Histochemical and Morphological Studies of the Lipids in Oogenesis. I. *Periplaneta americana*

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With one plate (fig. 6)

SUMMARY

1. Three kinds of lipid bodies have been described in the oogenesis of the cockroach, *Periplaneta americana*: (i) L_1 bodies, present in the earliest oocyte, which persist till the oocyte measures approximately 0·5 mm and contain phospholipids only, possibly having more lecithins than cephalins; (ii) L_2 bodies, which first arise in the oocyte measuring 0·4 mm and have a complete or incomplete sheath of phospholipids surrounding a medulla of triglycerides (rather highly saturated); (iii) L_3 bodies, which are the only type of lipids present in the oocytes measuring more than 0·65 mm and consist of triglycerides only (rather highly saturated).

2. Some of the larger L_3 bodies give a 'ringed' or 'crescentic' appearance in Sudan black when used at room temperatures (12° C to 40° C) but appear mostly solid when this colouring agent is used at 60° C.

3. Mitochondria, which remain as fine granules throughout the course of oogenesis, contain proteins and phospholipids. They seem to have some lipids which are masked normally but are unmasked after acetone extraction, with a resulting increase in sudanophilia.

4. Yolk globules appear in the oocytes measuring approximately 0·5 mm. They contain a protein-carbohydrate complex.

5. The bacterioid objects described by earlier workers have been shown to contain phospholipids and free fatty acids. They possibly play an active role in the lipid synthesis of the cell.

INTRODUCTION

NATH and Mohan (1929) studied the egg of *Periplaneta americana* by the techniques of Kolatchev, Mann-Kopsch, Champy-Kull, Da Fano, and Bouin-iron-haematoxylin, and by staining fresh coverslip preparations with neutral red, or osmicating them in 2% osmium tetroxide. They arrived at the following conclusions, which were subsequently fully confirmed by Gresson (1931).

The 'Golgi elements' can occasionally be seen in the young oocytes without the aid of any vital dye. Neutral red stains them weakly. They darken slightly after short periods of immersion in 2% osmium tetroxide. In form the so-called Golgi elements are described as hollow vesicular bodies with a distinct osmiophil rim and a central osmiophobe substance. With the growth of the oocyte a large number of these bodies grow enormously, store up fat in their interior, and give rise to the fatty yolk. In this process of enlargement the rim of the so-called Golgi vesicles becomes more and more attenuated. These authors also describe mitochondrial granules, and nucleolar extrusions which form the so-called albuminous yolk.

[Quarterly Journal of Microscopical Science, Vol. 99, part 3, pp. 315–332, Sept. 1958.]

In the present investigation a large number of modern histochemical techniques have been employed to work out the chemistry of the so-called Golgi bodies, mitochondria, and yolk. The most important conclusions are (1) that at no stage in the course of oogenesis do the so-called Golgi bodies appear in the form of an 'apparato reticolare' as originally described by Golgi (1898), and (2) that the so-called Golgi bodies are in reality lipid spheres of at least three types.

Material and Technique

Specimens of *P. americana* were collected locally from Hoshiarpur. Only sexually mature specimens were used. The material was available all the year round except during the months of severe winter. It was found that the cytoplasmic contents of the oocytes showed considerable variations in their size, amount, and chemical composition according to the season in which the material was fixed.

The animals were dissected alive in a wax dish. On opening the body-cavity the fixative to be used was poured into it to minimize the post-mortem changes. The ovarioles were then removed along with their terminal filaments by means of fine forceps and transferred to the fresh fixative in a glass-stoppered capsule. The details of the various fixatives employed, the embedding media used, as well as the various staining techniques tried, have been summarized in table 1 on page 328.

In addition to the routine methods, various lipid extractions were also tried. The solvents used were cold acetone, cold ethanol, cold ether, cold methanol plus chloroform, cold methanol plus ether, hot acetone, hot ethanol, and hot ether. The last three were used in a Soxhlet extractor. The following three distinct methods have been employed for these extractions:

(1) Fresh ovarioles were treated directly for 24 h with each of the above solvents, the material was brought to water quickly through the descending grades of ethanol and fixed in formaldehyde calcium (Baker, 1946) for 6 h, postchromed as usual, and embedded in gelatine.

(2) The ovarioles were first fixed in formaldehyde calcium for 6 h and embedded in gelatine without postchroming; sections were cut at 10 μ. These sections were subsequently treated for 24 h with each of the above solvents and coloured either with Sudan black B or acid-haematein test of Baker (1946).

(3) The gelatine sections of the material fixed by the formaldehyde calcium postchroming technique were also treated with all the above solvents and coloured with Sudan black B, Nile blue (Cain, 1947), or acid-haematein (Baker, 1946).

Results

General structure of the ovariole

The ovaries of *P. americana* contain 8 ovarioles each, which are panoistic, that is, there are no definite nurse-cells. The ovariole begins as a thin thread containing nuclei and lipid granules, but the cell boundaries are not discernible in this region (fig. 1, A). It continues as a region in which the cells, although

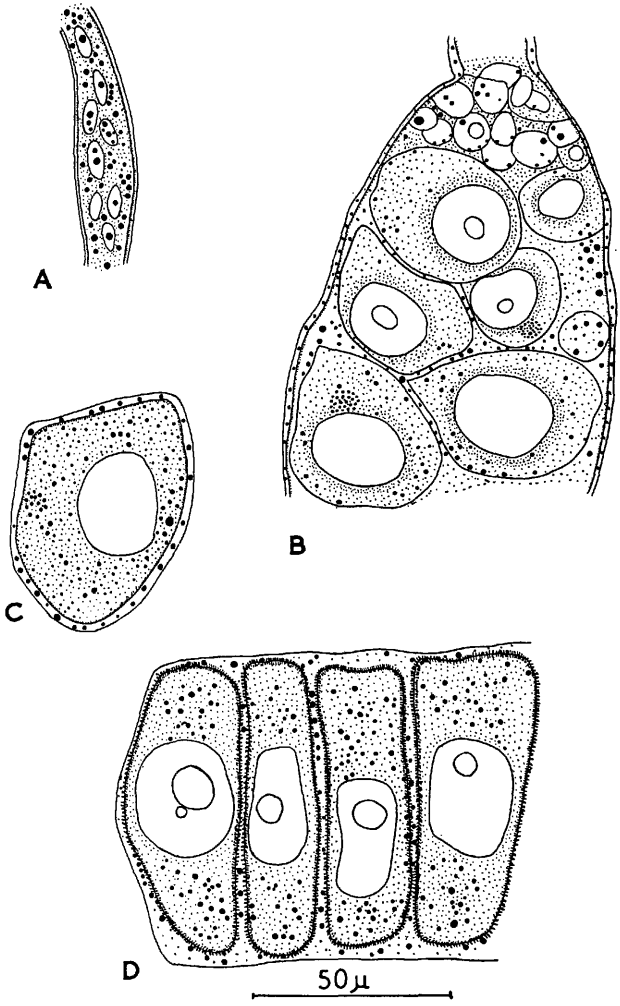

FIG. 1. Camera lucida diagrams drawn from gelatine sections of formaldehyde calcium material, postchromed; coloured with Sudan black B. The distribution of mitochondria, lipid bodies (L_1), and 'bacterioid forms' is shown. A, anterior end of germarium; B, distal end of germarium; C, a very young oocyte from the vitellarium; D, a portion of the anterior region of the vitellarium.

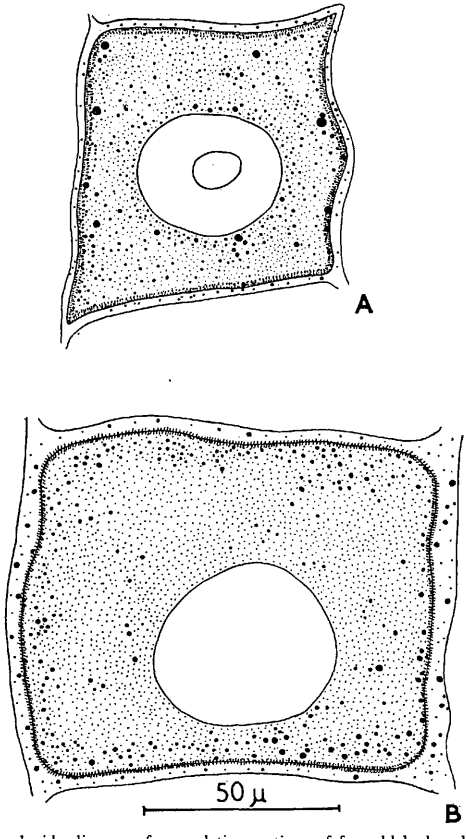

FIG. 2. Camera lucida diagrams from gelatine sections of formaldehyde calcium material, postchromed; coloured with Sudan black B. The drawings show the growth and distribution of the mitochondria, lipid bodies, and 'bacterioid forms' in the growing oocytes. A, oocyte measuring 0·075 mm; B, oocyte measuring 0·095 mm.

arranged in two or three rows, show distinct cell boundaries (fig. 1, B). Both these regions constitute the germarium. This is followed by a long vitellarium. It has been noticed that the vitellarium generally terminates in two large 'yolky' oocytes throughout the year except in March and April, when the number of these terminal 'yolky' oocytes is three.

The present investigation reveals that the lipid contents of the oocytes

gradually increase till the oocyte attains a length of approximately 0·1 mm, when the lipid bodies migrate towards the follicular epithelium and show a decline both in size of the individual granules and in number (figs. 1, A–D, and 2, A, B). When the oocyte attains a size of approximately 0·2 mm, the number and size of the lipid bodies start increasing, and they start migrating once

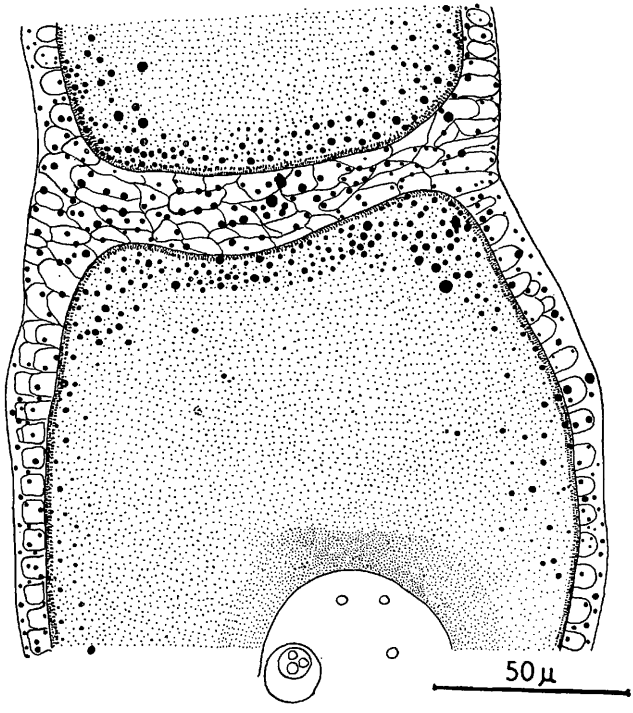

FIG. 3. Camera lucida diagram from gelatine section of formaldehyde calcium material, postchromed, coloured with Sudan black B. A part of two adjoining oocytes and the interfollicular region are shown. The lipid bodies (L_1) are mainly concentrated at the ends of the oocytes (size of oocyte approximately 0·28 mm).

again towards the centre of the oocyte (fig. 3). The increase in the amount of lipid granules continues without any detectable change in their chemical composition till the oocyte attains a size of approximately 0·4 mm (fig. 4, A). During these stages the larger lipid spheres are generally concentrated towards the periphery of the oocyte. Their distribution at the periphery of the oocyte also becomes more uniform (fig. 4, B) as compared with the earlier stages of

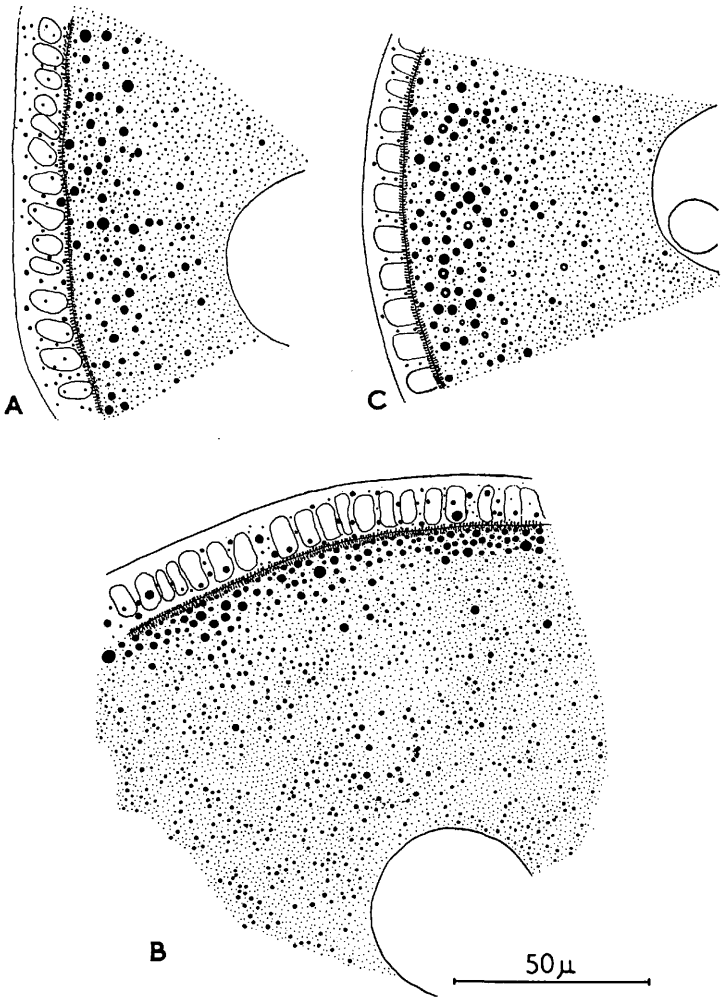

FIG. 4. A and B, portions of oocytes from gelatine sections coloured with Sudan black. The drawing shows the homogeneous nature of the L_1 and L_2 bodies. C, a portion of the oocyte from an acid-haematein preparation. The drawing shows the duplex nature of the L_2 bodies. Unchanged L_1 bodies are homogeneous (size of oocyte: A, 0·42 mm; B, 0·36 mm; C, 0·45 mm).

growth, when they are mainly concentrated near the interfollicular regions of the oocyte.

The lipid bodies of the oocytes continue to increase in number. It appears that lipid synthesis occurs mainly near the follicular epithelial cells and the new bodies continue to migrate towards the centre. The oocyte measuring 1·5 mm or more shows the concentration of the lipid bodies in the central region, the periphery being occupied by the growing yolk globules (albuminous yolk). Some of the lipid bodies now attain a considerable size.

The oocytes measuring approximately 0·6 mm generally show the first signs of yolk bodies (albuminous yolk), which appears in the form of minute granules in epithelial cells as well as in the cytoplasm adjacent to the epithelial layer. These show a quick increase in number and size of the individual bodies. Ultimately the larger yolk spheres come to occupy the whole of the cytoplasm of the oocyte. Lipid bodies in these stages become sandwiched between the yolk globules.

The follicular epithelium is always one cell thick and is the only source of supply of the raw material to the developing oocyte. In the interfollicular region, however, the epithelium is many cells thick (fig. 3). The cytoplasm of the follicular epithelial cells always contains large quantities of lipid bodies of various sizes.

It must be mentioned here that an ovariole always reveals a fairly thick layer of small rodlets at the extreme periphery of the cytoplasm and immediately below the follicular epithelium (figs. 1, D; 2; 3; 4, A, B). These rodlets are sudanophil except in very late oocytes, where they remain uncoloured by most of the techniques employed. They correspond to the 'bacteria' described by the earlier authors (Blochmann, 1884, 1887; Nath and Mohan, 1929). Since they appear to contain large amounts of free fatty acids in early stages of vitellogenesis (see below) and since they are sudanophil up to quite a late stage, they seem to be concerned with the lipid synthesis of the oocytes.

Histochemical observations

The various histochemical tests tried and their results have been tabulated on page 328.

Mitochondria. The mitochondria, which are always in the form of minute granules, are present in the cytoplasm of all stages of oogenesis, from the germarium to the oldest oocyte in the vitellarium. Their distribution in the cytoplasm is also more or less uniform except in very young oocytes, where some of them are arranged in the form of a ring round the nucleus. Their size and chemical composition remain unchanged throughout oogenesis.

The various histochemical tests listed in table 1 on page 328 show that the mitochondria in this material are rich in phospholipids and proteins (AH+, PE– and Hg–BPB+); they do not contain any triglycerides or carbohydrates. They do not seem to play any part in the process of vitellogenesis.

When the ovarioles are extracted with cold acetone or boiling acetone,

either fresh or after short fixation in formaldehyde calcium, the intensity of colour of the mitochondria with Sudan black B either remains unchanged or in some cases is actually enhanced. This is especially the case when fresh ovarioles are extracted with the solvents. The increase in the intensity of colour is also noticeable even when an ethanol solution of Sudan black B is employed. This increase in colour might be attributed to unmasking by the action of solvents, as described by Lovern (1955); that is, to the release of certain protein-bound phospholipids.

Lipid bodies. The various histochemical tests show that the lipid bodies in this material can be divided into three categories, L_1, L_2, and L_3.

The lipid bodies of the first category, L_1, are present in oocytes of diameters up to approximately 0·4 mm; these give a uniform histochemical reaction irrespective of the size of the individual granules, which varies from the granules only slightly larger than the mitochondria to 5 μ (figs. 1–3; 4, A).

The L_1 bodies contain phospholipids only, as they give a strong AH+ and PE– reaction (Baker, 1946) and are stained blue by Nile blue (Cain, 1947).

Feyrter's (1936) 'enclosure method' as given by Pearse (1954) stained these bodies metachromatically rose-red. This method, although originally recommended for phosphatides and cerebrosides, has been used to indicate the presence or absence of neutral lipids (triglycerides in this case), as discussed by Pearse (1954). The metachromatic rose-red coloration of these bodies excludes the possibility of the presence of triglycerides in them.

The various extraction tests give a very clear picture of the chemical composition of these lipid bodies (L_1). After cold or hot acetone extraction performed on fresh material, these bodies can be intensely coloured by Sudan black B or acid-haematein. Some of the larger bodies show a clear sign of displacement and aggregation at the two ends of the oocytes (fig. 6, A, opposite p. 326). This is obviously the result of the action of the solvent, which penetrates the oocytes from all sides except the two ends adjoining the interfollicular regions. This displacement of the lipids is not noticed in the sections of the material previously fixed in formaldehyde calcium. On the other hand, when gelatine sections of such material are treated with hot ethanol and ether, the L_1 bodies are mostly dissolved, except that very thin rims appearing in the form of fine crescents, sudanophil and AH-positive, are left over.

When fresh ovarioles are extracted with cold ethanol, the L_1 bodies appear in early stages as very small sudanophil and AH-positive granules, but in larger oocytes the sudanophil granules of fairly large size become aggregated at the ends of the oocytes. A similar picture is given by the treatment with cold and hot ether, except that after cold ether extraction the size of the individual granules is larger than after cold ethanol treatment. These lipid bodies are completely soluble in hot ethanol, methanol / chloroform and methanol / ether.

From these observations it is perhaps reasonable to infer the presence of lecithins (insoluble in ether) and cephalins (insoluble in ethanol), the former being more abundant. But since the solubility tests are not very reliable (Cain,

1950; Lovern, 1955), such a conclusion may be considered more or less conjectural.

The second category of sudanophil lipid bodies (L_2) first appear in oocytes measuring approximately 0·4 mm. They differ from L_1 bodies inasmuch as they show a colour between pink and blue in the Nile blue test; this suggests that they have developed some neutral lipids in their contents along with some acidic lipids.

A further elucidation of the chemical nature of the L_2 bodies is provided by the AH test with its PE control. In this test these bodies do not give a homogeneous solid appearance but appear instead as dark blue rings or crescents or even irregular bodies with a completely negative reaction in PE (figs. 4, C; 6, C). This appearance of the L_2 bodies in the AH test is in direct contrast with their appearance in various Sudan colouring agents, which colour them homogeneously (fig. 4, B). The co-existing L_1 bodies in these oocytes are homogeneously coloured, even in AH preparations. Thus, it can safely be concluded that at this stage the L_2 bodies consist of some neutral lipids, completely or incompletely enveloped in a sheath of phospholipids. The sheath gives these bodies a crescentic or ringed appearance in the AH test.

Further confirmation of this change in chemical composition is provided by the extraction tests. The neutral lipid contents of the L_2 bodies are dissolved by cold or hot acetone extractions either of fresh tissue or of tissue simply fixed in formaldehyde calcium. Consequently when Sudan colouring agents are applied to such sections, these bodies give a picture exactly corresponding to their appearance in the acid-haematein test. Extractions performed on fresh tissues, however, do not give such a clear picture of L_2 bodies as in the sections of material fixed in formaldehyde calcium. This is due to the displacement of these bodies in the oocytes.

The solubility of the neutral lipid contents of the L_2 bodies in cold acetone suggests that they are either glycerides or cholesterol and/or cholesteryl esters. The possibility of the latter is excluded as these bodies (L_2) give a completely negative reaction to Schultz's (1924, 1925) test for cholesterol and its esters (Gomori, 1952) and to Romieu's modification of Schultz's reaction (Pearse, 1954). Some sections were placed in the sun for 10 days in formaldehyde calcium solution and then immersed for 48 h in 2·5% iron alum; they were then subjected to Schultz's test. The reaction remained negative. The presence of triglycerides is inferred from the above tests.

The L_2 bodies are completely dissolved even in cold ethanol and cold ether, in spite of their phospholipid contents.

Thus the L_2 bodies have a medullary region rich in rather fully saturated triglycerides, surrounded by a sheath of phospholipids.

Although in oocytes measuring 0·4 mm both L_1 and L_2 bodies are present, the change in chemical composition of the total lipids of the oocytes is rapid, and within a very short period of growth the L_1 bodies completely disappear from the oocytes. In later stages all the lipid bodies, which still show a wide

range in size, are of the L_2 category. Soon, however, the phospholipid sheaths of L_2 bodies attenuate, and simultaneously there is an increase in the number and size of these bodies. Most of these bodies, especially the larger ones, now move towards the periphery of the oocytes.

The L_2 bodies now lose their phospholipid sheaths and thus become converted into L_3 bodies. This change occurs rapidly. The size of most of the lipid bodies increases considerably and they begin to move towards the central region of the oocyte. This change resulting in the attenuation of the phospholipid rims of the L_2 bodies as well as the increase in size is very clearly illustrated in figs. 4, C; 5, A–C; 6, C–E.

It may be noted that L_3 bodies are the only type of lipids present in the oocyte measuring 0·65 mm or more in diameter.

The lipids of the L_3 category generally appear in larger oocytes in the form of spheres ranging in size from very small granules up to about 0·04 mm. Their appearance in Sudan preparations is interesting. When Sudan black B or some other general lipid colorant is applied to the sections of tissue fixed in formaldehyde calcium and postchromed (Baker, 1946), the large L_3 bodies do not colour homogeneously but appear as 'rings' or 'crescents' (fig. 5, D). The smaller L_3 bodies as well as a few of the larger spheres colour homogeneously.

As Bradbury (1956) suggests, this appearance of lipid bodies may be due either to the presence in these bodies of lipids that are solid at room temperature (which varies in Hoshiarpur from 12° C to 40° C during the year), or due to the loss of certain lipids from the spheres during fixation. Bradbury (1956) further showed that in the adipose cells of the leech, *Glossiphonia*, such an appearance of 'fat globules' was due to incomplete fixation in formaldehyde saline, since no 'rings' and 'crescents' appeared in material fixed in formaldehyde calcium.

The appearance of 'rings' and 'crescents' in this material cannot be due to any incomplete fixation, as the ovarioles were always fixed in formaldehyde calcium and postchromed as recommended by Baker (1946, 1956). It is, therefore, reasonable to conclude that this incomplete colouring of larger L_3 bodies was due to the presence of lipids that are solid at room temperature (12° C to 40° C).

That this conclusion is correct has been shown by colouring the gelatine sections of formaldehyde calcium / postchromed tissue in Sudan black B at 60° C for 30 min. Such a procedure eliminated to a very great extent this incomplete colouring of L_3 spheres. Some of them, however, still retained their crescentic appearance, which might be due to the presence of some lipids having a melting-point even higher than 60° C.

As regards the chemical nature of L_3 bodies, they appear bright pink with Nile blue, are completely negative to AH test, and, do not give any metachromatic staining in Feyrter's enclosure method. They are completely soluble in even cold acetone, whether used on fresh or fixed material. All tests for other lipids being negative, the L_3 bodies seem to be composed of

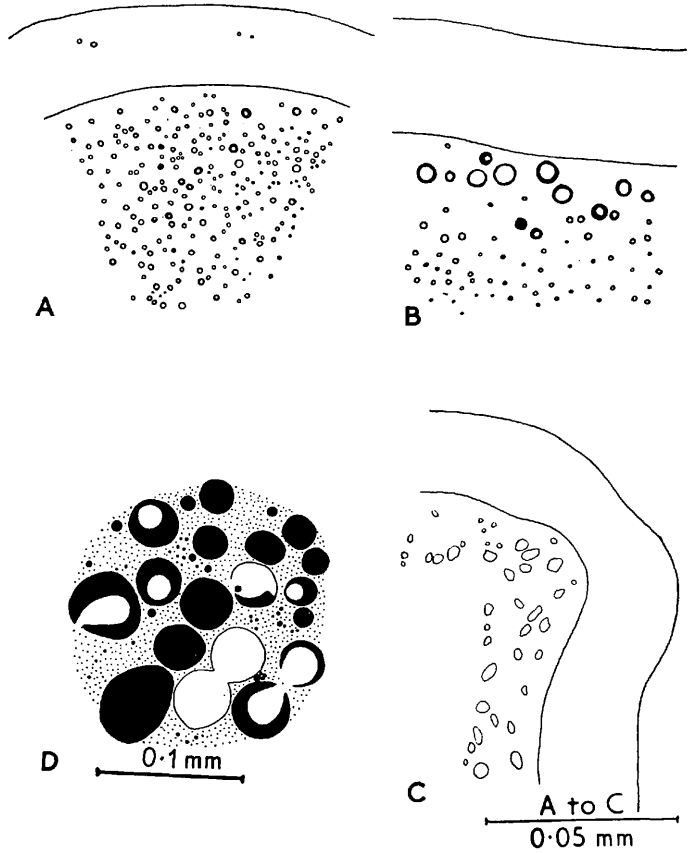

FIG. 5. A to C, portions of the oocytes from acid-haematein preparations. The drawing shows the gradual attenuation of the phospholipid rims of the lipid bodies. D, a very small portion of an oocyte from a Sudan black preparation. The incomplete colouring of the L_3 bodies is shown. (Size of oocytes: A, 0·5 mm; B, 0·63 mm; C, 0·66 mm; D, 1·65 mm).

triglycerides, which are not very unsaturated as their reaction to PFAS is feeble and they do not become insoluble in fat-solvents after postchroming.

Yolk globules

When L_3 bodies migrate to the more central areas of the oocytes, some globules of various sizes still appear in the peripheral region. The smallest of

these are minute granules; the largest recorded measures about 0·04 mm in diameter. The youngest oocyte in which these globules have been seen measured approximately 0·6 mm.

These globules appear for the first time in the form of minute PAS-positive granules in the follicular epithelial cells; thence they migrate to the periphery of the oocyte through the bacterial layer. Suddenly they grow and invade the entire cytoplasm of the oocyte. These granules are negative to all the lipid tests tried. Morphologically they are strictly homologous with the 'albuminous yolk' of Nath and Mohan (1929).

The strong PAS-positive reaction of these yolk globules indicates their carbohydrate nature, and this is confirmed by the acetylation and KOH-reversal controls (McManus and Cason, 1950). The intense blue colouring in Hg–BPB (Mazia and others, 1953) shows the presence of proteins in them.

A very interesting picture is presented by these globules in material fixed in weak Bouin's fluid and extracted with pyridine in the control to Baker's AH test. These globules give a strong blue-black coloration in acid-haematein after this method, but they appear frothy, showing a number of round vacuoles in their interior. This frothy appearance is conspicuous even after the PAS and Hg–BPB tests have been applied to these PE sections (fig. 6, F). This frothy appearance is also prominent in Carnoy-fixed material stained with PAS. Since the frothy appearance of these globules is not seen after formaldehyde calcium / postchroming technique, it is possible to attribute it to the loss of some alcohol- or pyridine-soluble material, which, however, is not lipid. We are unable to throw further light on this.

'*Bacteria*' *and lipid synthesis*

Nath and Mohan (1929) and Blochmann (1884, 1887) have described certain 'bacterioid forms' surrounding the oocytes of *P. americana* and lying immediately below the follicular epithelium. We have also seen these structures, which appear as small rodlets even in the earliest oocytes in the vitellarium.

The various histochemical reactions show that these 'bacterioid forms' contain phospholipid mixed with free fatty acids. These reactions are positive on

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FIG. 6 (plate). Photomicrographs of gelatine sections of the cockroach ovary subjected to various histochemical reactions.

A, a portion of an oocyte from material extracted with acetone and coloured with Sudan black. Note the accumulation of lipid bodies in the corner and the strong positive reaction of the 'bacterioid layer'.

B, a portion of an oocyte from material extracted with hot acetone and coloured with Sudan black B. The enigmatic sudanophil bodies in the nucleus are shown. L_1 bodies are not in sharp focus.

C–E, portions of oocytes from acid-haematein preparations. The gradual transformation of L_1 and L_2 bodies into L_3 bodies is shown. Note the attenuation of the phospholipid rims. Many homogeneous dark bodies in C have become negative to AH in figures D and E.

F, a portion of an oocyte from material extracted with pyridine (control to acid-haematein test). The frothy nature of the yolk globules is shown.

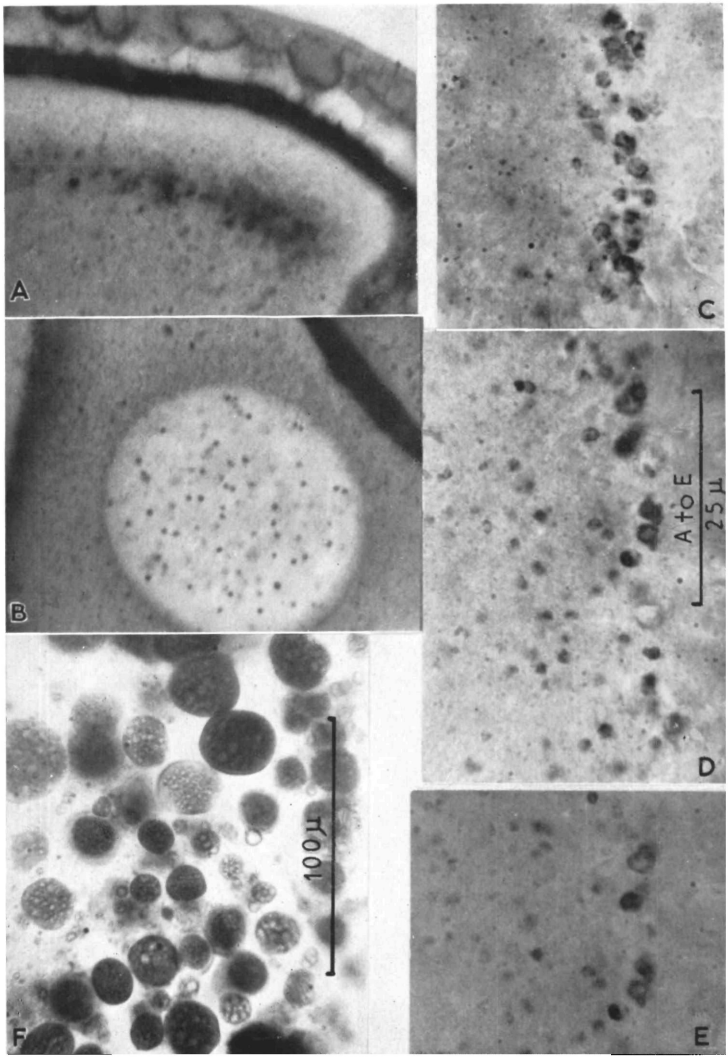

FIG. 6
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the bacterioid forms only up to that stage of vitellogenesis where the L_3 bodies have not yet fully developed. But once the L_3 bodies reach their maximum development and come to occupy the centre of the oocyte, these 'bacteria' become negative to all the lipid tests.

We have not tried any experimental work on lipid synthesis, but the available evidence clearly points towards the conclusion that these bacterioid forms are a centre of lipid synthesis in the oocytes. This conclusion is further supported by the fact that the lipid bodies of all categories found in the oocytes of the cockroach generally first appear and mature near this bacterial region. Moreover, the presence of free fatty acids also suggests the process of lipid synthesis being centred in these bodies. It may be repeated that when all the lipid material of the oocytes has been synthesized, these bacteria lose their lipid contents.

It may also be pointed out that lipid synthesis in the ovarioles of *P. americana* may be divided into three distinct phases:

(1) Phase of phospholipid synthesis, which continues till the oocyte attains a size of 0·4 mm (approximately). In this phase the L_1 bodies are formed, and they increase in number as well as in size.

(2) Phase of triglyceride synthesis, which starts in oocytes measuring approximately 0·6 mm and continues till they attain a size of approximately 1·5 mm. During this period all the lipids synthesized are triglycerides, which go to form the L_3 bodies.

(3) Intermediate phase in oocytes measuring 0·4 mm to 0·6 mm. This phase anticipates the phase of triglyceride synthesis, and during it the phospholipids of the L_1 bodies are either converted into or replaced by triglycerides.

Another important point noticed during the present investigation is that the formation of the L_3 bodies and of other lipid bodies in the oocytes varies considerably according to the season in which the material is fixed. Very large lipid bodies of the L_2 category have been recorded in oocytes measuring approximately 0·045 mm in material fixed during the month of April 1957.

Miscellaneous remarks

It has been noticed that the cytoplasm of oocytes measuring more than 1·65 mm does not show any definite globules, but is coloured uniformly by various tests, including lipid colorants as well as PAS and Hg–BPB, in sectioned material. This is due to the general diffusion of the material of the various globules under the incomplete and delayed action of the fixatives, which cannot penetrate quickly on account of the development of the chorion. This is confirmed by the fact that when the contents of such oocytes are smeared on a slide and then subjected to the various fixatives and colorants, both types of globules are clearly revealed.

Another enigmatic phenomenon is the appearance of certain well defined sudanophil bodies in the nucleus of comparatively young oocytes after extraction of fresh ovarioles by cold and hot acetone (fig. 6, B), cold ether, cold

TABLE I

Table showing the various histochemical reactions of the oocytes of P. americana

Technique	*Fixative*	*Embedding medium*	*Thickness of sections*	*Reference*	*Diameter of oocytes*	*Nucleus*	*Cytoplasm*	*Mitochondria*	L_1	L_2	L_3	*Bodies in the follicular epithelium*	*Albuminous yolk*	*'Bacteroid forms'*
Sudan black B:			(μ)		(mm)									
in 70% ethanol	FCa and FCa+PC	G	10	Baker, 1949, 1956	0·02–0·40	–	+	+	+++	A	A	+++	A	++
					0·40–0·60	–	+	+	+++	+++	A	+++	A	+
					0·60–1·65	–	+	+	A	A	+++(p)	+++	–	–
in 70% ethanol at 60° C	,,	G	10		0·02–1·65	–	+	+	+++	+++	+++	+++	–	++
in propylene glycol	,,	G	10	Chiffelle and Putt, 1951	,,	–	++	++	+++	+++	+++(p)	+++	–	++
— after treatment with:														
cold acetone	Fresh or FCa	G	10	Krishna, 1950 Pearse, 1954	,,	+	+	++	+++	++(p)	O	+++	–	+
hot acetone	,,	G	10	,,	,,	+	+	++	+++	++(p)	O	+++	–	+
cold ethanol	,,	G	10	,,	,,	+	–	–	+	O	O	++	–	O
hot ethanol	,,	G	10	,,	,,	–	–	–	O	O	O	O	–	O
cold ether	,,	G	10	,,	,,	+	–	–	++	O	O	++	–	O
hot ether	,,	G	10	,,	,,	–	–	–	+	O	O	++	–	O
methanol / chloroform	,,	G	10	,,	,,	–	–	–	O	O	O	O	–	O
methanol / ether	,,	G	10	,,	,,	–	–	–	O	O	O	O	–	O
Sudan III and IV:														
in 70% ethanol / acetone	FCa and FCa+PC	G	10	Kay and Whitehead, 1941	,,	–	+	+	+	++	+++(p)	++	–	+
in 1% gelatine suspension	,,	G	10	Govan, 1944	,,	–	+	++	++	++	+++(p)	++	–	++
Fettrot in 70% ethanol	,,	G	10	Pearse, 1954	,,	–	+	+	++	++	+++(p)	++	–	+
Nile blue sulphate	,,	G	10	Cain, 1947, 1948	0·02–0·40	–	B	B	B	A	A	B	A	B
					0·40–0·60	–	B	Nc	B	V	A	B	A	Nc
					0·60–1·65	–	Pk	Nc	A	A	Pk	B	–	–
Acid haematein (AH)	,,	G	10	Baker, 1946	0·02–1·65	–	–	+	+++	+++(p)	–	+++	–	+++

Acid haematein (AH) after pyridine ext. (PE)	WB+PE	G	10	Baker, 1946	0·02–1·65	+	−	−	O	O	O	O	++(p)	O
Plasmal reaction	FCa+PC	G	10	Hayes, 1949	,,	−	−	−	−	−	−	−	−	−
Fischler's reaction	Fs	G	10	Pearse, 1954	,,	−	−	−	−	−	−	−	−	+++
— after treatment with ether / ethanol	FCa or Fs	G	10	,,	,,	−	−	−	−	−	−	−	−	O
Feyrter's enclosure	FCa+PC	G	10	,,	,,	−	−	N	+++(R)	++(R)	−	Nc	−	N
Periodic acid / Schiff (PAS)	FCa and FCa+PC	G	10	Hotchkiss, 1948, Pearse, 1954	,,	−	+	−	−	−	−	+	+++	−
,, ,,	C and Z	P	5	,,	,,	−	−	−	−	−	−	+	+++	O
— after treatment with:														
ethanol	FCa	G	10	Pearse, 1954	,,	−	−	−	O	O	O	+	+++(p)	O
pyridine	WB+PE	G	10	Baker, 1946	,,	−	−	−	O	O	O	O	+++(p)	O
acetylation	FCa+PC	G	10	McManus and Cason, 1950	,,	−	−	−	−	−	−	−	−	−
restoration by 0·1N KOH	,,	G	10	,,	,,	−	−	−	−	−	−	+	+++	−
Mercuric bromophenol blue (Hg-BPB)	,,	G	10	Mazia and others, 1953	0·02–0·60	+	+	++	−	−	A	−	A	−
,, ,,	C and Z	P	5	,, ,,	0·60–1·65	+	+	++	A	A	−	−	+++	−
— after treatment with:					,,	+	+	++	−	−	−	−	+++	O
ethanol	FCa and FCa+PC	G	10	,, ,,	,,	−	−	+	O	O	O	O	+++(p)	O
pyridine	WB+PE	G	10	,, ,,	,,	−	−	+	O	O	O	O	+++(p)	O
Performic acid / Schiff (PFAS)	FCa and FCa+PC	G	10	Pearse, 1951 Lillie, 1952	,,	−	+	+	−	−	+	−	−	−
Ciaccio's technique	FCa+PC (1 day)	P	5	Bradbury, 1956	,,	−	−	+	+++	++	N	+++	−	Nc
Cholesterol reactions	FCa and FCa+PC	G	10	Schultz, 1924, 1925 Gomori, 1952 Romieu, 1927 Pearse, 1954	,,	−	−	−	−	−	−	−	−	−

Key: A = absent; B = blue; C = Carnoy; FCa = formaldehyde calcium; Fs = formaldehyde saline; G = gelatine; N = not observed; Nc = not clear; P = paraffin; (p) = partially or ringed; PC = with postchroming; Pk = pink; (R) = rose-red; V = violet; WB+PE = weak Bouin's fluid followed by pyridine extraction; Z = Zenker's fixation for 3 h; + = weak reaction; ++ = moderate reaction; +++ = strong reaction; − = negative; O = dissolved.

ethanol. These sudanophil granules are never seen after fixing in formaldehyde calcium and postchroming, nor do they appear after hot ethanol, hot ether, methanol / chloroform, or methanol / ether extractions. We are unable to explain this phenomenon, but it might be due to the presence in the nucleus of lipid material that is not detectable in ordinary Sudan black or AH preparations. Such a possibility has been pointed out recently by Chayen and others (1957) in a number of plant and animal cells.

Discussion

The selective histochemical colouring and solubility tests, carried out on gelatine sections of the ovarioles of the cockroach that have been fixed in formaldehyde calcium with or without postchroming, reveal the chemical composition and distribution of mitochondria, lipid bodies, yolk globules, and the 'bacterioid forms', which are discussed below.

Mitochondria

These are very minute granules, concentrated round the nucleus in the young oocytes, but distributed evenly throughout the cytoplasm in older oocytes. This confirms the observations of Nath and Mohan (1929) in the oocytes of the cockroach.

Nath and Mohan did not work out the chemical composition of the mitochondria, but the present investigations have clearly brought out that the mitochondria of these cells resemble ordinary mitochondria in being composed largely of protein and phospholipid.

Lipid bodies

The lipid bodies are abundant and widely distributed in the cytoplasm in all the oocytes of the ovarioles. By differential histochemical staining and solubility tests (Krishna, 1950; Pearse, 1954) the following three types of lipid bodies have been recorded, which differ from one another in their size and chemical composition.

(1) The first category of lipids or L_1 bodies, which contain phospholipid and are homogeneous spheres or granules.

(2) The second category of lipids or L_2 bodies, which are composed of triglyceride with a phospholipid sheath.

(3) The third category of lipids or L_3 bodies, which are composed of triglyceride only.

The first type of the lipid bodies correspond to the 'Golgi bodies' of Nath and Mohan (1929), but they have not the duplex structure formerly attributed to them. The duplex structure of the 'Golgi bodies' of this cell appears to be due to incomplete reduction of osmium tetroxide.

The second category of lipid bodies, which appear in the oocyte after it has attained a size of 0·4 mm, have undoubtedly a duplex structure and correspond to the duplex 'Golgi vesicles' of Nath and Mohan (1929).

A hint about their chemical nature is provided by the observations of Nath and Mohan (1929), who have shown that their 'Golgi vesicles' appear solid on prolonged osmication, as in Kolatchev preparations, but they again appear duplex if such slides are treated with turpentine. This shows that whereas the neutral fats of the medullary region are decolorized in turpentine, the lipids of the cortical region are not.

The third type of lipid bodies (L_3) correspond to the 'fatty yolk' of Nath and Mohan (1929). They are completely washed out in all the fat solvents, even after postchroming. That the L_3 bodies are composed of triglycerides only is strongly indicated by the selective staining and solubility tests employed by us. These observations are in harmony with those of Nath and Mohan (1929), who state that in Kolatchev preparations decolorized in turpentine the 'fatty yolk' spheres appear as clear vacuoles of various sizes, giving the cytoplasm a frothy appearance.

Nath and Mohan (1929) claimed that the 'fatty yolk' in the egg of the cockroach comes directly from the 'Golgi vesicles'. The present investigations are in complete harmony with this claim, as we have already shown that the L_3 bodies come directly from the L_2 bodies, and these from the L_1 bodies ('Golgi bodies').

Finally, it must be emphasized that there is no homology between the lipid bodies of the cockroach egg and the classical reticular 'Golgi apparatus', as a reticulum is conspicuous by its absence in this material and the lipid bodies do not show any fixed chemical composition.

Yolk globules

In addition to the lipid bodies there are present in the egg of the cockroach some non-lipid globules or spheres, which appear in later stages of vitellogenesis and seem to be homologous with the 'albuminous yolk' of Nath and Mohan (1929) and Gresson (1931). We have shown that these globules contain a protein-carbohydrate complex. This is indicated by their positive reactions to the PAS and Hg–BPB tests.

These yolk globules seem to arise suddenly as small, PAS-positive granules in the follicular epithelial cells and then migrate into the peripheral regions of the oocyte. Soon they invade the whole of the cytoplasm, increasing in size considerably. There is no evidence of the origin of yolk globules from the 'nucleolar extrusions' of Nath and Mohan (1929) and Gresson (1931), or from mitochondria.

Bacterioid forms

We have studied the rodlets called 'bacterioid forms' by Nath and Mohan (1929) and Blochmann (1884, 1887). These are situated just below the follicular epithelium. They contain free fatty acids and phospholipids. We have not tried any RNA or DNA tests. It seems that the 'bacterioid forms' participate in lipid synthesis in the developing oocyte. This is suggested by the

fact that all the lipid bodies grow in size and number near the layer of bacterioid forms, and when the synthesis of lipids is over the bacterioid forms are no longer positive to any lipid tests.

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