966). Broadly speaking, two kinds of yolk have been described, *viz.* lipid yolk, consisting of triglycerides, and compound yolk, which consists mainly of proteins but very often containing lipids and carbohydrates in addition.

The compound yolk according to a few workers is derived from extraovarian source (Anderson 1964, in cockroach, Roth and Porter 1964, in *Aedes*, Nath *et al.* 1958b in *Culex*) while Nath *et al.* 1958a in cockroach and Nath *et al.* 1959a, in *Chrotogonous* and *Grylloides* showed that carbohydrate granules (the yolk precursors) migrate from the follicular epithelium cells into the cortical ooplasm. On the contrary, Bonhag (1956) in *Anisolabis* has derived the compound yolk from the circumnuclear carbohydrate granules.

Nath and his collaborators have assigned the lipid yolk (=fatty yolk) to the lipid bodies of the first and second category (L_1 and L_2 bodies) (Nath *et al.* 1958a, b and 1959a, b, c, Aggarwal 1962, 1964). Bonhag (1958) derives fatty yolk from 'lipochondria'.

The present study was undertaken to throw some light on the origin of compound and lipid yolk in oocytes of *Locusta migratoria*. Evidence has been furnished that 1) the Golgi bodies take their origin from the mitochondria (*cf.* Hirsch 1939, Nath 1965, etc.), 2) the lipids of the first two categories (L_1 and L_2 bodies) are strictly homologous with the Golgi complex, 3) the lipid yolk is derived from L_2 bodies (*cf.* Nath 1960), and 4) nuclear RNA is transported to the ooplasm through nucleolar emissions, where it plays an important role in the synthesis of the protein constituent of the compound yolk (Gupta 1966).

Material and methods

Locusta migratoria (with a panoistic ovary) was selected for the present investigation on account of the economic importance of this species and the ease with which its culture can be maintained in the laboratory throughout the year.

A few pairs of *Locusta migratoria* were kindly supplied by the Field Station on Locusts Investigations, Bikaner (India), these were reared in the laboratory at a temperature of 25°-30°C throughout the year and fed on cauliflower leaves.

Ovaries were dissected out of living specimens and fixed in various fixatives for cytochemical study. The usual morphological techniques like Champy-iron haematoxylin, Lewitsky saline-iron haematoxylin, Kolatchev, Aoyama, Hermann's post osmication (HPO), and 2% osmic acid were also employed.

The extraction of lipids with pyridine (Baker 1946), RNA with perchloric acid (Erickson *et al.* 1949) and with salivary RNase (Bradbury 1956) and both DNA and RNA with trichloroacetic acid (Schneider 1945) were carried out. Unmasking of lipids was carried out with 1% phenol (Gupta 1958).

Observations

Golgi bodies and mitochondria. The young oocytes of *Locusta migratoria* reveal a prominent juxtanuclear mass, consisting of Golgi bodies and mitochondria.

In addition, there is a loose basophilic substance distributed throughout the ooplasm.

When oocytes are fixed with formol calcium (F/Ca), postchromed or unpost-chromed, and coloured with Sudan black B

(SBB), the juxtanuclear mass in the earliest

oocytes appears to be a dense structure, not revealing any granulation

(Fig. 1). But in more advanced

oocytes (Fig. 2) two types of granules can be easily

observed, *viz.* larger, irregular and dark granules, the Golgi bodies, embedded in a mass of much smaller and much less dark granules, the mitochondria.

In Baker's acid-haematein (AH) technique the juxtanuclear mass of mitochondria and Golgi bodies stains deeply and uniformly blue, revealing no

Figs. 1-5. Drawn with camera lucida. 1, 2, figures drawn from the young oocyte of *Locusta migratoria*, fixed in formaldehyde-calcium, postchromed in dichromate-calcium and coloured in Sudan black B. 1, showing homogeneously coloured juxtanuclear mass in the youngest oocyte. 2, an advanced oocyte showing Golgi bodies (G) embedded in the mitochondrial mass (M). 3, circumnuclear ring of the mitochondria (M) and Golgi bodies (G) is showing by employing Hermann's post osmication method. 4 and 5, figures drawn from the oocytes during previtellogenetic phase, fixed in formaldehyde-calcium, postchromed in dichromate-calcium and stained in acid haematein. 4, Golgi bodies (G) and mitochondria (M) scattered in the ooplasm. 5, the duplex Golgi bodies (G_1) and mitochondria (M) in the ooplasm.

granulations (as in SBB preparations) showing a concentration of phospholipids in the juxtannuclear mass.

When fresh oocytes are treated with 2% osmic acid run under the coverslip, the Golgi bodies become dark within 5 m., showing that they

consist of highly unsaturated lipids.

With Nile blue sulphate (NBS) both the Golgi bodies and the mitochondria appear blue; this confirms the presence of acidic lipids (phospholipids) and complete absence of neutral lipids.

On further growth of the oocytes the juxtannuclear mass becomes circumnuclear (Fig. 3) and a little later it fragments throughout the ooplasm (Fig. 4).

Figs. 6-9. 6, camera lucida drawings of the forms of duplex Golgi bodies (G_1) magnified, prepared as in Fig. 5. 7, photomicrograph of an advanced oocyte fixed in formaldehydecacium, postchromed and stained with Sudan III and IV showing the fatty yolk (FY) in the ooplasm. 8, photomicrograph of an early oocyte fixed in Carnoy and stained with methyl green/pyronin G technique, illustrating RNA positive granules (BG) in the ooplasm and in germinal vesicle (N). 9, photomicrograph of an early oocyte fixed in ethanol and stained with PAS technique, showing carbohydrate granules (CYP) in the vicinity of germinal vesicle (N).

The Golgi bodies in early oocytes seem to contain phospholipids; they have been called lipids of the first category (L_1 bodies). But with growth the Golgi bodies seem to develop some neutral lipids also and assume a duplex appearance (Figs. 5 and 6). In NBS the cortex of the duplex Golgi body

stains blue and the sphere attached to it violet ; from this it may be concluded that the cortex contains phospholipids only while the sphere contains both phospholipids and triglycerides. These duplex Golgi bodies are the lipids of the second category (L_2 bodies). The duplex structure of the L_2 bodies is confirmed in AH-technique in which the cortex is strongly AH-positive but the sphere is completely negative (Figs. 5 and 6).

With further growth of the oocytes the phospholipids in the L_2 bodies are completely transformed into triglycerides (L_3 bodies), as revealed by their uniformly pink coloration in NBS and orange in mixture of Sudan III and IV (Fig. 7). Thus arises the fatty yolk from the Golgi bodies.

There are no proteins in the Golgi bodies at any stage of oogenesis as revealed by their completely negative reaction to coupled tetrazonium (CTZ), mercuric bromphenol blue (Hg-BPB), ninhydrin-Schiff, and Himes and Moriber.

On the contrary, the mitochondria react strongly for proteins in these techniques. It may be added that the mitochondria remain granular throughout oogenesis.

Basophilic material. The basophilic material in *Locusta migratoria* comes in the ooplasm from the nucleolus in the form of granules as reported earlier by the author (Gupta 1966). This basophilic material contains RNA, bound lipids and proteins. The basophilic granules are completely devoid of carbohydrates and DNA.

The basophilic granules (=nucleolar extrusions) form a circumnuclear ring (reference Fig. 2, Gupta 1966) which soon disperses in the ooplasm during previtellogenesis (Fig. 8). During growth of the oocyte, these granules disappear in the ooplasm, which nevertheless now shows a strong pyroninophilia in methyl green/pyronin G technique. In vitellogenesis the RNA contents of the ooplasm decrease as protein yolk is synthesized.

Vitellogenesis. With the appearance of yolk, the oocyte enters the second phase of growth, *i.e.* vitellogenesis. During this phase, the cytochemical techniques demonstrate two types of yolk bodies, *viz.* 1) compound yolk and 2) fatty yolk in the oocytes of *Locusta migratoria*.

The compound yolk in *Locusta migratoria* consists of carbohydrates and proteins but no glycogen. To begin with, there is a circumnuclear mass of carbohydrate granules present in the early oocytes (Fig. 9). As the oocyte grows the carbohydrate granules are dispersed more or less uniformly in the ooplasm but they quickly concentrate in the cortical ooplasm (Fig. 10), where they acquire proteins under the influence of RNA and grow into prominent compound yolk globules (Figs. 11 and 12). Gradually the compound yolk globules migrate to the inner ooplasm till they are uniformly dispersed. In advanced oocytes the whole of the ooplasm is loosely packed with such yolk globules (Fig. 13).

Cytochemically, the compound yolk reveals strongly positive reaction for carbohydrates, particularly 1:2-glycol group as indicated by Himes and Moriber and PAS (with its controls). The intensity of this reaction is almost

the same in various fixatives which dissolve lipids (such as ethanol, Bouin, Carnoy, Zenker) and in those which do not contain lipid solvents (such as formol calcium, Lewitsky-saline and Helly). This shows that the strong

Figs. 10-13. Photomicrographs of advanced oocytes fixed in Bouin's and stained with Himes and Moriber's method. 10, showing the carbohydrate granules (CYP) in the cortical ooplasm. 11, the carbohydrate granules grow and form the compound yolk globules (CY). 12, fully formed compound yolk, the dark globules (CY), at the cortical region; negative picture of fatty yolk appears as empty spaces (FY) in between compound yolk globules. 13, photomicrograph of an advanced oocyte, fixed in Carnoy and stained in bromphenol blue, showing the compound yolk globules.

positive PAS and Himes and Moriber reactions are mainly due to carbohydrates and not to the presence of lipids and/or glycolipids, which also take stain with PAS (Hale 1957, Cain 1960).

The yolk globules show the presence of proteins as demonstrated by Hg-BPB, CTZ, ninhydrin-Schiff and Himes and Moriber techniques, but are completely devoid of lipids or lipoproteins. Further, the compound yolk globules give negative

reaction for acid mucopolysaccharides in toluidine blue preparations and also for glycogen in Bauer-Feulgen and Best carmine methods (references in Pearse 1960, Gurr 1960).

It has been already shown that the fatty yolk globules (L_3 bodies), consisting of triglycerides only, are derived from duplex L_2 bodies which consist of phospholipids in their cortex and triglycerides in their medulla and which, in their turn, are derived from purely phospholipid bodies, the L_1 bodies, *i.e.* the Golgi bodies of earlier oocytes. In this process the cortex of the L_2 bodies gets very much attenuated.

Discussion

Two types of yolk bodies, *viz.* the compound yolk and fatty yolk, can be demonstrated by employing various cytological and cytochemical techniques during vitellogenesis in various insect species (see Raven 1961, Nath 1967).

Compound yolk. In the oocytes of *Locusta migratoria* the nucleolus of the germinal vesicle emits strongly RNA-positive granules in the nucleoplasm, which subsequently migrate into the ooplasm where they form a prominent ring of RNA-positive granules. These are the nucleolar extrusions which soon disperse into the general ooplasm.

The role of nuclear RNA, transported into the ooplasm through nuclear extrusion, in the synthesis of protein yolk has now been well established in the oogenesis of a large number of animal species, particularly the insects (*e.g.*, Gresson and Threadgold (1962) in *Blatta orientalis*; Anderson (1964) and Bonhag (1959) in *Periplaneta americana*; Gupta (1966) in *Dysdercus cingulatus*, *Acheta domesticus*, *Periplaneta americana*, *Chrotogonus* sp.; Hsu (1953), Sharma (1966), King (1960) in *Drosophila*; Aggarwal (1962), Colombo (1955) in *Bombyx*; Bonhag (1956) in *Anisolabis*; Krainska (1961) in *Cynips*; Gupta (unpublished) in *Paratelphusa*, and *Macrobrachium* (Crustacea)). There can, therefore, be hardly any doubt that the nuclear RNA in *Locusta* plays a prominent role in the synthesis of proteins in the compound yolk.

The precursors of the polysaccharide moiety of the compound yolk in *Locusta migratoria* first appear in the circumnuclear position as fine granules, as revealed by Himes and Moriber and PAS (with its controls); later they are scattered in the whole of the ooplasm but quickly concentrate in the cortical ooplasm. Here they acquire proteins under the influence of nuclear RNA and grow to form the compound yolk. Bonhag (1956) in *Anisolabis* also describes a circumnuclear concentration of carbohydrate yolk precursors. Contrary to this Nath *et al.* (1958a) and Anderson (1964) in cockroach derive the polysaccharide precursors from the follicular epithelial cells of the oocyte.

Golgi bodies and lipid yolk. Sotelo and Porter (1959) are the only workers on oogenesis (rat) who have made 'parallel observations on light and electron microscope preparations with attempted correlations'. These workers have very ably shown that their Golgi dictyosomes of electron microscopy, showing the typical fine structure with parallel membranes, has the same distribution as the Golgi dictyosomes of earlier light microscopists (references in Sotelo and Porter 1964).

Likewise Nath (1960) showed that the lipid bodies (L_1 and L_2) in the oogenesis of a large number of animal species were strictly homologous with the Golgi apparatus of earlier classical workers who used silver and osmium impregnation methods.

Further, Nath (1967) has brought about a very close homology between the Golgi dictyosomes of electron microscopy on the one hand and the Golgi apparatus of classical workers and the lipids of cytochemists on the other, in various forms of oogenesis.

In the somatic cells a correlation has been admirably brought about by Baker (1963) between the larger lipid inclusions of the neurones of *Locusta* and *Schistocerca* of light microscopy on the one hand and the Golgi dictyosomes with parallel lamellae of electron microscopy (Ashhurst and Chapman 1962) on the other.

Hirsch (1939) was the first worker to show very clearly that the Golgi bodies were built up from the Golgi substance derived from the mitochondria; further the fully formed Golgi body had a duplex structure with an externum (which could be impregnated with silver and osmium) and a pale internum. At the level of electron microscopy Hirsch (1961, 1962) describes the duplex Golgi body as the "lamellar vacuolar field". Nath (1965) in sperm-forming cells of *Anadenus* also clearly showed the origin of Golgi bodies from the mitochondria. There is thus no doubt that the identity of Golgi body, as seen with light and electron microscopy, is now well established.

In *Locusta migratoria* the origin of fatty yolk from Golgi bodies follows exactly the same pattern as in *Periplaneta americana* (Nath *et al.* 1958a). To begin with the Golgi body consists of phospholipids only (L_1 bodies); gradually triglycerides develop in the medulla and the Golgi body assumes the characteristic duplex appearance (L_2 bodies). Still later the cortical phospholipids of the Golgi body are also converted into triglycerides with the simultaneous attenuation of its cortex, thus forming the fatty or lipid yolk (L_3 bodies) (*cf.* Nath *et al.* (1959a) in *Chrotogonus* and *Grylloides*; Nath *et al.* (1959b) in *Labidura*; Nath *et al.* (1959c) in *Laccotrephes*; Aggarwal (1962, 1964) in *Bombyx* and *Tenebrio*).

Droller and Roth (1966), in the oogenesis of *Lebistes reticulatus*, have actually demonstrated in their micrograph (ref. Fig. 4) duplex L_2 bodies, each showing a cortex (consisting of Golgi cisternae and vesicles) circumscribing their lipid droplet, which corresponds to the medulla of the L_2 body. The lipid droplet of the authors probably contains unsaturated neutral fat in addition to phospholipids (Chopra 1958a, b, 1960), as is shown by its blackening by the osmic acid used by the authors in fixation. But Droller and Roth (1966) have not been able to explain the 'precise physiological relationship' between the Golgi cisternae and vesicles forming the cortex and the lipid body forming the medulla, as they have not employed any cytochemical technique. Fig. 4 of Droller and Roth (1966) also admirably illustrates the attenuation

of Golgi cisternae and vesicles forming the cortex of the duplex Golgi complex to give rise to pure triglyceride yolk.

Bonhag (1958), while reviewing the work of Ries and van Weel (1934), states that lipochondria, which he considers comparable with the Golgi vesicles of Nath, enlarge and multiply during the growth of the oocyte and gradually develop fatty material in them and form the lipid yolk in *Pediculus*.

Mitochondria. The mitochondria in the oogenesis of *Locusta migratoria* do not take any demonstrable part in yolk formation; they remain granular throughout oogenesis. At no stage they contain either RNA or triglycerides (cf. Nath *et al.* 1958a and 1959a, in cockroach, and *Chrotogonus* and *Gryllodes*).

Summary

1. In the present work, the origin and cytochemistry of both the types of yolk (fatty and compound) in the oogenesis of *Locusta migratoria* are discussed.

2. The fatty yolk globules (L_3 bodies), consisting of triglycerides only, are derived from the Golgi bodies (L_1 bodies), consisting of phospholipids only, through an intermediate stage, the duplex Golgi bodies (L_2 bodies) which contain phospholipids in their cortices and triglycerides in their medullae. In this process the thick cortex of duplex Golgi bodies becomes greatly attenuated.

3. The compound yolk globules have their origin from the fine circum-nuclear carbohydrate granules of the early oocyte which arrange themselves at the cortical ooplasm in the late stages of oogenesis, where they grow and form the compound yolk globules.

4. The protein moiety of the compound yolk is formed under the influence of nucleolar RNA which plays an important role in protein synthesis.

5. Cytochemically, the compound yolk consists of carbohydrates (1:2 glycol groups) and acidic proteins; it is devoid of mucopolysaccharides and glycogens.

6. The mitochondria, throughout oogenesis, remain in the form of small granules and do not take part in yolk synthesis.

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