Egg-Follicle of Culex.

By

Vishwa Nath, M.Sc.,

of the Mohindra College, Patiala, India, and Trinity Hall, Cambridge.
(From the Zoological Laboratory, Cambridge.)

With Plates 7 and 8.

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1. INTRODUCTION.

In 1900 Lecaillon (14) gave a very brief and meagre account of the general structure and development of the ovary of Culex pipiens, and from a study of his paper it appears that he examined the ovaries mostly in fresh material.

In 1901 Professor N. Kulagin of Moscow (13) made an attempt
to work out the development of the egg-follicle in Culex pipiens and Anopheles bifurcatus, but he stopped at the synizesis stage of the oocyte nucleus, apparently on account of the great difficulties in section cutting caused by the presence of brittle yolk-granules and very hard chorion which develop in the older oocytes.

In 1911 S. R. Christophers (5) described briefly the development of the egg-follicle in Anophelines.

In 1921 A. J. Nicholson (19) took up the work of Kulagin and Christophers (although Nicholson does not make any reference to Kulagin’s work) and completed the development of the egg-follicle in Anopheles maculipennis.

In 1914 and 1916 Monica Taylor (25 and 26) worked on the somatic, spermatogonial and oogonial mitoses of Culex pipiens, but to quote her own words, ‘the ultimate fate of egg and nurse-cells has still to be worked out’.

In the present paper an attempt has been made to work out in detail the development of the egg-follicle in Culex fatigans, the common species of Culex in Patiala, till the laying of the eggs, with particular reference to (1) the behaviour of the oocyte nucleus, (2) the supposed connexion between the germinal vesicle and the nutrition of the oocyte, (3) the structure and development of the egg-membranes, (4) the micropylar apparatus, and (5) the striated collar attached to the anterior end of the laid egg.

I must take this opportunity to express my deep indebtedness to that munificent Prince of India, His Highness the Maharaja Dhiraj Mohinder Bahadur of Patiala, who, at my request, provided facilities for research in the Mohindra College. To Lieut.-Colonel S. R. Christophers, I.M.S., I am particularly grateful, not only for his suggesting to me this line of research, but for his extreme kindness and ever-ready help in this work. My thanks are also due to Lieut.-Colonel W. F. Harvey, I.M.S., the Director of the Central Research Institute, Kasauli, for allowing me unstinted use of reagents in his laboratory during the summer. I am also indebted to Mr. J. Gray, M.A., for finding time to read and correct the
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2. Technique.

A satisfactory cytological study of mosquito ovaries is rendered difficult for the following reasons:

Firstly, the anterior ends of the follicular tubes are very small, and it is here that the oogonial mitoses and the differentiation of the oogonia into follicle cells take place. To study these processes in detail very thin sections must be cut, but this is a task which even with the best available method I found quite impossible to perform; for lower down in the follicular tubes there are oocytes in various stages of development with brittle yolk and very hard chorion, both of which are serious impediments in the way of cutting very thin sections. Nicholson (19) experienced a similar difficulty in Anopheles maculipennis.

Brittle yolk and hard chorion are great impediments in the study of both the germinal vesicle and the post-germinal vesicle stages of the oocyte nucleus. The usual paraffin method is not at all satisfactory, but I obtained satisfactory sections, however, by the paraffin-celloidin method.

For younger follicles Bliss (acetic acid, alcohol, and formaldehyde), Flemming, and Bouin proved the most satisfactory fixatives. Gilson was found most valuable when the oocytes had developed hard chorion.

Heidenhain's iron haematoxylin was used as the principal stain. Since it also stains the yolk-granules deeply, it would appear to be unsatisfactory for differentiating the nucleus from yolk. But at the commencement of yolk-formation the nucleus passes into the germinal vesicle stage in which chromatin is present in the form of very fine granules which do not stain readily. Eosin was used as a counter-stain in many cases.

For the study of younger follicles the best results were obtained when the ovaries were dissected out of the abdomen and fixed. But when the eggs have developed chorion and yolk
it is better to fix the whole abdomen. The advantage of this is that the eggs, being held in position by the other viscera and the wall of the abdomen, undergo much less distortion in cutting than if they are taken out and fixed. Much useful study was made in fresh material in normal salt solution and also in whole mounts.

3. **Female Genital Organs in the Adult Mosquito.**

There are two ovaries, oval in form, situated ventrolaterally in the hinder region of the abdomen. Posteriorly each ovary is continued into an oviduct. The two oviducts unite to form a common oviduct which opens at the genital opening on the eighth segment. There are three brownish pear-shaped spermathecae opening into the common oviduct. When the ovary is dissected out of the abdomen and studied in normal salt solution, the outer surface of each spermatheca is seen to be covered by bristles, each bristle arising from a pore. In sections each spermatheca is seen to be lined by a thick chitinous layer and covered on the outside by large cells which do not take much haematoxylin stain.

Each oviduct is lined by a single layer of columnar cells and covered on the outside by a fibrous sheath on which a number of nuclei are seen. The common oviduct has got a bigger lumen and the columnar cells lining it are also much bigger.

Each ovary (fig. 1, Pl. 7) is invested by a thin limiting membrane which is continued anteriorly into a suspensory ligament fixed to the inner side of the wall of the abdomen. The limiting membrane consists of a thin membrane with nuclei distributed at intervals on its inner face. The lumen of each ovary is lined by a similar membrane with nuclei. Between the limiting membrane and the lining of the lumen is a number of follicular tubes. The wall of each follicular tube is made of a membrane similar to the limiting membrane with this difference, that its nuclei are distributed on its outer face. In *Anopheles maculipennis* Nicholson describes a number of striped muscle-fibres in the ovary. These join the follicular tube-membranes with each other on the one hand, and the limiting
membranes and the follicular tube-membranes on the other. In *Culex fatigans* I have not observed any striped muscles. It is true that there is a number of fibres with nuclei, but they appear to be the cut follicular tube-membranes. It is possible that they are non-striped muscle-fibres like those of most insect ovaries (see Gross, 9).

When the living ovary is studied in normal salt solution a large number of dark tubes are seen entering the ovary through the limiting membrane. These are the tracheae. Inside the ovary they branch profusely into finer and finer branches. It is easy to see, in the larger branches at any rate, the usual spiral lining on the inside of the tracheal wall which also shows some local thickenings.

Inside each follicular tube is a row of egg-follicles. Each egg-follicle (figs. 1 and 2, Pl. 7) consists of a number of cells surrounded by an epithelium—the follicular epithelium which is surrounded by a thin structureless basement membrane. In young follicles it is impossible to make out the cell boundaries of the follicular epithelium. Immediately after the mosquito emerges from the pupa all the cells of a follicle are similar to each other in structure. Each has a prominent nucleolus embedded in a lightly staining matrix of linin fibres. At this stage the nucleolus stains deeply with haematoxylin but does not stain with eosin. As will appear later, the nucleolus, however, is an amphinucleolus, being made of a ground substance of plastin impregnated with some basophil substance. The cytoplasm of each follicle cell takes a deep haematoxylin stain. In the anterior region of each follicular tube is a number of cells. These are the oogonia which are gradually differentiated into the follicle cells as the eggs lower down in the follicular tubes become mature and are laid.


Can eggs develop by artificial food? This is a question which has engaged the attention of many researchers and, to the best of my knowledge, the conclusions arrived at are that feeding
on blood is essential for the development of eggs in mosquitoes. S. K. Sen (22), however, succeeded in inducing Stegomya scutellaris to oviposit by feeding the mosquitoes on artificial food in the form of peptone sweetened with cane-sugar or milk. I fed a large number of imagines of Culex fatigans on milk, peptone sweetened with cane-sugar and also fruits, but I found that eggs do not develop. My observations, however, show that, although the eggs do not develop completely on artificial food, development of the egg-follicle does proceed to a certain extent.

It will be remembered that immediately after emergence from the pupa the nuclei of all the cells of a follicle are similar with a lightly staining linin network and a prominent nucleolus (fig. 2, Pl. 7). But when the imagines are fed on artificial food the little development of the egg-follicle that takes place is absolutely the same as during the first few hours after the mosquitoes have fed on blood.

Fig. 20, Pl. 8, shows a follicle of an eight hours old imago fed on artificial food. The nucleolus which was lying in the centre of the linin network in the follicle of a newly emerged imago (fig. 2, Pl. 7) is here lying on one side of the contracted network. This is the synizesis stage. It is interesting to note that all the cells of a follicle are in this stage, and as yet there is no differentiation between the oocyte and the nurse-cells. In fig. 21, Pl. 8, is shown a follicle of a ninety-six hours old mosquito fed on artificial food. The nucleus of one cell, the oocyte, is in the synizesis stage, whereas in the nuclei of the other cells, the nurse-cells, the chromatin is more or less uniformly distributed. The amount of chromatin in the nucleus of a nurse-cell of an imago fed on artificial food is much less, however, than when the mosquito has fed on blood. The cells of the follicular epithelium also have grown bigger and their nuclei have become more prominent. Consequently the follicle as a whole has grown larger. The follicle does not develop further on artificial food.

In the literature I have studied I have not discovered any instance (except of course Stegomya scutellaris, in
which the eggs develop completely on artificial food) in which the egg-follicles develop up to the synizesis stage on artificial food.

In other words the development of the oocyte of an artificially fed imago stops short of the germinal vesicle stage, when deposition of yolk and secretion of membranes is active and for which blood is necessary. The nurse-cells, however, as might be expected, behave in the same way as when the mosquitoes are fed on blood.

During the examination of the ovaries of confined females I also observed that as a rule copulation does not take place in captivity. In two cases, however, I observed that the spermatheceae of confined imagines contained a large number of sperms.

5. The Structure of the Laid Egg.

The egg, when laid (fig. 17, Pl. 8), is elongated in shape with a broad anterior and a narrow posterior end. It is surrounded by two walls. The outer wall is the chorion and the inner wall is the vitelline membrane. The chorion consists of a large number of hard processes situated between an outer thin membrane, the basement membrane of the follicular epithelium and a similar inner membrane which appears in the course of development on the inside of the follicular epithelium (fig. 14, Pl. 7, and fig. 16, Pl. 8). The chorion at the broad anterior end of the egg is different in structure from the rest. Its processes are much thicker and radiate outwards from the micropylar apparatus, which is situated at the middle of the broad end of the oocyte in the form of about thirty-five ridges (fig. 18, Pl. 8).

The micropylar apparatus (figs. 12, 13, Pl. 7, and fig. 16, Pl. 8) consists of a broad supporting ring whose outer margin is thin and wavy and whose inner margin is very thick and stains deeply. The prominent ridges of the chorion at the anterior end of the egg radiate outwards from the outer margin of the supporting ring. The inner thick margin of the supporting ring is continued inwards into a very thin disc which does not stain.
Piercing through this disc is a funnel, the micropylar funnel, whose broad end opens into the interior of the egg just outside the vitelline membrane. The cavity of the funnel is the micropyle. The narrow end of the micropylar funnel is continued outwards into a long straight tube running through the degenerating nurse-cells. This is the micropylar tube.

It is interesting to note here the difference between the micropylar apparatus of *Culex fatigans* and that of *Anopheles maculipennis*. In the latter the inner margin of the supporting ring is not so thick as in *Culex fatigans* and is regularly 'scalloped', whereas in *Culex fatigans* it is circular. Also the disc of the micropylar apparatus in *Anopheles maculipennis* has eight ridges radiating outwards from the micropyle to the 'scalloped' inner margin of the supporting ring, thus dividing the disc into eight areas. In *Calliphora erythrocephala* (15) also the micropylar disc is similarly divided into eight areas. In *Culex fatigans* the disc does not have any such ridges and is quite structureless. There is no micropylar tube in *Anopheles*.

In certain zoological text-books a striated collar has been very briefly described in *Culex pipiens*, but its development, function, and detailed structure have, to the best of my knowledge, not been known. I have studied this collar in detail in *Culex fatigans*.

When the egg is laid (fig. 17, Pl. 8) there is a hollow collar attached at its broad anterior end. Its connexion with the egg is very slender, and it is detached from the egg by the slightest pressure. The collar is surrounded on all sides, except at its anterior end, by two membranes, the outer striated membrane which is the basement membrane of the follicular epithelium and an inner membrane which is the cell-membrane of degenerated nurse-cells. The striations of the outer membrane run parallel, except at the base of the collar, where they make a network. The base of this collar is pierced by the micropylar funnel. It has been said that the collar is open at the anterior end. It is at this place that the micropylar tube, running through the collar, opens.
The micropylar tube develops a very short time before fertilization, which takes place in the common oviduct (fig. 12, Pl. 7, and figs. 16 and 19, Pl. 8). It degenerates immediately after the egg is laid and is absent in the laid egg (fig. 17, Pl. 8). It is thus that the tube has escaped notice so long. The micropylar tube becomes distorted by the action of fixatives and is best studied in fresh material in normal salt solution.

When it first develops the anterior end of the micropylar tube is covered by the basement membrane of the follicular epithelium and the cell-membrane of nurse-cell. In other words the collar is not open at the anterior end. But just before fertilization the collar opens at the anterior end, and so exposes the micropylar tube to the sperm.

Discussing the rôle of the micropylar apparatus in Anopheles maculipennis during fertilization, Nicholson gives a rather interesting theory. When the eggs are passing down the common oviduct, they will be pressed very much by its muscular wall. Consequently the supporting ring will press on the disc. The latter will be pulled out somewhat and will lie flush with the supporting ring. But when the greater portion of the egg has passed out at the genital opening the pressure on the disc will be released and it will be once more pulled down. As a result of this the spermatic fluid will be sucked into the micropylar funnel along the grooves of the disc.

The same theory might well be applied to the case of Culex fatigans with this difference, that the sperm will be directed along the grooves on the outer surface of the striated collar and not along the disc, which is quite plain. Besides the sperm will first enter the micropylar tube and, descending down the micropylar funnel, pass into the interior of the egg just outside the vitelline membrane. In Anopheles the sperm will enter the micropylar funnel at once, because there is no micropylar tube.

The vitelline membrane is a transparent, gelatinous membrane on the inside of the chorion (fig. 16, Pl. 8).

The cytoplasm of the egg is granular and contains a large number of densely packed yolk-granules. Each yolk-granule
consists of a central more deeply staining and less deeply staining cortical portion. A little patch of protoplasm at the broad anterior end of the egg is free from yolk. The nucleus of the egg lies in this patch. I have not found it possible to cut sections of the laid eggs showing the nucleus, because the chorion of the laid egg is very hard, much harder than that of an anopheline egg, and even the paraffin-celloidin method proved unsatisfactory. But I have been able to get very good sections of the eggs just before oviposition (fig. 16, Pl. 8). In such eggs the nucleus is a small structure with a very definite membrane and a central, darkly staining mass of chromatin which is not yet defined into chromosomes. In some cases, however, I observed on extraction that the nucleus shows a number of ill-defined, minute, and round chromosomes tightly packed together.

Fig. 16, Pl. 8, shows the nucleus of an oocyte a very short time before oviposition. The nucleus has pushed the vitelline membrane from inside and is ready for the formation of the polar bodies, which process, it is highly probable, takes place immediately after oviposition.

6. The Development of the Egg-follicle.

The description of the development of the egg-follicle can be divided into (a) the behaviour of the oocyte nucleus, (b) the formation of yolk, (c) the relation between the germinal vesicle and the nutrition of the oocyte, (d) the formation of the vitelline membrane, (e) the formation of the chorion, and (f) the development of the micropylar apparatus.

(a) The Behaviour of the Oocyte Nucleus.

In a newly emerged imago the nuclei of all the cells of an egg-follicle are similar, each with a lightly staining reticulum of linin fibres and a prominent amphinucleolus (figs. 1 and 2, Pl. 7). When the mosquito feeds on blood the nucleus of one cell of the egg-follicle, the oocyte, quickly becomes differentiated from the rest. The chromatin threads stain more readily, contract, and, intertwining each other closely, form a some-
what round structure. The nuclei of the other cells of the follicle, the nurse-cells, greatly enlarge so that their nuclear membranes lie near the cell membranes. Their chromatin is distributed in the form of irregular fragments on a fine network of linin fibres. No nucleolus can be seen in the nuclei of the nurse-cells (fig. 3, Pl. 7).

The amphinucleolus of the oocyte nucleus now undergoes a remarkable change. Its plastin portion, in the middle of which is embedded the circular basophil portion (fig. 3, Pl. 7), first becomes ovoid (fig. 4, Pl. 7) and then branches in an irregular fashion (fig. 5, Pl. 7). These branches fill up the whole nucleus and ultimately get detached from the nucleolus, which now consists of the circular basophil portion only (fig. 6, Pl. 7). A number of vacuoles now appear in the basophil nucleolus and its staining properties also change. It no longer stains with haematoxylin but stains readily with eosin. It has a very thin membrane and is granular in structure (fig. 8, Pl. 7).

We know that nucleoli contain proteins and nucleinic acid. It would thus appear that the amount of nucleinic acid in the nucleolus of the oocyte of Culex fatigans immediately after its emergence from the pupa is so great that it will stain with basic dyes only. As the oocyte grows the nucleinic acid seems gradually to leave first the outer portion of the nucleolus, which therefore begins to stain with acid dyes, and ultimately the central portion also. This seems to me to be the right explanation of the changes in the staining capacity of the amphinucleolus of Culex fatigans.

When the amphinucleolus is passing through the above changes the contracted loops of chromatin begin to stain less readily and the round structure formed by the chromatin loops gradually disappears. The chromatin seems to be present at this stage in the form of lightly staining fine granules distributed throughout the oocyte nucleus. A prominent eosin-staining nucleolus is present. This is the beginning of the germinal vesicle stage. The oocyte nucleus has now increased in size (fig. 8, Pl. 7).
About the time the above changes are completed the yolk-granules begin to appear in the cytoplasm of the oocyte (fig. 7, Pl. 7). The egg-follicle grows in size and becomes elongated with a broad anterior and a narrow posterior end. The nurse-cells lie at the broad end. The germinal vesicle continues to increase in size till it occupies the greater portion of the oocyte. Its membrane becomes thicker and stains more readily with haematoxylin (figs. 8, 9, and 10, Pl. 7).

When the vitelline membrane and the chorion have been finally secreted, the germinal vesicle, shrinking considerably in size, moves towards the anterior end of the oocyte and takes its position at the extreme end just below the vitelline membrane in a patch of protoplasm free from yolk (fig. 14, Pl. 7, and fig. 15, Pl. 8). The chromatin, which at the height of the germinal vesicle stage was present in the form of lightly staining fine granules, reappears now in the form of a large number of intensely staining small irregular fragments. A very interesting structure now arises in the centre of the germinal vesicle. This structure is circular in shape and has a very definite membrane within which is a mass of chromatin which stains deeply with haematoxylin (fig. 15, Pl. 8). The whole structure is the 'definitive nucleus' from which the polar bodies and the female pronucleus probably arise after the egg is laid. The membrane of the germinal vesicle slowly disappears and the intensely staining fragments of chromatin contained in its peripheral portion merge into the protoplasmic mass at the anterior end of the oocyte (fig. 16, Pl. 8).

Before the egg is laid no amount of extraction reveals any structure in the deeply staining mass of the 'definitive nucleus', except in some cases in which minute and ill-defined structures can be seen. The 'definitive nucleus' now moves anteriorly and takes its position immediately below the vitelline membrane (fig. 16, Pl. 8). It pushes out the vitelline membrane and so lies just below the micropylar funnel. It is now ready for the formation of the polar bodies, which process, it is highly probable, takes place immediately after oviposition.

When the oocyte is densely packed with yolk-granules so
that it is difficult even to make out the cytoplasmic granules
(and before the germinal vesicle has moved to the anterior
end of the oocyte in a patch of protoplasm free from yolk),
there is a short period when it is impossible to make out any
nuclear structure if thin sections are cut. If, however, sections
as thick as 12\(\mu\) are cut the nuclear membrane can always be
identified (fig. 11, Pl. 7). When thin sections are cut the
course yolk-granules are so badly displaced that they destroy
the very much swollen germinal vesicle.

St.-Hilaire in his paper on the oogenesis of Dytiscus,
published in a Russian journal (21), states that the oocyte
nucleus becomes a mass of fine granules from a small portion
of which the 'definitive nucleus' is later produced. Unfortu-
nately I have not been able to consult this journal, but Will
(27), in his paper on the oogenesis of Colymbetes fuscus,
mentions the above fact. It is therefore clear that the
behaviour of the oocyte nucleus of Culex fatigans is
similar to that of Dytiscus.

Up to the appearance of the intensely staining irregular
fragments of chromatin in the germinal vesicle, when the
latter takes its position at the anterior end of the oocyte, the
behaviour of the oocyte nucleus of Culex fatigans is
quite normal. As Doncaster (8) has stated, 'when the egg
becomes full-sized, and approaches the final stages of matura-
tion, the nucleus shrinks to a very much smaller volume,
sometimes becoming so small as to be found only with diffi-
culty. The chromosomes then reappear often tightly packed
together and the egg is ready for the maturation.' In Culex
fatigans, however, the 'definitive nucleus' arises from only
a portion of the germinal vesicle, while the rest of the chromatin
merges into the cytoplasm of the egg.

(b) Yolk-formation.

Soon after the mosquito has fed on blood the oocyte nucleus
passes into the germinal vesicle stage. At the same time
particles of yolk begin to be deposited in the cytoplasm of the
oocyte. When first formed the yolk-granules are very small
and stain intensely with haematoxylin (fig. 7, Pl. 7). After it has enlarged, each yolk-granule shows a central more deeply staining and a cortical less deeply staining portion. The yolk-granules do not arise necessarily first in the neighbourhood of the nucleus; they arise simultaneously throughout the cytoplasm of the oocyte. When the oocyte becomes elongated the deposition of yolk proceeds towards the broad anterior end.

The nutritive material appears to come from the nurse-cells and from the surrounding fluid. The cytoplasmic boundaries of the nurse-cells disappear, but the outer portions of their cell membranes unite to form one membrane containing the nurse-cell nuclei in one continuous sheet of protoplasm (fig. 11, Pl. 7). It is this membrane which forms the inner of the two membranes of the striated collar. The chromatin of the nurse-cell nuclei becomes less and less prominent, and appears to be used up in the formation of the yolk granules. Gradually the whole of the chromatin is absorbed by the oocyte along with the cytoplasm of the nurse-cells. Lastly, the nuclear membranes also of the nurse-cell nuclei disappear (fig. 16, Pl. 8).

The vitelline membrane at the broad anterior end of the oocyte is not secreted till yolk-formation is complete (figs. 8, 9, 10, and 11, Pl. 7). The absence of the vitelline membrane between the oocyte and the nurse-cells during the period of yolk-formation probably facilitates the passage of nutritive material from the nurse-cells.

The appearance of the vitelline membrane between the oocyte and the nurse-cells at a stage later than the rest has been observed by Korschelt (1889) in other insects also. Agnes Mary Clappole (7) has observed the same fact in Anurida maritima.

(c) Relation between the Germinal Vesicle and the Nutrition of the Oocyte.

In many cases it has been shown that chromatin is extruded from the chromosomes or the nucleolus into the cytoplasm of the oocyte, and this chromatin is supposed to be used up either directly in the formation of nutritive material or indirectly exert a formative influence on the cytoplasm. The
extruded chromatin may be present in the cytoplasm either in a diffused form or may condense to form definite bodies, the 'yolk-nuclei', like the corpuscles of Balbiani, which are supposed to control in some way or other the nutrition of the oocyte.

Bambeke in his paper on Pholeus phalangioides (2) says that the 'corps vitellin', which lies near the germinal vesicle, is nuclear in origin. According to him, this body grows very much in size and controls the nutrition of the oocyte.

In Antedon Chubb (6) describes the passage of a large number of deeply basophil granules from the nucleolus into the cytoplasm of the oocyte. These are aggregated round the nucleus and ultimately unite together to form the 'yolk-nucleus'. Chubb, however, does not think that the 'yolk-nucleus' in any way, directly or indirectly, controls the nutrition of the oocyte. He regards the 'yolk nucleus' as a waste product. The yolk-granules, according to him, arise from the material which enters the cytoplasm from the surrounding fluid. Thus he says, 'The process of yolk-formation, that is, the actual appearance of definitive yolk-spherules, is unaccompanied by increased nuclear or nucleolar activity, and consists simply in the rapid and automatic conversion of the accumulated material into a form sufficiently stable to survive the period of quiescence which succeeds the completion of the egg's growth'.

In Calliphora erythrocephala Lowne (15) describes the passage of granules into the cytoplasm of the oocyte, and these, according to him, are directly converted into food material.

McGill (16) describes granules close to the germinal vesicle of the oocyte of the dragon-fly and, although she has not been able to show their nuclear origin, she thinks that probably they originate from the nucleus. In support of her theory she says that 'Hennegay believes that the corpuscles of Balbiani (a type of yolk-nuclei) in Vertebrates are either parts of the nucleolus or the entire nucleolus which passes through the nuclear wall into the cytoplasm'.
In cases in which the passage of granules from the nucleus or the nucleolus into the cytoplasm of the oocyte has actually been observed it is possible that they control, directly or indirectly, the nutrition of the oocyte, particularly if this passage synchronizes with the formation of the yolk-granules.

It is difficult, however, to associate the extruded chromatin of the oocyte nucleus of *Culex fatigans* with the nutrition of the oocyte. It will be remembered that in *Culex fatigans*, when the germinal vesicle takes its position at the anterior end of the oocyte in a patch of protoplasm free from yolk, the chromatin, which did not stain in younger oocytes, reappears in the form of a very large number of deeply basophil fragments of irregular form (figs. 14 and 15, Pls. 7 and 8). After the ‘definitive nucleus’ has arisen in the centre of the germinal vesicle, the nuclear membrane of the germinal vesicle slowly disappears and the deeply staining fragments of chromatin contained in its peripheral portion merge into the cytoplasm (fig. 16, Pl. 8). It may be argued that these fragments of chromatin are similar in function to the granules described in other cases and take part in the nutrition of the oocyte. But it should be carefully noted that these granules merge into the cytoplasm at a time when the nutrition of the oocyte is practically over and the oocyte has almost reached its full size.

In *Culex fatigans*, therefore, there is no evidence to show that the nucleus controls the nutrition of the oocyte beyond the fact that it increases in size during the period of yolk-formation, and its chromatin ceases to take any stain.

It has also been suggested that the nutrition of the oocyte may be controlled not by the ‘yolk-nuclei’ or extruded chromatin particles only, but in many cases by the amoeboid activities of the germinal vesicle itself. These amoeboid movements are interpreted as indicating a direct participation of the nucleus in the formation of food material.

The branched condition of the oocyte has been described by various authors (Soyer (23), Stuhlmann (see Korschelt, 12), Barnbeke (2), Will (27)).

Quite recently Nicholson (19) has described an elaborate
and altogether unique form of branching of the oocyte nucleus of *Anopheles maculipennis*. In this mosquito the oocyte nucleus in the synizesis stage contains a prominent nucleolus close to which lie the contracted loops of chromatin which unite together to form the 'chromatin residue'. In the germinal vesicle stage the nucleus increases very much in size and branches in an elaborate fashion. The nucleolus also branches and its branches are continued into the branches of the nucleus itself. Later the nucleus is absorbed in the cytoplasm of the oocyte, and is used up in the formation of yolk-granules. This is the vegetative portion of the nucleus. The 'chromatin residue', the germinal portion, is lost for some time in the yolk-granules, and Nicholson believes that the definite nucleus of the oocyte which appears at a later stage in the patch of protoplasm free from yolk at the anterior end of the oocyte is the 'chromatin residue'.

There are three main theories concerning the irregularity of the nuclear membrane in the germinal vesicle stage. Korschelt (1889), Bambeke (2), Nicholson (19), and others interpret this nuclear irregularity as indicating a direct participation of the nucleus in the formation of food material.

Nussbaum (1882) and Conklin (1897) hold that the nuclear irregularity results from the pressure of the yolk-laden cytoplasm on the nuclear membrane. This view is also shared by Champy and Carleton (3). Thus they say, 'The study of our material has convinced us that nuclear shape is often due to pressure exerted on it by various cell inclusions. ... Again, in the early segmentation stages of ova, containing much yolk, the nuclei are indented by the large inert yolk-discs.'

Thirdly, Chubb (6) regards nuclear irregularity as an artifact due to the action of fixatives. It may be mentioned here that Korschelt himself in 1898 suggested that nuclear irregularity might be due to rapid osmosis resulting from fixation by a quickly penetrating fixative.

Jordan (11), on the other hand, in his paper on Newt observes that he has never noticed any nuclear irregularity when hot water is used as a fixative. With other fixatives, however, which reach the nuclear membrane slowly, the membrane
appears amoeboid. He therefore regards nuclear irregularity as due to the action of slowly penetrating fixatives. He further suggests that the nucleus may have a potential capacity of throwing out pseudopodia which might influence the nutrition of the oocyte. In the Newt, however, this capacity is not exercised.

In some sections of the ovary of Culex fatigans the germinal vesicle assumes an amoeboid appearance. This cannot have any relation with the nutrition of the oocyte. If the nucleus by its amoeboid activities controls the nutrition of the oocyte one would expect the amoeboid condition to be assumed at the commencement of yolk-formation. In fig. 8, Pl. 7, it will be seen that the formation of yolk has reached a fairly advanced stage, and yet the nuclear membrane is perfectly entire and does not even show a 'coarse wrinkling'.

It seems fairly certain, on the other hand, that the amoeboid appearance results from the pressure of yolk-granules on the nuclear membrane. Fig. 8, Pl. 7, shows an oocyte in which the yolk-granules, although present in fairly large numbers, are not situated immediately round the nucleus. They cannot, therefore, press upon the nuclear membrane, which is quite regular in outline. In an older oocyte (fig. 9, Pl. 7) the yolk-granules have increased in number and are closely surrounding the nuclear membrane, which appears amoeboid on account of the pressure exerted upon it. In a still older oocyte (fig. 10, Pl. 7) the yolk-granules are more tightly packed, so that in the process of sectioning the nuclear membrane has broken at many places and the nuclear contents have been much displaced, thus giving an amoeboid appearance to the nucleus. Some of the yolk-granules are actually lying in the nucleus. In a still older oocyte (fig. 11, Pl. 7) the nucleus can be made out only in sections of 12μ in thickness. In thinner sections the nucleus is completely destroyed by the yolk-granules. The nuclear membrane of the germinal vesicle after this stage is absolutely entire. As it has taken its position in a patch of protoplasm free from yolk at the anterior end of the oocyte (figs. 14 and 15, Pls. 7 and 8), it is no longer subject to disruption.
Nor can the nuclear irregularity in *Culex fatigans* be explained as an artifact due to fixation. In fig. 8, Pl. 7, the nuclear membrane is entire, and in fig. 9, Pl. 7, it is amoeboid; yet the same fixative was used in both cases. The pressure of yolk-granules explains both such cases.1

(d) The Formation of the Vitelline Membrane.

When the oocyte nucleus has passed into the germinal-vesicle stage the vitelline membrane appears as a thin structureless membrane between the oocyte and the follicular epithelium at the sides and the narrow end of the oocyte (fig. 8, Pl. 7). At the broad end of the oocyte the membrane appears at a much later stage when yolk-formation is practically over (fig. 12, Pl. 7). This doubtless facilitates the passage of nutritive material from the nurse-cells to the oocyte. The membrane has a strong affinity for stains.

The membrane is very much distorted by the action of fixatives and by sectioning. At places it protrudes into the oocyte and at others towards the chorion (fig. 14, Pl. 7). Its gelatinous contents may spread out and assume the appearance of radial fibres connecting the margins (fig. 11, Pl. 7).

The membrane is secreted by the oocyte and not by the follicular epithelium. At the broad end of the oocyte there is no follicular epithelium between the nurse-cells and the oocyte, and it would therefore appear that at this place at least the oocyte secretes the membrane. It is reasonable to suppose that the oocyte secretes the membrane at the sides and the narrow end also where there is follicular epithelium.

It is interesting to note some of the more important differences between the development of the vitelline membrane of *Culex fatigans* and that of the ‘inner wall’ of *Anopheles maculipennis* (Nicholson, 19).

1. In *Anopheles maculipennis* the ‘inner wall’ is

1 The author also possesses sections of the oocytes of *Stegomya albopicta* in which the nuclear membrane is perfectly entire even at a very late stage of vitellogenesis.
thicker just below the micropylar funnel, and Nicholson calls this thickening the 'stopper'. No such structure is present in Culex fatigans.

2. In Anopheles maculipennis the 'inner wall' is continued round the nurse-cells except at the top where they are covered by the follicular epithelium only, so that at this place a hole is left. It is through this hole that the nurse-cells are squeezed out later on, and afterwards this hole is occupied by the micropylar funnel with the 'stopper' immediately below it. In Culex fatigans the vitelline membrane is not continued round the nurse-cells and lies below them between them and the oocyte, so that at no stage the nurse-cells are included within the membrane. This explains the necessity of a micropylar tube passing through the degenerating nurse-cells (fig. 19, Pl. 8).

(c) Formation of the Chorion.

In young follicles (fig. 2, Pl. 7) the follicular epithelium is surrounded on the outer side by a thin basement membrane; on the inside there is no membrane. After yolk-formation is complete a thin darkly staining membrane appears on the inside of the follicular epithelium (figs. 14 and 16, Pls. 7 and 8). No such membrane develops on the inside of the follicular epithelium covering the nurse-cells because they protrude inwards to secrete the micropylar apparatus (fig. 12, Pl. 7).

With the elongation of the oocyte the epithelial cells between the two membranes are subject to very great strain. They elongate in the longitudinal direction. The cells lose their individuality, and their nuclei, which become spindle-shaped, appear to lie in one continuous sheet of protoplasm. Small processes of the chorion now appear on the outside of the inner membrane and gradually grow in size towards the basement membrane (fig. 14, Pl. 7). Ultimately the follicular epithelium disappears, having obviously been used up in the secretion of the processes.

From a study of figs. 8, 9, 10, and 11, Pl. 7, it will be clear that there is no follicular epithelium between the nurse-cells
The epithelial cells which are situated all round the point of contact of the nurse-cells and the broad end of the oocyte grow inwards between the oocyte and the nurse-cells and secrete the chorion at the broad end except in the middle where the micropylar apparatus develops (fig. 12, Pl. 7).

(f) The Development of the Micropylar Apparatus.

When the chorion is being secreted, the follicular epithelial cells covering the nurse-cells protrude inwards towards the broad end of the oocyte between the nuclei of the degenerating nurse-cells (fig. 12, Pl. 7), and secrete the micropylar apparatus including its funnel, tube, disc, and the supporting ring. Ultimately the follicular epithelial cells disappear. A transverse section through the nurse-cells at this stage shows the inwardly projecting epithelial cells radiating outwards from the transversely cut micropylar apparatus (fig. 13, Pl. 7).

7. Summary.

1. When a newly emerged mosquito is fed on food other than blood, the nuclei of all the cells of an egg-follicle pass through the synizesis stage. After three to four days feeding on artificial food the nuclei of the nurse-cells become differentiated from the nucleus of the oocyte. The egg-follicle does not develop further on artificial food.

2. A feed on blood is essential for the deposition of yolk and the secretion of membranes.

3. The nucleolus of the oocyte is an amphinucleolus and consists of proteids and nucleinic acid as shown by the very interesting changes in its staining capacity.

4. The irregularity of the nuclear membrane of the germinal vesicle is due to the pressure of coarse yolk-granules and cannot be interpreted either as indicating a direct participation of the nucleus in the formation of yolk or as an artifact due to
5. The yolk-granules do not arise necessarily first in the neighbourhood of the nucleus.
6. In the germinal-vesicle stage the nucleus enlarges considerably in size, and its chromatin is present in the form of very small granules.
7. When the oocyte has almost reached its full size the germinal vesicle shrinks considerably in size and takes its position at the anterior end. The ‘definitive nucleus’ from which the polar bodies and the female pronucleus doubtless arise appears in the centre of the germinal vesicle. The ‘definitive nucleus’ has a very definite membrane within which is a deeply basophil mass of chromatin.
8. The outer portion of the germinal vesicle merges into the cytoplasm of the oocyte. It is difficult to associate this with the nutrition of the oocyte as it takes place when the oocyte has almost reached its full size and yolk-formation is practically complete.
9. The structure and the development of the chorion, the vitelline membrane, the striated collar, and the micropylar apparatus have been described.
10. The micropylar tube develops a short time before fertilization. It degenerates immediately after the egg is laid and is absent in the laid egg. It is thus that it has escaped notice so long.
11. As a rule copulation does not take place in captivity, but in two cases I observed that the spermathecae of females confined with males contained a large number of spermatozoa.

8. List of Literature.
1. Agar, W. E.—‘Cytology with special reference to the Metazoan cell.’
EGG-FOLLICLE OF CULEX


7. Claypole, Agnes Mary.—"The Embryology and Oogenesis of Anurida Maritima", 'Journ. of Morph.', vol. xiv, 1898.


EXPLANATION OF PLATES 7 AND 8.

REFERENCE LETTERS.

B.M., basement membrane of the follicular epithelium; ch., chorion; ch.*, chorion at the anterior end of the oocyte; chr., chromatin reappearing in the germinal vesicle; cy., cytoplasm of the oocyte; C.T., chromatin threads of the oocyte; D., disc of the micropylar apparatus; D.K., definitive nucleus; F.c., follicle cell; F.C.N., follicle cell-nucleus; F.E., follicular epithelium; F.E.N., follicular epithelium nucleus; F.T.M., follicular tube-membrane; I.ch., inner membrane of the chorion; I.M., inner membrane of the supporting ring of the micropylar apparatus; L.M., limiting membrane of the ovary; L., lumen of the ovary; M.F., micropylar funnel; M.T., micropylar tube; N., nucleus; N.C., nurse-cell; N.C.N., nurse-cell nucleus; N.C.M., nurse-cell membrane; O., oocyte; O.M., outer margin of the supporting ring of the micropylar apparatus; O.N., oocyte nucleus; O.N.M., oocyte nucleus-membrane; S.C., striated collar; Tr., tracheae; Vit., vitelline membrane; Y., yolk; Y.F., younger follicle.

PLATE 7.

Fig. 1.—Transverse section of the ovary of a mosquito immediately after it has emerged from the pupa. All the cells of an egg-follicle are similar. x 320.
Fig. 2.—An egg-follicle of a mosquito newly emerged from the pupa. x 550.
Fig. 3.—An egg-follicle with the oocyte nucleus in the synizesis stage. Nucleolus clearly shows its double nature. x 550.
Fig. 4.—Oocyte nucleus. Acidophil portion of the nucleolus becoming ovoid. Synizesis stage. x 550.
Fig. 5.—Oocyte nucleus. Acidophil portion of the nucleolus beginning to branch. Chromatin threads staining less readily. x 550.
Fig. 6.—Oocyte nucleus. Acidophil portion of the nucleolus detached from the nucleolus and filling up the nucleus. Basophil portion of the
nucleolus becoming granular and vacuolated. Chromatin threads faintly staining. \( \times 550 \).

Fig. 7.—An egg-follicle, oocyte nucleus in the same stage as in fig. 6. Yolk beginning to appear. \( \times 230 \).

Fig. 8.—An egg-follicle, oocyte nucleus in the germinal vesicle stage, chromatin not staining. Vitelline membrane has appeared. \( \times 590 \).

Fig. 9.—An egg-follicle. Yolk-formation has reached an advanced stage. Nuclear membrane of the germinal vesicle indented by the pressure of yolk-granules. \( \times 320 \).

Fig. 10.—A more advanced follicle. Nuclear membrane of the germinal vesicle broken in sectioning by the pressure of yolk-granules. \( \times 320 \).

Fig. 11.—A still more advanced follicle. Germinal vesicle has increased considerably in size. Nuclear membrane broken at places in sectioning. 12\( \mu \) thick. \( \times 230 \).

Fig. 12.—An older follicle. Follicle has become longer and narrower. Development of the micropylar apparatus. \( \times 320 \).

Fig. 13.—Transverse section through the anterior end of a follicle of the same age as shown in fig. 12. Development of the micropylar apparatus. \( \times 320 \).

Fig. 14.—An advanced oocyte. Germinal vesicle has shrunk considerably in size and has moved to the anterior end. Chromatin intensely staining. Definitive nucleus has arisen and is ready for the formation of the polar bodies. \( \times 230 \).

**PLATE 8.**

Fig. 15.—Transverse section through the anterior end of an oocyte of about the same age as shown in fig. 14. \( \times 320 \).

Fig. 16.—A still more advanced oocyte. The chromatin of the germinal vesicle has merged into the cytoplasm after the disappearance of the nuclear membrane. Definitive nucleus pushing out the vitelline membrane preparatory to the formation of polar bodies. \( \times 230 \).

Fig. 17.—Anterior end of the laid egg showing the striated collar. Highly magnified.

Fig. 18.—Chorion at the anterior of the oocyte detached in whole mounts. Showing thirty-five ridges of the chorion radiating outwards from the micropylar apparatus. \( \times 320 \).

Fig. 19.—An oocyte studied in normal salt solution just before oviposition. Showing the striated collar with the micropylar tube and the degenerating nurse-cell nuclei. Highly magnified.

Fig. 20.—A follicle of an eight hours old mosquito fed on artificial food. In the nuclei of all the cells chromatin threads are lying on one side of the nucleolus. \( \times 550 \).

Fig. 21.—An egg-follicle of a ninety-six hours old mosquito fed on artificial food. Oocyte nucleus only in the synizesis stage. \( \times 550 \).