Yolk-Formation in Periplaneta Orientalis.

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With Plates 12 and 13.

CONTENTS.

	PAGE
I. INTRODUCTION AND PREVIOUS WORK	257
II. MATERIAL AND METHODS	259
III. OBSERVATIONS	259
1. Golgi vacuoles and fatty yolk-formation	259
2. Golgi vacuoles in follicle-cells	260
3. Nucleolar extrusions and albuminous yolk-formation	260
4. Feulgen's 'Nuclealreaktion'	263
5. Bacteria	263
IV. DISCUSSION	263
V. SUMMARY AND CONCLUSIONS	270
VI. ACKNOWLEDGMENTS	271
REFERENCES	272
EXPLANATION OF PLATES	274

I. INTRODUCTION AND PREVIOUS WORK.

IT has been shown by Nath and his co-workers (**18, 21, 22,** and **23**) that fatty yolk in certain invertebrates is formed by the deposition of free fat inside the oocyte Golgi vacuoles, and by the present writer (**9**) that the fatty yolk of the Tenthredinid egg is formed from the Golgi vacuoles of the oocyte and nurse-cell.

Hogben (**14**), for the eggs of Periplaneta americana, described remarkable nucleolar extrusions which migrated into the ooplasm and there gave origin to ordinary yolk. He did not investigate the part played by the Golgi elements in vitellogenesis. Consideration of the above facts led the present writer to suppose that Periplaneta would be a suitable form for

investigation of the phenomena associated with yolk-formation, and one which might be expected to shed light on the function of the Golgi elements. As previously pointed out by the writer (**9**) there is much difference of opinion at present, so that further work is necessary to establish the part played by the Golgi bodies during yolk-formation in the different groups. For although several workers ascribe a Golgi origin for the fatty yolk, others claim that the albuminous yolk is formed from these bodies.

After the present work on the yolk-formation of Periplaneta orientalis was commenced the writer received a reprint of Nath and Piare Mohan's (**23**) recent study of Periplaneta americana. The investigations on Periplaneta orientalis were continued in order (i) to compare yolk-formation in Periplaneta orientalis with that of the related form Periplaneta americana; (ii) to determine the staining reactions of the oocyte nucleolus and nucleolar extrusions; (iii) to determine by means of Feulgen's method the history of the chromatin in follicle-cell and oocyte nuclei.

Nath and Piare Mohan (op. cit.) show that the Golgi vacuoles of the oocytes of Periplaneta americana consist of 'an osmiophilic rim and a central osmiophobic substance'. During the growth of the egg they become distributed throughout the ooplasm and, by the deposition of fat inside them, are converted into fatty yolk-spheres. Hence the structure and behaviour of these bodies in the egg of Periplaneta are similar to those of the Golgi vacuoles in the other forms described by Nath and his co-workers and by the present writer. Those of Periplaneta appear to be slightly different, however, in that they tinge slightly with neutral red, whereas no staining was observed in those previously described by Nath and his co-workers and by the present writer. Further the Golgi vesicles (or vacuoles) of the follicular epithelial-cells of Periplaneta are not so intensely darkened by osmic acid as those of the oocytes, thus indicating that the former contain much smaller quantities of fat.

The albuminous yolk is formed from vacuolated nucleolar extrusions which break up into small homogeneous bodies, the latter being subsequently transformed into the yolk-spheres.

II. Material and Methods.

The material for this paper was obtained in November and December 1929, and in January and February 1930, from female specimens of Periplaneta orientalis in various stages of growth.

For an examination of the Golgi vacuoles the ovaries were dissected out in saline solution, stained in neutral red and subsequently mounted in a drop of saline or stain and examined. Ovaries were also fixed by the standard Mann-Kopsch and Kolatschev methods, while fixation for a short time in 2 per cent. osmic acid produced good results.

The studies on oocyte nucleolar extrusions and albuminous yolk-formation were carried out with ovaries dealt with by the following methods: fixed in Bouin's picro-formol and stained in iron haematoxylin; fixed in Flemming (Gatenby's modification without acetic acid) and stained in iron haematoxylin and in Auerbach's stain; fixed in corrosive acetic fixative and subsequently stained in Mann's methyl-blue eosin.

The ovaries dealt with according to Feulgen's technique were dissected out in saline solution and fixed in corrosive acetic fixative.

In all cases sections were cut 5μ in thickness.

III. Observations.

1. Golgi vacuoles and fatty yolk-formation.

The Golgi elements of the youngest oocytes towards the proximal end of the ovarioles were revealed by osmic methods; in Mann-Kopsch and Kolatschev preparations they appear as small dark spherical bodies situated chiefly in the vicinity of the nucleus (fig. 4, Pl. 12). With the growth of the oocyte these bodies spread through the ooplasm towards the periphery of the cell, and, at the same time, increase in size (fig. 5, Pl. 12). Later they become evenly distributed, and, in the eggs at the posterior end of the ovarioles, store up fat in their interior, increase greatly in size and become transformed into the fatty yolk-spheres (fig. 7, Pl. 12).

In ovaries fixed in 2 per cent. osmic acid the dark spheres

described above are clearly shown to consist of an osmophilic rim and a central clear substance (fig. 8, Pl. 12). In neutral red preparations clear vacuoles occupy similar positions to the dark spheres of the Mann-Kopsch and Kolatschev material, and to the dark rings shown by fixation in 2 per cent. osmic acid. Furthermore, the clear vacuoles shown in oocytes treated with neutral red are seen to develop dark rims if a few drops of osmic acid are introduced under the cover-slip and the preparation examined at intervals. Hence it is evident that these bodies are Golgi vacuoles with an osmophilic rim, similar to those previously described as occurring in the oocytes of certain other invertebrates.

The Golgi vacuoles do not stain with neutral red but remain as clear vesicles easily distinguishable from the surrounding ooplasm. At the time of their great increase in size, prior to their conversion into yolk-spheres, they are much more rapidly darkened by immersion in 2 per cent. osmic acid than are those of the earlier oocytes. This shows clearly that at a certain stage there is a great increase in the amount of fat within the vesicles, and that the fatty yolk-spheres are formed by the deposition of fat within the original Golgi vacuoles.

2. Golgi vacuoles in follicle-cells.

Golgi vacuoles were revealed in the follicle-cells of Kolatschev, neutral red and 2 per cent. osmic preparations. They are small and are situated chiefly in the vicinity of the nucleus, although many occur scattered through the ooplasm (figs. 4 and 5, Pl. 12). They do not stain with neutral red and are not rapidly darkened by immersion in 2 per cent. osmic acid.

3. Nucleolar extrusions and albuminous yolk-formation.

The nucleoli of the earliest oocytes are spherical in shape, and, as revealed by Mann's methyl-blue eosin, are faintly basophil; or consist of a central basophil portion with a slightly oxyphil margin; at this stage they may contain small vacuoles (fig. 1, Pl. 12). While the oocytes are still small and situated towards the anterior end of the ovarioles the nucleoli become irregular in

outline, are non-vacuolated and are strongly basophil in staining reaction. That the nucleoli of the older oocytes are basophil was confirmed by examination of ovaries fixed in Flemming's fixative (Gatenby's modification) and subsequently stained in Auerbach's stain. In this material the nucleoli were stained by the basic dye, but it was noted that their reaction was not so strongly basophil as in Mann's methyl-blue eosin preparations.

Soon afterwards small basophil nucleolar buds are liberated; these pass out into the nucleoplasm and towards the nuclear membrane (fig. 9, Pl. 13). Small slightly basophil bodies were observed in the ooplasm; these, although more faintly staining, closely resembled the nucleolar buds within the nucleus.

An examination of Bouin-fixed material stained in iron haematoxylin revealed the nucleolar emissions as small darkly-stained bodies situated in the nucleoplasm. Numerous small bodies, closely similar in size and staining properties, occurred scattered through the ooplasm (fig. 2, Pl. 12).

In a certain few slightly older oocytes the nucleolus appeared to be more active than in the earlier cells. In these cases the nucleolus was irregular in outline and more faintly basophil than in the other oocytes; it contained, however, several deeply basophil granules. The latter, in all probability, are buds which have not yet been liberated (fig. 10, Pl. 13).

In the older oocytes the first type of extrusion is no longer given off from the nucleolus, and, at the same time, the latter has become more spherical in outline and is vacuolated. This stage is marked by the appearance of a second type of basophil emission which has origin in the vacuoles of the nucleolus (fig. 3, Pl. 12; figs. 12 and 13, Pl. 13). These bodies pass towards the nuclear membrane and migrate to the ooplasm. They are but slightly basophil after liberation from the nucleolus and in the ooplasm become oxyphil. After extrusion they could only be distinguished with difficulty in Mann's methyl-blue eosin preparations, but in material treated with Auerbach's stain they were revealed as oxyphil bodies. It was found, however, that for the study of the nucleolar emissions ovaries stained in iron haematoxylin were the most satisfactory.

The first type of extrusion seems to disappear before the

nucleolus becomes vacuolated. This point, however, is difficult to determine with certainty, for although in many cases the ooplasm appears to become free of such bodies, in others the nucleolus develops vacuoles at an earlier stage than is usual. In a certain few young oocytes the nucleolus appeared to be breaking up to form several bodies, and, in one instance, one of the resulting masses contained small vacuoles (fig. 11, Pl. 13). It seems probable that this vacuolated portion would form the nucleolus of the later oocyte, the other parts of the original nucleolus being broken up and liberated as extrusions to the ooplasm. It would appear that the condition described above is not normal, as, in the majority of oocytes examined, the nucleoli remained whole although giving rise to numerous buds.

Several of the buds within the nucleus are vacuolated in a similar manner to the nucleolus, while many others appear to be homogenous (fig. 3, Pl. 12; fig. 13, Pl. 13). After extrusion to the ooplasm they pass towards the periphery of the oocyte, increase in size and become highly vacuolated. Later they are evenly distributed through the egg and undergo fragmentation to form small deeply-stained homogenous granules (figs. 14 and 15, Pl. 13).

The next stage is clearly demonstrated by Kolatschev material stained in acid fuchsin. The ooplasm is seen to contain a number of vesicles or globules which consist of a clear non-staining substance. These globules are apparently formed from the nucleolar extrusions which have undergone a chemical change; the presence of small dark granules within the clear substance of the former producing evidence in favour of this supposition (fig. 16, Pl. 13). The vacuoles make their appearance at the periphery of the oocytes but rapidly spread through the ooplasm. As they increase in size their staining properties change, for they now stain with acid fuchsin, and rapidly form the albuminous yolk-spheres (fig. 17, Pl. 13).

From the above account it will be seen that the first type of oocyte nucleolar extrusion disappears at an early stage. These extrusions are, in all probability, dissolved in the ooplasm, their substance, at a later stage, contributing in some way towards yolk-formation. Those of the second type appear later; they

become vacuolated and undergo fragmentation to form small dark granules which are dissolved, their substance forming clear vesicles which increase greatly in size and form the albuminous yolk-globules.

4. Feulgen's 'Nuclealreaktion'.

In material treated according to Feulgen's technique (**5**) chromatin was not observed in the nuclei of the oocytes, nor did the nucleolus or nucleolar extrusions contain any substance which gave the chromatin reaction. The preparations, however, did not show oogonia, consequently the present observations were confined to the growing oocytes.

The chromatin of the follicle-cells (as revealed by Feulgen's method) is in the form of granules scattered through the nuclei. Many of the granules are connected by threads which gave the chromatin reaction but were more faintly stained than the chromatin granules (fig. 6, Pl. 12).

In the follicular epithelial-cells chromatin granules were observed scattered through the nuclei (fig. 6, Pl. 12).

5. Bacteria.

The presence of bacteroid forms within the oocytes was noted. These bodies are numerous and occur at the periphery of the eggs. Similar bodies have been noted by Nath and Piare Mohan (**23**) in Periplaneta americana and more recently have been isolated and cultivated by Glaser (**7**). The latter worker states that the penetration of the egg by bacteria occurs after oviposition; the present writer, however, believes that in Periplaneta orientalis the bacteria are present in the older oocytes. However, this matter is beyond the scope of this paper.

IV. Discussion.

It is evident from the above account that the behaviour of the oocyte Golgi vacuoles or vesicles of Periplaneta orientalis is similar to that described in the related species, Periplaneta americana, by Nath and Piare Mohan (**23**). In both species the fatty yolk-globules are formed by the deposition and accumulation of fat inside the original Golgi vesicles of the young

oocytes. Consequently, further evidence is furnished in favour of the view that the fatty yolk of certain invertebrates is formed by the transformation of the original Golgi vacuoles into yolk-spheres.

Nath (**19**) has demonstrated very clearly that the Golgi vacuoles in the eggs of Culex consist of 'an osmiophilic or argentophilic rim and an osmiophobic or argentophobic central area'. More recently the same has been shown to be true for the Golgi vacuoles observed by the writer (**9**) in the oocytes of certain Tenthredinids, by Nath and Piare Mohan (op. cit.) for Periplaneta americana, and by Nath for the earthworm, Pheretima (**20**). The present investigations show that the Golgi vacuoles of Periplaneta orientalis are similar in form to those of the above-mentioned types.

The work of Covell and Scott (**3**) on smear preparations of ventral horn and spinal ganglion cells of mice and young rabbits, stained with neutral red and subsequently treated by osmic and silver methods, is worthy of note. These workers claim to have seen the actual process of osmication and silver impregnation of granules previously stained with neutral red.

During the investigations recorded here 2 per cent. osmic acid was added to neutral red preparations of Periplaneta ovarioles, and subsequent examination at intervals showed that the Golgi vacuoles developed a dark rim. This demonstrated clearly that the rim of the Golgi vacuoles is osmophilic.

Gatenby (**6**) in a paper reviewing the work of Hirschler, Monné, Voinov, and others, shows that during the spermatogenesis of certain forms, the vacuoles become separated from the Golgi elements. Gatenby demonstrates that a similar condition exists in the male cells of Cavia, Helix, and Abraxas, and points out that 'the vacuole is not the Golgi apparatus, but the associate or derivative of the Golgi cortex'. He believes 'that in such examples of oogenesis as that of Daphnia, the Golgi element is a cortex on the vacuole'. In a recent paper on saw-fly oogenesis the present writer (**9**) supports Gatenby's view; furthermore, the findings of Nath and his co-workers and the present observations on the Golgi vacuoles of Periplaneta orientalis demonstrate clearly that during oogenesis the

Golgi cortex remains in contact with the vacuole. Hence it is evident that in the male the Golgi apparatus and vacuolar system may become separated in some way, while during oogenesis (of invertebrates at least) the Golgi cortex and vacuole remain in association.

That the Golgi bodies and vacuoles may become separated during vertebrate oogenesis is suggested by a recent note by Bhattachyra and Das (**2**). Small pieces of ovary from a very young pigeon, treated with neutral red, revealed the Golgi elements as spherical bodies with a clear core, and also as crescent-shaped structures. Similar bodies occupied the same position in material fixed by silver or osmic methods. The neutral red material contained 'prominent and highly refractive bodies each surrounded by two or three crescents of the nature of Golgi bodies'. These were identified as 'Golgi yolk'. Gatenby's 'vacuole' or Parat's 'vacuome' occurred as groups of vesicles, stainable with neutral red, dispersed between the Golgi elements. As the vesicles are not osmophil these workers conclude that they are 'something totally different from the Golgi bodies'.

The above account indicates that during pigeon oogenesis Golgi body and vacuole are separate as in spermatogenesis. If this be true the condition appears to be very different from that described by Hibbard for the vertebrates, Pygosteus (**12**) and Discoglossus (**13**). Until Bhattachyra and Das publish a full account of their findings no useful purpose can be served by further discussion of their claim.

The Golgi vacuoles as described by Nath and Piare Mohan for Periplaneta americana are slightly tinged by neutral red. In this they differ from the clear non-stainable vacuoles previously recorded by Nath (**18**) for the spider, Crossopriza, by Nath and Husian (**21**) for the Chilopod, Otostigmus, by Nath and Mehta (**22**) for the fire-fly, Luciola, by Nath (**19**) for Culex and the earthworm, Pheretima (**20**), by the present writer for Tenthredinids (**9**), and in the present contribution for Periplaneta orientalis. It seems probable, however, that this is of no great significance as the Golgi vacuoles of Periplaneta americana are but faintly tinged.

In a previous contribution (**9**) the writer drew attention to the fact that the Golgi vacuoles, which give rise to albuminous yolk in the vertebrates, Pygosteus (**12**) and Discoglossus (**13**), are stainable with neutral red until a late stage of oogenesis. Whereas those described by Nath and his co-workers and by the present writer, as giving origin to fatty yolk in certain invertebrates, do not stain but remain as clear vacuoles. Hence there is a chemical difference between the two types of Golgi vesicles even at an early stage of oogenesis. The above-mentioned facts suggest that the Golgi vacuoles in the early cells of the vertebrate ovary are similar to those described for male and other tissues, the chemical change which precedes their conversion into albuminous yolk-globules not taking place until late in the growth stage. On the other hand the Golgi vacuoles of the invertebrate egg are chemically different (as revealed by neutral red) in the earliest cells in which they have been observed.

Nath in his recent paper on Pheretima (where the Golgi vesicles contain fat) points out that in the amphibian, Discoglossus, and the invertebrate, Carcinus (where the Golgi elements are stated to give origin to albuminous yolk), the fatty-yolk is said to arise independently in the cytoplasm, and further that Hibbard (**12** and **13**) and Harvey (**11**) 'are dealing with "fat globules" and "fat droplets" respectively, while I am dealing with a vesicle having a definite membrane containing fat'.

The Golgi vacuoles of the follicle-cells are not so rapidly darkened in osmic acid as those of the oocytes. This agrees with Nath and Piare Mohan's findings for the Golgi vacuoles of the follicular epithelial-cells of Periplaneta americana. It should be noted that the cells termed 'follicular epithelium' by Nath and Piare Mohan are called follicle-cells in the present contribution.

The nucleoli of the oogonia of Periplaneta americana, Hogben states (**14**), are plasmosomes. In the oocytes, according to 'methylene blue eosin staining the plasmosome and nucleolar particles' are basophil after acid fixation, but are acidophil after fixation in Flemming (Gatenby's modification).

In Periplaneta orientalis all the preparations did not

reveal oogonia. In the earliest oocytes seen in Mann's methyl-blue eosin material the nucleoli are faintly basophil or consist of a basophil part surrounded by a slightly oxyphil margin; later, they become deeply basophil, as indicated by Mann's methyl-blue eosin, and by ovaries fixed in Flemming (Gatenby's modification) and subsequently stained in Auerbach's fuchsin methyl-green.

From the above account it would appear that the nucleoli of the oogonia and early oocytes of Periplaneta orientalis are amphiphil, or else that the nucleoli of the oogonia are oxyphil and change later as a whole from oxyphil to basophil in a manner somewhat similar to that recently described by the writer (**8**) for the oocyte nucleoli of a certain Tenthredinid.

In Periplaneta orientalis the nucleoli of many of the early oocytes were observed to contain small vacuoles; these disappear and are not connected in any way with the marked vacuolated condition observed in the older oocytes. This primary vacuolation has not been recorded for Periplaneta americana.

The occurrence of two types of nucleolar extrusions in Periplaneta orientalis agrees with Hogben's and with Nath and Piare Mohan's observations on the oocyte nucleolus of Periplaneta americana. It would seem, however, that in the former species the nucleolus undergoes a period of more marked activity during the liberation of the first kind of nucleolar emission. This is clearly demonstrated by the manner in which certain of the nucleoli were observed to break up into several bodies. Moreover, in one instance, one of these resulting masses was vacuolated before the cessation of the first type of extrusion. This latter condition has not been recorded for Periplaneta americana.

The writer's findings for the second type of nucleolar extrusion agree closely with those of Nath and Piare Mohan for Periplaneta americana. Many of the emissions within the nuclear membrane were observed to be vacuolated while the remainder appeared to become so on extrusion to the ooplasm. Later they break up into homogenous granules which give rise to the albuminous yolk-globules. The latter, in Kolatschev

preparations stained with acid fuchsin, at first resemble clear vesicles but rapidly undergo a change in staining properties and are stained by acid fuchsin. This last-mentioned phenomenon is not recorded by Nath and Piare Mohan for Periplaneta americana.

Koch (**15**) has shown that chromatin as revealed by Feulgen's technique, although present in the oogonia of Chilopods, is absent from the nuclei of the oocytes. He concludes that during oogenesis the chromatin undergoes a profound chemical change. In Limnaea stagnalis Ludford (**16**) finds that scarcely any chromatin is distinguishable in the oocytes. He is 'inclined to believe that the chromatin is so finely dispersed in the nucleus as to render its detection' by Feulgen's method impossible. It may be, he states, as Koch has suggested, that the chromatin of the oocytes undergoes profound chemical changes. Ludford could not detect chromatin in the oxyphil or basophil nucleoli. In the oocyte of the mouse (Ludford, **16**) small granules of chromatin are present; the nucleolus, however, did not give the chromatin reaction. The present writer in a recent contribution to Tenthredinid oogenesis (**10**) has shown that the early oocytes of Allantus pallipes contain chromatin which disappears in the older cells. In the more highly developed ovarioles of Thrinax mixta, chromatin, although present in the nurse-cells and follicle-cells, was not detected in the oocytes. In neither species is chromatin extruded from the nuclei of the oocytes, nurse-cells, and follicle-cells. The writer pointed out that the above-mentioned observations appear to support Koch's view.

Since the findings on the chromatin of the saw-fly oocytes were sent for publication a paper on Apanteles by Mukerji (**17**) has appeared. Mukerji finds that the secondary nuclei and germ-cell determinant of the egg of Apanteles give a negative reaction with Feulgen's technique, and thus fail to reveal any relationship with the chromatin of the nucleus. He states that the nuclei of the early oocytes contain a few grains of chromatin, but none is present at a later stage. Before maturation the chromosomes stain purple.

The presence of chromatin granules in the young oocytes, its disappearance from the older cells, and the absence of chromatin

emissions to the ooplasm, agree with the present writer's findings for saw-flies. Mukerji states that 'it is perhaps safe to say' that the nucleolar extrusions of such forms as Saccocirrus, Patella, and Limnaea will be found to give a negative reaction with Feulgen's method. This is precisely what the present writer has found to be true in the case of the Tenthredinid nucleolar extrusions, and in the case of Periplaneta orientalis described in the present contribution.

In Periplaneta orientalis chromatin was not detected in the oocytes examined; its absence would seem to support Koch's view that during the growth of the oocytes the chromatin undergoes chemical changes. The absence of chromatin from the nucleolus and nucleolar extrusions agrees with the findings of Ludford for the mouse and Limnaea stagnalis, Mukerji for Apanteles, and the writer for Tenthredinids.

Faulkner (**4**) in a recent paper doubts the accuracy of Feulgen's method. This author states that during the early growth phases of the oocytes of the Coelenterate, Obelia geniculata, as revealed by observations on the living oocyte, the nucleolus elongates and fragments. 'Each fragment has been identified as a pair of homologous chromosomes indistinguishably united; later each of the bivalent elements divides in half, and the individual chromosomes are thus separated.'

Attempts to stain the nucleolar fragments by means of Feulgen's 'Nuclealreaktion' proved unsuccessful. Faulkner, however, does not consider this negative evidence of much significance as Bělař (**1**) says it is doubtful whether the test is specific for all nuclear phases, and Harvey (**11**) finds that in the oocytes of Carcinus moenas the chromosomes do not show the reaction after the 'bouquet' stage.

It seems that Bělař's doubt regarding the accuracy of Feulgen's technique for all nuclear phases refers to the diffuse stage, as described by Koch (**15**), Ludford (**16**), and the present writer (**10**). Bělař considers Feulgen's method more trustworthy than any of the former so-called chromatin reactions.

In Carcinus (**11**) the chromatin is stained up to and including the 'bouquet' stage, but after becoming diffuse it no longer gives the reaction. Clearly Harvey's observations do not support

the view that chromosomes or chromatin granules may not give a positive reaction. Furthermore, Mukerji has recently shown that the chromatin granules of the early oocytes and the chromosomes before maturation give the correct reaction. It is worthy of note that the writer has obtained a positive reaction with chromosomes in the spermatocytes of a certain Tenthredinid.

V. Summary and Conclusions.

1. The Golgi vacuoles and fatty yolk-formation in Periplaneta orientalis were studied by means of Mann-Kopsch, Kolatschev, 2 per cent. osmic acid and neutral red preparations.

2. The Golgi vacuoles of the young oocytes are situated in the vicinity of the nucleus; later they pass to the periphery of the cell. In the older oocytes, towards the posterior end of the ovarioles, they become evenly distributed in the ooplasm, store up fat, increase greatly in size, and give rise to the fatty yolk-spheres. In the older oocytes they darken much more rapidly in 2 per cent. osmic acid.

3. In neutral red preparations clear non-stained vacuoles are seen to occupy similar positions to those of the dark bodies of the osmic preparations; on introducing a few drops of 2 per cent. osmic acid under the cover slip the vacuoles develop an osmophilic rim. These Golgi vacuoles are not stained by neutral red.

4. In 2 per cent. osmic acid preparations the Golgi vacuoles are seen to consist of an osmophilic rim and a central clear substance.

5. The Golgi vacuoles of the follicle-cells are similar to those of the egg, except that they do not increase greatly in size and are not so rapidly darkened in 2 per cent. osmic acid.

6. The nucleoli of the early oocytes are spherical in shape and are amphiphil or slightly basophil in staining reaction; they may contain small vacuoles. In slightly older oocytes the nucleoli are non-vacuolated; they become strongly basophil, irregular in outline, and, at the same time, give rise to emissions which pass through the nuclear membrane to the ooplasm, where they ultimately disappear. In a certain few oocytes the nucleolus

was seen to have broken up into several masses, some of the latter, in all probability, fragmenting to form nucleolar extrusions. In a certain oocyte one of the masses was observed to be vacuolated before the first type of extrusion had ceased.

7. In the more highly developed oocytes the first type of nucleolar emission ceases, and the nucleolus becomes more spherical in outline. Numerous vacuoles appear which give origin to nucleolar extrusions. The latter become vacuolated, either before extrusion through the nuclear membrane, or later in the ooplasm.

8. The second type of nucleolar extrusions pass to the periphery of the egg. Later they become evenly distributed in the ooplasm, where they fragment to form homogeneous granules. The latter form clear spheres (Kolatschev material) which rapidly increase in size to form the albuminous yolk-globules.

9. Chromatin was not observed in the oocyte nuclei, nucleoli, or nucleolar extrusions (Feulgen's technique). The chromatin of the follicle-cells is in the form of granules connected by threads (which give the chromatin reaction). The chromatin of the follicular epithelial-cells was observed as granules scattered through the nuclei.

10. Bacteroid forms were observed in the ooplasm at the periphery of the older oocytes.

11. The method of yolk-formation is similar to that of *Periplaneta americana* as described by Nath and Piare Mohan.

12. The writer's conclusions regarding the shape and character of the Golgi vacuoles agree with the findings of Nath and his co-workers and with the former conclusions of the present writer for oocyte Golgi vacuoles.

VI. Acknowledgements.

The work recorded in this paper and in a series on the Tenthredinidae (Gresson, **8, 9,** and **10**) has been prosecuted under Professor A. D. Peacock in the Zoology Laboratory of University College, Dundee, and has been assisted by a grant from the Royal Society of London. I have pleasure, therefore, in giving acknowledgements and thanks for these facilities.

Addendum.

Since this paper was completed Miss M. 'OBrien and Professor J. Brontë Gatenby have published a note on the oogenesis of Lumbricus (Nature, vol. 125, p. 891, 1930). These workers show that in the oocytes of Lumbricus, globules, which stain with neutral red, are present; these globules, however, are not related to the Golgi elements. They state that 'it does not seem possible entirely to dismiss the idea that these globules might be segregation vacuoles and not pre-existing structures'. They succeeded in staining a 'vacuome' in coelomic epithelial cells and in nerve-cells.

The present writer has recently carried out further examinations of Periplaneta ovarioles stained in neutral red. In no case were vacuoles or granules stainable by neutral red detected, although the same sample of neutral red stained the vacuolar system of male germ-cells and oocytes of Helix.

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EXPLANATION OF PLATES 12 AND 13.

Lettering.

Ay, albuminous yolk; *Ay*^{1}, clear spheres formed from nucleolar extrusions; *cgr*, chromatin granules; *Fc*, follicle-cell; *Fe*, follicular epithelium; *Fy*, fatty yolk; *G V*, Golgi vacuole; *Nu*, nucleolus; *Nue*, nucleolar extrusion; *Nuf*, nucleolar extrusion undergoing fragmentation; *oo*, ooplasm.

Plate 12.

Fig. 1.—Early oocyte nucleus at anterior end of ovariole; nucleolus contains a few small vacuoles. Mann's methyl-blue eosin.

Fig. 2.—Young oocyte. Nucleolar emissions in nucleus and in the ooplasm. Iron haematoxylin.

Fig. 3.—Nucleus of older oocyte. *Nucleolus vacuolated;* numerous nucleolar emissions in nucleus and in ooplasm. Iron haematoxylin.

Fig. 4.—Young oocyte. Golgi vacuoles in oocyte and follicle-cells. Kolatschev.

Fig. 5.—Older oocyte. Golgi vacuoles have increased in size and are situated towards the periphery of the egg. Kolatschev.

Fig. 6.—Follicle-cells and follicular epithelium. Chromatin granules in follicle-cell nuclei; many of the granules are connected by threads. Chromatin granules in follicular epithelial cell nuclei. Feulgen's technique.

Fig. 7.—Golgi vacuoles, fatty and albuminous yolk-spheres. Mann-Kopsch.

Fig. 8.—Golgi vacuoles showing osmophilic rim. 2 per cent. osmic acid.

Plate 13.

Fig. 9.—Showing nucleolus and first type of nucleolar extrusion. Mann's methyl-blue eosin.

Fig. 10.—Nucleolus appears to be breaking up into numerous buds. Mann's methyl-blue eosin.

Fig. 11.—Nucleolus breaking up; one of the resulting masses contains small vacuoles. Mann's methyl-blue eosin.

Fig. 12.—Older oocyte showing vacuolated nucleolus and second type of nucleolar extrusion. Mann's methyl-blue eosin.

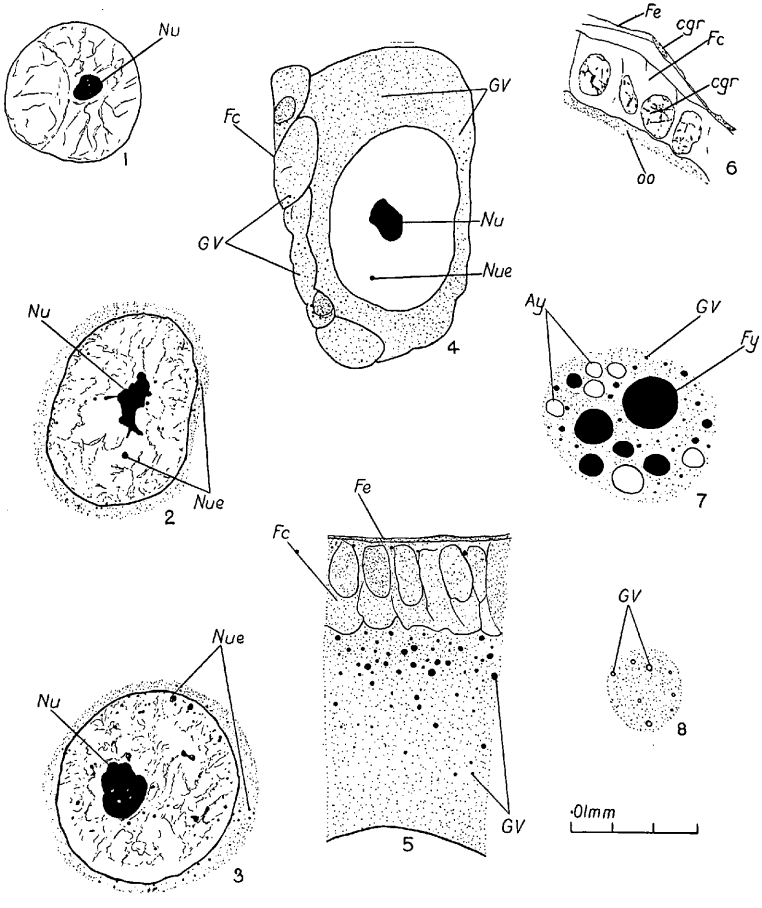
Fig. 13.—Showing vacuolated nucleolus and vacuolated nucleolar extrusions in the nucleoplasm. Mann's methyl-blue eosin.

Fig. 14.—Showing vacuolated nucleolar extrusions in the ooplasm. Iron haematoxylin.

Fig. 15.—Nucleolar extrusions undergoing fragmentation in the ooplasm. Iron haematoxylin.

Fig. 16.—Clear spheres formed from nucleolar extrusions. Kolatschev.

Fig. 17.—Showing Golgi vacuoles and albuminous yolk-globules. Kolatschev.



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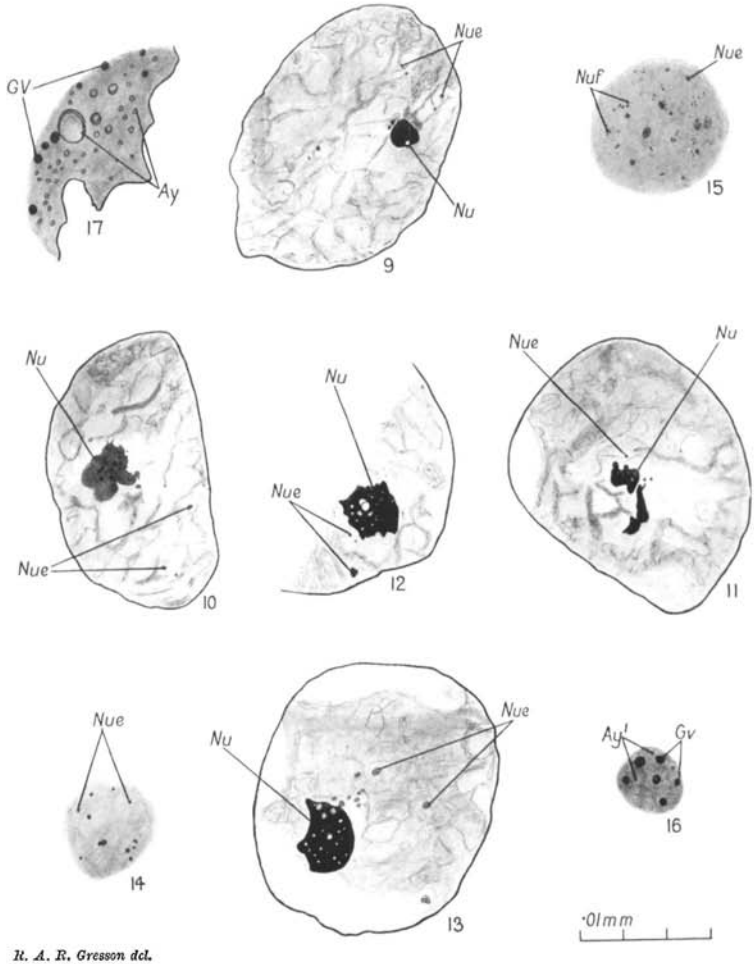

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