The relationship between oocyte and follicle in the hen's ovary as shown by electron microscopy

by Ruth Bellairs

From the Department of Anatomy and Embryology, University College London

WITH EIGHT PLATES

INTRODUCTION

In the adult hen each oocyte is surrounded by a capsule of follicle cells and all the raw materials that enter the oocyte must pass through this capsule. It is not surprising, therefore, that the morphological relationships between the follicle and the oocyte are of a highly specialized nature. Several workers have studied them, mainly by light microscopy, but their findings have not been unanimous, largely because of difficulties in resolving fine details. For instance, although it has frequently been suggested that certain structures pass from the follicle cell into the oocyte, these structures have been interpreted by different authors as Golgi bodies, as mitochondria or as fat drops. Similarly, there have been several different theories about the relationship between the cell membrane of the oocyte, the zona radiata and the vitelline membrane. Other problems that have not been solved are that of whether the follicle cells actively synthesize any materials for secretion into the oocyte, and that of what happens to the oocyte cell membrane just before ovulation.

The present study is an attempt to solve these problems by an electron microscopical investigation of the follicular-oocyte region throughout the entire course of oogenesis. Recently, two other groups of workers using electron microscopy have reported on certain aspects of the early stages of oogenesis (see Discussion).

MATERIAL AND METHODS

Material was fixed from the ovaries of twenty-eight adult laying hens, which were either Rhode Island Reds or White Leghorns. The ovary of a laying hen contains oocytes varying in size from about 0.05 mm. up to about 3.5 cm. in maximum diameter (the full-grown oocyte is not spherical but ellipsoidal).

1 Author's address: Department of Anatomy, University College London, Gower Street, London, W.C.1, U.K.
The hens were killed by injecting about 10–15 ml. of air intravenously, and pieces of ovary were removed from the body and placed in fixative within about 1 min. of death. Oocytes of different sizes were dissected from the ovarian stroma immediately afterwards, and fixative was injected beneath the surface of the larger oocytes with a hypodermic syringe.

The fixatives used for electron microscopy were ice cold, and were osmium tetroxide (Palade, 1952; Millonig, 1961), potassium permanganate (Luft, 1956) or glutaraldehyde (Sabatini et al., 1963). The pH of the fixative was between 7.2 and 7.8 and the tissue remained in it for between $\frac{1}{2}$ to $1\frac{1}{2}$ hr., except in the glutaraldehyde, in which it was left for 2–3 weeks. After dehydration in graded ethanols, the material was embedded in araldite (Glauert & Glauert, 1958), though some of it was first stained in bulk with 1 per cent. phosphotungstic acid made up in absolute ethanol. The remainder was stained on the sections with uranyl acetate (Watson, 1958), with potassium permanganate (Lawn, 1959) or with lead citrate (Reynolds, 1963). Sections were examined with a Siemens Elmiskop Ib electron microscope.

An attempt was made to trace the passage of material from follicle to oocyte by injecting five hens with 10–20 ml. of colloidal gold (Gurr's). Oocytes from these hens were fixed at intervals ranging from 2 hr. to 1 week after the injection.

Material from two hens was fixed in formal-saline, and embedded in paraffin wax. Sections were stained with methyl-green pyronin for RNA. The method was that given by Pearse (1953) and the ribonuclease used for the control sections was obtained from the Sigma Chemical Company (Type XII–A).

GENERAL MORPHOLOGY OF THE OVARIAN FOLLICLE

This brief account is based largely on the work of van Durme (1914) and of Marza & Marza (1935), but is in agreement with the present electron microscopical findings.

The follicle wall is composed of several layers (see Text-fig. 1). Immediately around the oocyte is the follicular epithelium (or membrana granulosa) which consists of a single layer of cubical cells in the smallest and largest oocytes, but has a stratified, or pseudostratified, appearance in oocytes of intermediate size (say about 0.4 mm. to about 4 mm.). The depth of the epithelium can be correlated with events in the oocyte (see Table 1). Distal to the epithelium is a thin layer of extra-cellular material, the membrana propria, which is about 1 $\mu$ deep. This, in turn, is covered by the closely packed cells of the theca interna and the theca externa. Blood vessels and bundles of collagen fibres are present in these layers as well as the so-called luteal cells which are highly specialized and will not be considered in this paper.

In the early stages the cell membranes of the follicle cells and oocyte lie close together. Subsequently, according to light microscopists, two further 'membranes' appear between them, viz. the zona radiata (or zona striata) and the
TEXT-FIG. 1. Diagram to show the various layers surrounding the oocyte. The cell membrane of the oocyte is extended into villous projections, the zona radiata.

**TABLE 1**

**Table of events in yolk formation**

(partially after van Durme, 1914, and Marza & Marza, 1935)

<table>
<thead>
<tr>
<th>Phases</th>
<th>Diameter of oocyte (mm.)</th>
<th>Cytology of oocyte</th>
<th>Histochemistry</th>
<th>Average depth of follicular epithelium</th>
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<tbody>
<tr>
<td>1st phase</td>
<td>0.05–0.2</td>
<td>Balbiani body</td>
<td>Fats penetrate oocyte in larger amounts than protein</td>
<td>4.0 µm</td>
</tr>
<tr>
<td></td>
<td>0.2–0.3</td>
<td>Fat drops in oocyte</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.3–1.0</td>
<td>Dispersal of Balbiani body</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mitochondria lie peripherally</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2nd phase</td>
<td>2.0–3.0</td>
<td>Vacuoles</td>
<td>Proteins penetrate oocyte in larger amounts than fats</td>
<td>17.0 µm</td>
</tr>
<tr>
<td></td>
<td>3.0–6.0</td>
<td>First yolk appears</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.0–9.0</td>
<td>Some white yolk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3rd phase</td>
<td>10.0–35.0</td>
<td>White and yellow yolk, latebra, nucleus of Pander</td>
<td>More fats than proteins enter oocyte</td>
<td>3.0 µm</td>
</tr>
<tr>
<td>(rapid growth phase)</td>
<td></td>
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vitelline membrane. Recent electron microscopical studies have shown that the zona radiata is derived from the oöcyte cell membrane (see Discussion). There have been many investigations by light microscopy of the events taking place in the oöcyte itself (see reviews by Marza & Marza, 1935; Needham, 1950; Bellairs, 1964). An abridged summary is provided by Table 1.

RESULTS

The Follicle Cells

General

In the early stages of oogenesis (up to about 0.3 mm.) the follicle cells lie close together, their cell membranes being separated by a gap of about 200 Å. Desmosomes and lining bodies (see below) are infrequent at this stage. By the time the follicle cells have become columnar (i.e. when the oöcyte is about 1 mm. in diameter) short processes have begun to extend out from them into the intra-cellular spaces between adjacent follicle cells. During the final growth phase, long processes develop on all aspects of the follicle cells and some of these carry desmosomes.

In many of the follicles, two types of cell are present (Plate 2, Fig. B). This observation confirms that of certain light microscopists (Brambell, 1926; Marza & Marza, 1935) and also that of Press (1964) who used electron microscopy. The major differences between the types are that one is considerably more electron opaque than the other and contains more vacuolated structures. These latter are characteristics of degenerating cells (see Bellairs, 1961a). In most follicles, the opaque cells are relatively uncommon. Occasionally, however, a whole follicular epithelium appears to be composed of them, and it seems probable that these are atretic follicles, for it is known that small oöcytes, together with their follicle cells, occasionally stop developing and degenerate (see Romanoff & Romanoff, 1949).

The normal follicle cells are characterized by the presence of many vesicles. The most common are small and vary in size from about 300–450 Å, and are found in all parts of the cytoplasm (sv, Plate 1, Fig. A, inset). They are surrounded by smooth-surfaced membrane, and hence may be derived from the Golgi apparatus. Large smooth-surfed vesicles are also present which measure about $\frac{1}{4} - \frac{1}{3} \mu$ in diameter and are of two types. The first (lvg) contain a fine granular material and closely resemble the rather smaller vesicles (vg) found in the cortex of the oöcyte (Plate 5, Fig. K). The second (vdp) contain electron dense patches and are not unlike lysosomes, except that they sometimes contain within them structures resembling lining bodies (described below) (Plate 5, Fig. K). In addition, patches of lightly staining amorphous material (nf in Plate 1, Fig. A) are present and these are probably neutral fat, for in specimens fixed in potassium permanganate the vesicles of this type are empty (see Bellairs, 1961b, 1963, on the failure of potassium permanganate to fix neutral fat).

By the time the vitelline membrane has begun to form (see below) large vacuoles
have become conspicuous in some of the follicle cells. Some at least of these are in communication with one another (Plate 2, Fig. C) and it seems possible that they form a continuous, tortuous channel throughout the cell. Most of the vacuolar profiles appear empty at this time, though occasionally a fine fibrillar material is visible within them. It is not known whether these vacuoles are new structures or whether they have formed by modification of the vesicles present in the younger stages.

Fine filaments are present throughout the cytoplasm of some follicle cells at all stages, though they appear to be especially common from the stage of about 1–3 mm. They are about 40–50 Å in diameter but at irregular intervals have dark granules attached to them, each about 60 Å in width. The filaments run in all directions, frequently crossing one another. In a few cells a dense tangle of filaments is visible (Plate 2, Fig. D).

The mitochondria of the follicle cells are oval or round in transverse section, appearing usually about two or three times as long as they are wide (Plate 1, Fig. A; Plate 2, Fig. C). They are comparable in size with those of the oöcyte, although they do not stain so intensely with potassium permanganate. Also present in the follicle cells of the early stages, however, are sections of mitochondria which are often three or four times larger, and which may measure as much as ½ μ in diameter. These profiles are usually round or oval, and like the smaller mitochondria they contain a lot of densely staining material between the cristae (Plate 3, Fig. H). Occasionally a constricted region is present at one side which has the dimensions of one of the smaller mitochondria. This suggests that the two types are not necessarily separate entities, but that some at least of the mitochondria are irregular in diameter and swell out at intervals along their length. The large mitochondrial profiles have not been seen in the immediately pre-ovulatory stages.

Two types of granule lie freely in the cytoplasm. The first is about 100 Å in diameter and thus corresponds in size with ribonucleoprotein particles (Palade, 1955; Palade & Siekevitz, 1956). The second is present as small clusters of particles of the type characterized as glycogen (see Luft, 1956; Revel et al., 1960; Drochmans, 1960, 1962). They are especially conspicuous in material fixed in potassium permanganate or glutaraldehyde (Plate 2, Fig. E).

The complex mass

One of the most characteristic features of the follicle cells in the early stages of oögenesis is the presence of certain complex masses, each measuring 3–5 μ in section. They appear to correspond with the 'true Golgi' described by a number of light microscopists (Brambell, 1926; Ikeda, 1928; Das, 1931; Sluiter, 1940; Guraya, 1957, 1962). They are probably spherical or ovoid and, in the early stages, lie between the nucleus and the oöcyte cell membrane. By the time the oöcyte is about 0·3 mm., however, the complex mass and nucleus have changed places, the nucleus now lying nearer the oöcyte. When examined by electron
microscopy (Plate 4, Fig. J) the complex mass appears to consist of several elements:

(i) Paired centrioles. These lie at the centre of the complex mass and are therefore not visible in all sections. They have not been specifically investigated but appear to resemble centrioles in other tissues described by a variety of authors (e.g. Sorokin, 1962) in that they each possess a central cylinder surrounded by a series of filaments orientated parallel to the long axis (Plate 3, Fig. G).

(ii) Microtubules. Most of the microtubules present in the cell run from the basal to the distal border (Plate 4, Fig. J) but in the complex mass they appear to radiate from the centriole (Plate 3, Fig. G), often extending out to the cell membrane (Plate 3, Fig. F). Each microtubule has a diameter of about 200 Å. The individual walls are about 50 Å and do not have a unit membrane structure. The gap between the walls contains a less dense material and is about 100 Å. The microtubules have been seen only in material fixed in glutaraldehyde.

(iii) Stacked membranes. These are profiles of endoplasmic reticulum with granules of about 200 Å diameter attached to their outer surfaces. A fine granular material lies in the cavity. The profiles are about $\frac{1}{10} - \frac{1}{20} \mu$ apart and they are frequently, though not invariably, arranged in a manner radial to the centrioles (Plate 4, Fig. J).

(iv) Patches of Golgi membranes. These are the usual smooth-surfaced profiles of endoplasmic reticulum characteristic of the Golgi apparatus. Like the stacked membranes of the granular endoplasmic reticulum, they lie at the periphery of the complex mass (Plate 4, Fig. J).

(v) Vesicles. The small vesicles mentioned above appear to be especially numerous in the complex mass (Plate 3, Fig. F).

In the final growth phase (see Table 1) the complex masses are no longer visible as such, although patches of stacked lamellae of endoplasmic reticulum are still present.

The lining bodies

Another characteristic feature of the follicle cells is the presence of certain specializations that are closely associated with the cell membrane (Plate 1, Fig. A; Plate 4, Fig. I; Plate 6, Fig. L). These specializations have been called ‘pre-mitochondria’ by Schjeide & McCandless (1962) who have suggested that they give rise to mitochondria. Since I regard this supposition as unlikely (see Discussion), I introduced the descriptive term ‘lining body’ (Bellairs, 1964). Almost simultaneously, a third term ‘transosome’ was suggested by Press (1964).

A lining body may be at the junction of one follicle cell and another, or of the

1 'Unit membrane' is the term introduced by Robertson (1959) to describe the appearance of normal cell membranes in electron micrographs. He defined it as being about 75 Å wide and consisting of two dark layers separated by a light layer. Recently, Yamamoto (1963) has shown that the thickness is not always 75 Å.
TEXT-FIG. 2. A: Structure of a lining body in longitudinal section (cf. Plate 6, Fig. L). Membrane \( b \) is derived from the follicle cell membrane, but since the exact relationship has not been established the latter is shown by dotted lines. B: Structure of a lining body in transverse section (cf. Plate 6, Fig. M). C: Structure of a body within the oocyte that appears to be a derivative of a lining body (cf. Plate 6, Figs. N & O). Membrane \( a \) remains intact, but \( b, c \) and \( d \) are less easy to distinguish. D: Structure of a mitochondrion. E: Structure of a lining body according to the interpretation of Press (1964). T.O.M. is the ‘transosome outer membrane’ and corresponds with membrane \( a \) in Figs. A & B; and T.I.M. is the ‘transosome inner membrane’ and corresponds with the membranes \( b, c \) and \( d \) in Figs. A & B. (After Press, 1964.) F: Diagram to show the widths of the individual membranes of the lining bodies as found in this investigation. G: Diagram to show the widths of the mitochondrial membranes.
follicle cell and the oocyte. In some ways each lining body resembles a desmosome in that its most conspicuous feature is a thickening beneath the cell membrane. But lining bodies differ from desmosomes in that they are not arranged in pairs that are mirror images of one another. They are also much bigger than desmosomes, being frequently about $\frac{1}{2} \mu$ or more in length. The shape of the lining body follows that of the cell membrane, so that sometimes it appears to be flat whereas at other times it is curved and forms the lining for a finger-like projection from the follicle cell indenting the surface of its neighbour (Plate 4, Fig. I).

The total thickening is about 150 Å and consists of two layers ($c$ and $d$ in Text-fig. 2 and in Plate 6, Fig. 11) each about 50 Å wide with some dense granular material between them. Large granules, each about 150–250 Å, are attached at fairly regular intervals to membrane $d$. This thickening lies beneath the cell membrane, and the latter is modified in that region—that is it is no longer present as a unit membrane (see footnote, p. 220) but has become reduced to a single dense line about 30 Å wide. Unfortunately, it has not been possible to decide whether membrane $b$ is derived from the original cell membrane or not, hence the dotted line in Text-fig. 2. The complex $c$–$d$ stains densely with uranyl acetate. In sections from the same block which are not stained with uranyl acetate, the staining is less intense.

**Histochemistry**

Follicle cells of oocytes up to about 1 mm. in diameter exhibited a strong basophilic reaction when stained with methyl-green-pyronin. Both the cytoplasm and nucleolus stained deeply but it was not possible to distinguish by this method those cells (shown in Plate 2, Fig. B) which have an intensely electron dense character. In control sections which had been digested with ribonuclease the basophilia was markedly reduced throughout the follicular layer.

**The follicular–oocyte border**

In the early stages (up to about 0.3 mm.) the cell membrane of the oocyte lies close beneath the cell membrane of the follicle cell, and the two cells share the paired members of desmosomes (Plate 1, Fig. A). Strands of filaments stretch from one part of the egg surface to another and pinocytic-like indentations can be seen in the oocyte cell membrane; in places the surface of the oocyte is indented by a follicular projection bearing a lining body (Plate 4, Fig. J).

Even in the earliest stage examined, however, (0.06 mm.), the smooth surface of the oocyte is interrupted in places by isolated patches of specialized cell membrane which form protuberances. These patches are located opposite the junction of two follicle cells (Plate 7, Fig. Q) and appear to be the primordium of the zona radiata. Sometimes simple folds or crypts can be seen in the protuberances, but usually the region appears highly complex in transverse section and gives the impression that a mass of papillae are extending out in many directions.
At the base, or apparent base, of each crypt, strings of small vesicles become visible (Plate 7, Fig. P). Attempts to demonstrate the pinocytic nature of these vesicles by examining them in hens that had been injected with colloidal gold were unsuccessful; no trace of colloidal gold was seen. Vesicles also appear in the substance of the villi (Plate 8, Fig. T).

As development proceeds the indentations become more numerous until they have spread all around the oöcyte, being interrupted only where the lining bodies are insinuated between them (Plate 8, Fig. T). By the time the oöcyte is about 2 mm. in diameter the zona radiata has reached a depth of about \( \frac{1}{10} \mu \). By the time the oöcyte is about 1 cm. in diameter, the zona radiata may measure as much as 3 \( \mu \).

During the time that the zona radiata is forming, the lining bodies continue to project into the substance of the oöcyte, indenting the oöcyte cell membrane as they do so. They have been seen in association with oöcytes as large as 8 mm. in diameter but not in oöcytes of 1 cm. or more.

The first definite indications of the inner layer of the developing vitelline membrane were seen in an oöcyte of about 2-5 mm. diameter. The outer layer of the vitelline membrane is laid down after ovulation (Bellairs, Harkness & Harkness, 1963). The inner layer is visible in the intercellular space between follicle and oöcyte cells. It consists at this stage of fibrillae, each about 100 Å in diameter (Plate 7, Fig. R), and it is not until the oöcyte is about 8 mm. that the characteristic appearance of the inner layer of the vitelline membrane is visible. By the time the oöcyte has grown to about 1 cm. diameter the inner layer of vitelline membrane is about \( \frac{1}{2} \mu \) thick; when the oöcyte is about 2-5 cm., the inner layer of vitelline membrane is about 2-5 \( \mu \), and has reached its maximum size.

At first the developing vitelline membrane is present as a uniform layer lying close beneath the smooth-surfaced follicle cell membrane, although it appears to gradually fill the crypts between the oöcyte villi. By the time the oöcyte is about 1 cm. in diameter, however, the lower surface of each follicle cell has become covered with projections and these extend down into the vitelline membrane (Plate 8, Fig. U). It seems possible that the inner layer of vitelline membrane starts to develop its characteristic mesh-like appearance by being moulded against the template of these projections. Subsequently, however, the contact between vitelline membrane and follicle appears to be reduced and a fine granular matrix becomes visible in the cavities of the developing vitelline membrane (Bellairs, Harkness and Harkness, 1963).

In the final stages of oöcyte growth large vacuoles appear beneath the surface of the oöcyte. They vary in size and shape, though are usually between about \( \frac{1}{2} \) and 2 \( \mu \) in diameter; they are bounded by a unit membrane. They have been seen in oöcytes as small as 1 cm. diameter where they lie among the oöcyte villi. In the final stages of development (Plate 7, Fig. S and Plate 8, Fig. U) the oöcyte villi are less common and the vacuoles lie immediately beneath the vitelline membrane.
The cortical (i.e. peripheral) region of the oocyte

In the early oocyte (up to about 0.15 mm. in diameter) large lipid drops are distributed throughout much of the cytoplasm, including the cortical layer (Plate 7, Fig. Q). They may be as much as 4 \( \mu \) in diameter. As the oocyte grows, however, they come to lie further from the surface, both relatively and absolutely. Beaded filaments are present which are about 40 \( \AA \) in diameter and which run in all directions; lengths up to 2 cm. have been traced. No granular endoplasmic reticulum has been seen, though it may be present in small amounts. Vesicles are, however, present, containing fine granules (Plate 5, Fig. K). The vesicles vary in size from about 1000 \( \AA \) to 4000 \( \AA \), and the granules they contain are individually about 100 \( \AA \). Small patches of annulate lamellae are also present (illustrated by Bellairs, 1964, Fig. 11).

In oocytes of about 0.25 mm. the large granule-filled vesicles are the most conspicuous feature of the cortical region, the beaded filaments lying between them. About 5–15 \( \mu \) from the surface large numbers of rod-shaped mitochondria, which are usually about 3000 \( \AA \) wide, lie among the vesicles and Golgi bodies are spaced at intervals among, or slightly proximal to them. The layer of fat drops lies proximal to the Golgi bodies.

It has already been mentioned that lining bodies from the follicle cells indent the surface of the oocyte. The tip of each lining body is thus surrounded by the oocyte cell membrane (unit membrane \( a \) in Text-fig. 2). It is possible that some at least of the lining bodies lose contact with the follicle cell and become totally encapsulated in a vacuole formed from oocyte cell membrane, though there is no conclusive proof that this is so. Vesicles of this type have, however, been seen more than 5 \( \mu \) deep within the oocyte (lbd in Plate 5, Fig. K), although certain changes appear to have taken place within them (Plate 6, Figs. N and O). The layers \( c \) and \( d \) have usually lost their clear outlines and have become attached to membrane \( b \). The former intercellular space between \( a \) and \( b \) has become packed with granules, whereas the large granules have often disappeared from the inner layer.

When the oocyte has reached about 3 mm. in diameter, yolk spheres become visible. They will not be considered here.

DISCUSSION

Secretory activity of the follicle cells

One of the main problems to be considered is whether or not the follicle manufactures and secretes materials into the oocyte. It seems clear from the literature (see discussions by Marza, 1935; Bellairs, 1964) that in the final growth phase the follicle cells do not synthesize and secrete yolk; the yolk proteins and lipids are formed in the liver and transported to the oocyte in the blood plasma. Nevertheless, the follicle cells may indeed synthesize materials that are secreted into the oocyte, especially in the early stages. The evidence is based mainly on a consideration of their fine structure that is described in this paper.
In many cells known to be secretory, the secretion product is apparently synthesized by the ribosomes covering the membranes of granular endoplasmic reticulum. It is thought that it then moves to the Golgi region whence it is secreted, frequently as well-defined granules (Porter, 1961). Thus, a secreting cell may be expected to possess extensive amounts of granular endoplasmic reticulum often arranged in stacks (Kurosumi, 1961), secretion granules, and Golgi apparatus. Furthermore, Slautterback (1963) has pointed out that microtubules of about 120–200 Å in diameter are frequently found in secretory cells and has suggested that they may act as a transport system through the cell. All these features are present in the follicle cells, at least in the early stages.

It has been shown that these structures tend to be concentrated together into a 'complex mass'. The mass as such disappears in the later stages of oocyte development, and thus it would appear that if it is indeed secretory, then its activity occurs in the earliest stages.

Secretory activity is characteristic of follicle cells in general, as various light microscopical investigations have indicated (see review by Raven, 1961). Unfortunately, the structure of the follicle cells of other vertebrate classes does not seem to have been examined in great detail by electron microscopy apart from the relationships between their cell membranes and those of the oocyte. Nevertheless, the follicle cells of the guinea pig have been shown to be rich in endoplasmic reticulum by Anderson & Beams (1960) who have pointed out that these cells appear to be secretory in nature. Similarly, although Hope et al. (1963) do not draw attention to it, their electron microscopical pictures of the follicle cells of amphibians show highly complex cytoplasmic components with numerous vesicles.

It is pertinent to enquire about the function of this apparent secretory activity. It is possible that it serves mainly to nourish the oocyte as such, for this is an exceptionally large cell which is entirely encapsulated by follicle cells. Alternatively, or perhaps additionally, it may help to prepare the oocyte for the final phase of yolk formation.

The lining bodies

Various authors using light microscope techniques have reported that substances are secreted by the follicle cells into the oocyte. Loyez (1906) described the transfer of fatty materials from the follicle cells into the oocyte in reptiles, and Brambell (1926), Bhattacharya (1929) and Guraya (1957) reported the passage of Golgi bodies into the oocytes of birds. A similar suggestion was made for mitochondria in various Indian birds (Varma, 1954) and for fat drops in the hen (Konopacka, 1933). All these cytoplasmic structures are osmiophilic.

In the present study also it has been demonstrated that certain osmiophilic structures (the lining bodies) project from the follicle cell into the oocyte, and it is likely that it is these structures that have previously been identified as Golgi
bodies, mitochondria or fat drops. They have also been seen by electron microscopy (Schjeide & McCandless, 1962; Schjeide et al. 1963, 1964; Press, 1964), though the detailed descriptions of these authors do not agree with mine or indeed with those of each other. This can be seen by considering each membrane separately (see Text-fig. 2 and Table 2).

**Table 2**

*Comparison of the individual membranes of the lining bodies given by different authors*

Measurements are included whenever these have been made by the authors.

<table>
<thead>
<tr>
<th>Authors</th>
<th>a</th>
<th>b</th>
<th>c and d</th>
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<tbody>
<tr>
<td>Schjeide et al.</td>
<td>Plasma membrane</td>
<td>Not described</td>
<td>Double external membrane</td>
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<td>(1964)</td>
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<td>50 Å and 50 Å</td>
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<tr>
<td>Press (1964)</td>
<td>Unit membrane</td>
<td>Not described</td>
<td>Unit membrane</td>
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<tr>
<td>Bellairs</td>
<td>Unit membrane</td>
<td>30 Å</td>
<td>50 Å and 50 Å (total width 150 Å)</td>
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*Membrane a.* Schjeide et al. (1964) call this a plasma membrane and regard it as formed by invagination of the receiving cell. Press (1964) has described it as a unit membrane. The present results agree with both conclusions.

*Membrane b.* This does not appear to have been described either by Schjeide et al. (1964) or by Press (1964) who have probably included it in the complex c–d. In the present investigation, membrane b was found to be 30 Å wide.

*Membranes c and d.* Together these have been labelled 'double external membrane' by Schjeide et al. (1964), who have found them to be individually about 50 Å each. In the present investigation similar measurements were obtained. Press (1964) has, however, called this complex a unit membrane, but gives no measurements. The total width of 150 Å is somewhat high to be included within the term 'unit membrane', although it has recently been shown that not all unit membranes measure 75 Å (Yamamoto, 1963; Robertson, 1964).

*Granules.* These have been interpreted as ribonucleoprotein granules by Schjeide, McCandless & Munn (1963) but these authors do not appear to have measured them. In the present study, however, the granules were found to have a diameter of 150–250 Å, which is somewhat larger than the size of 100 Å generally considered to be characteristic for ribonucleoproteins (Palade & Siekevitz, 1956).

There is also some disagreement as to the fate of the lining bodies. According to Press (1964) they remain permanently attached to the follicle. His evidence is that he failed to find any lining bodies ('transosomes' in his terminology) deeper than the most superficial layer of the oöcyte. He does not appear to have examined the sub-cortical region, however. In contrast to his results, it has been shown in this
Oöcyte and follicle in the hen's ovary

paper that structures closely resembling the lining bodies lie deep within the oöcyte. Although there is no conclusive evidence that they are directly derived from the lining bodies, the similarity is so marked as to be highly suggestive. The fact that comparable structures are also found in vesicles within the follicle cells (vdp in Plate 1, Fig. A) may support the idea that lining bodies can be engulfed by a neighbouring cell (see also below).

As a result of the present investigation, I have also found myself unable to agree with a hypothesis put forward by Schjeide & McCandless (1962) that the lining bodies give rise to mitochondria. In particular, they proposed that the layers $c$ and $d$ (my terminology) form the unit membranes $i$ and $ii$ of the mitochondrion (see Text-fig. 2). This interpretation seems unlikely for two reasons:

(1) To the best of my knowledge lining bodies have never been reported in any other tissue examined by electron microscopy, not even in the ovaries of mammals (Adams & Hertig, 1964; Anderson & Beams, 1960; Odor, 1960; Franchi, 1960; Sotelo & Porter, 1959; Blanchette, 1961; Stegner & Wartenberg, 1961) or of amphibians (Wartenberg, 1962; Hope et al., 1963; Wischnitzer, 1963) where a similar increase in the numbers of mitochondria is taking place (Raven, 1961). It is difficult to accept that birds might possess a unique mode of mitochondrial formation, but even if that were so then it is curious that within them it should be restricted to the ovary.

(2) If Schjeide's hypothesis is correct it is still necessary to explain the fate of membrane $b$ (see Text-fig. 2 and Plate 6, Fig. L).

More recently, however, Schjeide, McCandless & Munn (1964) appear to have partially withdrawn this theory. The eventual fate of the lining bodies is thus still unsettled, though it seems probable that if a lining body enters the oöcyte it does so enveloped in a vesicle and then undergoes some changes. It may be that the lining bodies play a rôle in preparing the oöcyte for yolk formation. This idea is supported by the fact that lining bodies are apparently found only in large-yolked eggs, i.e. those of birds as shown here, and, judging by light microscopical studies, possibly also those of reptiles (Loyez, 1906; Bhattacharya, 1929).

It is not known how the lining bodies form, though Press (1964) has tentatively suggested that the lining bodies found within vesicles inside the follicle cells (vdp in Plate 1, Fig. A) may be in process of developing. An alternative explanation, however, could be that these lining bodies have been engulfed from a neighbouring follicle cell and are in process of transformation, that is, that they are undergoing similar changes to those in the oöcyte. Their appearance, which is strikingly similar to those in the oöcyte, supports this idea.

The pinocytic-like vesicles

The pinocytic-like vesicles, found around the periphery of the early oöcyte and apparently derived by invagination, have been described in mammalian oöcytes (Stegner & Wartenberg, 1961; Adams & Hertig, 1964; Anderson & Beams, 1960)
and in amphibian oocytes (Hope et al., 1963). Several of these authors have also drawn attention to the fact that these vesicles appear to come from the base of the villi at the surface of the oocyte.

It seems possible that the contents of these vesicles are derived ultimately from the follicle cells. The nature of the contents is, however, not known although since they consist of granules of about 100 Å in diameter it is possible they may be ribonucleoprotein particles. This suggestion is supported by the fact that the follicle cells have been shown by the methyl-green-pyronin test to be rich in ribonucleoproteins, at least in the early stages.

The relationship between the follicle cell membrane and the oocyte cell membrane

This has been studied by a number of investigators using electron microscopy in amphibians, mammals and birds. The chick resembles mammals and amphibians in that desmosomes are frequently present between the follicle cell membrane and the oocyte cell membrane (see Plate 1, Fig. A; Franchi, 1960; Adams & Hertig, 1964; Hope et al., 1963; Anderson & Beams, 1960). According to Kemp (1958) intercellular bridges exist between follicle and oocyte in amphibians, birds and mammals. With the benefits of improved electron microscopical techniques, however, all later investigators have been unable to substantiate these findings. In particular, the present results agree with those of Press (1959) in that no evidence for intercellular bridges of this type has been found in birds.

In all three classes of vertebrates, however, an extra-cellular material is laid down during the later stages of development. This is the zona pellucida in mammals (see Sotelo & Porter, 1959; Franchi, 1960) and the vitelline membrane in amphibians and birds. This extra-cellular material appears to have a very different structure in birds from that in mammals and amphibians. In all three types, however, processes from the follicle cells pass across it to make contact with the oocyte. It is of interest that in birds the vitelline membrane starts to appear at about the time when the laying down of the yolk begins. It seems likely that it is secreted by the follicle cells but there is no clear evidence on this point.

The present results agree with those of Schjeide, Wilkens et al. (1963) and Press (1964) in demonstrating that the zona radiata is formed from the surface of the oocyte and that the vitelline membrane is laid down distal to it. The oocyte cell membrane of birds thus resembles that of mammals (Franchi, 1960; Sotelo & Porter, 1959; Stegner & Wartenberg, 1961) and amphibians (Wischnitzer, 1963; Hope et al., 1963) in being raised into microvilli.

The fate of the cell membrane of the oocyte just before ovulation is of interest since it is probably from this structure that the cell membrane of the early cleavage stage cells develop. The large, membrane bounded vesicles at the surface of the oocyte are morphologically reminiscent of the cortical granules of fertilized Echinoderm (Afzelius, 1956; Balinsky, 1961; Mercer & Wolpert, 1962) and of
Oocyte and follicle in the hen's ovary

Xenopus (Balinsky & Devis, 1963) eggs which play an important role in forming the fertilization membrane. It is unlikely that the vesicles in the hen's oocyte are cortical granules, however, for polyspermy occurs in birds and no fertilization membrane is produced (see Romanoff, 1960). It is more likely that the cell membrane becomes discontinuous at this time and develops into a series of vacuoles lying at fairly regular intervals over the surface of the oocyte (a similar fragmentation of cell membranes has been obtained by Goldberg & Green (1960) using Ascites tumour cells under experimental conditions). This somewhat remarkable procedure seems more plausible when one considers that a continuous cell membrane may no longer be necessary at this stage, for the functions of selectivity have apparently been usurped by the follicle capsule, and those of support are probably carried out by the vitelline membrane.

SUMMARY

1. In the early stages of oogenesis, the follicle cells contain a 'complex mass' of organelles which from its size and position apparently corresponds with the region identified as Golgi body by authors using light microscopy. It contains paired centrioles, radiating tubules, patches of Golgi apparatus and an orderly series of stacks of rough-surfaced endoplasmic reticulum which contain a granular material. It is concluded that these cells are probably secretory. The 'complex mass' disappears in the final stages of oogenesis.

2. Using the methyl-green-pyronin test, the follicle cells have been shown to be rich in ribonucleoproteins.

3. Specialized structures termed 'lining bodies' are found at irregular intervals at the surfaces of the follicle cells where they abut on to each other or on to the oocyte. Long processes from the follicle cells indent the surface of the oocyte, bearing lining bodies with them. Although there is no direct evidence that these lining bodies become engulfed by the oocyte, nevertheless structures that are found several microns deep within the oocyte so closely resemble the lining bodies that it seems probable they are derived from them.

4. The available evidence does not support the ideas of Schjeide and his co-workers that the lining bodies give rise to mitochondria.

5. No evidence was found of intercellular bridges between follicle and oocyte.

6. At first, the follicle and oocyte membranes lie close together. Gradually, villous-like elevations arise on the oocyte cell membrane which increase in number and in height. These constitute the zona radiata.

7. Vesicles, containing a granular material, are common in the cortical region of the oocyte. It is possible that these are formed by pinocytosis, for pinocytotic-like invaginations of the oocyte cell membrane are visible. These are especially apparent in the zona radiata.

8. The inner layer of the vitelline membrane forms between the follicle and zona radiata, and at first appears to be moulded around projections of the follicle cells.
9. Just before ovulation the cell membrane of the ovum appears to become fragmented into a series of large vacuoles at the cell surface.

RÉSUMÉ

Des rapports de l’oocyte avec la follicule tels qu’ils apparaissent au microscope électronique dans l’ovaire de la poule

1. Au cours des premiers stades de l’oogénèse, les cellules de la follicule contiennent une masse complexe d’organelles qui d’après leur forme et leur situation correspondent, semble-t-il, à ce que les auteurs qui se servaient d’un microscope ordinaire avaient identifié au corps de Golgi. Cette masse contient des centrioles appariés, des tubules rayonnant, des fractions d’appareil de Golgi et une masse régulière, contenant des matières granuleuses. Ces cellules sont, donc, probablement secrétrices. La ‘masse complexe’ disparaît au cours des stades terminaux de l’oogénèse.

2. La réaction à la pyronine-vert de méthyle met en évidence la richesse des cellules folliculaires en ribonucléoprotéines.

3. On rencontre à intervalles réguliers des formations spécialisées désignées sous le nom de ‘corps de revêtement intérieur’ à la surface des cellules folliculaires où elles s’implantent l’une dans l’autre ou dans l’oocyte. De longs apophyses des cellules folliculaires indentent la surface de l’oocyte, portant avec elles les ‘corps de revêtement’. Bien qu’il n’existe pas de preuve directe que ces corps de revêtement soient enrobés par l’oocyte, néanmoins certains éléments que l’on trouve à plusieurs microns de profondeur dans l’oocyte ressemblent tant aux corps de revêtement interieurs qu’il semble probable qu’ils en dérivent.

4. Les preuves disponibles ne s’accordent pas avec les idées de Schjeide et collaborateurs, à savoir que les corps de revêtement interieurs donnent naissance aux mitochondries.

5. L’existence de ponts intercellulaires entre la follicule, et l’oocyte n’a pas été mise en évidence.


8. La couche interne de la membrane vitellique est formée entre la follicule et la zona radiata, et elle semble au premier abord être moulée autour des prolongements des cellules folliculaires.

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EXPLANATION OF PLATES

Abbreviations

c' centriole
cr crypt
d desmosome
f follicle cell
G Golgi membranes
lb lining body
lbd vesicles thought to be derivatives of lining bodies
lvg large vesicles containing granules
m mitochondrion
mt microtubules
n nucleus

nf neutral fat drop
o oöyte cell
p pinocytic-like indentation
pr protuberance
sm stacked membranes
sv small vesicles
v vesicle
va vacuole
vdp vesicles containing dense patches
vg vesicles containing granules
vm vitelline membrane
zr zona radiata

PLATE 1

Fig. A. Section through follicular-oöyte border (diameter of oöyte 0.25 mm.). Desmosomes (d) are present at the junction. Fixed in osmium tetroxide and stained with potassium permanganate. × 20,000. Inset. Enlargement of small vesicles. × 100,000.
PLATE 2

Fig. B. Section through portions of three follicle cells a, b and c (diameter of oocyte 0.4 mm.). Cell a is more electron opaque and vacuolated than its neighbours. Fixed in glutaraldehyde and post-fixed in osmium tetroxide. Stained with potassium permanganate. × 17,000.

Fig. C. Section through a follicle cell (diameter of oocyte 3.5 cm.). Note the large vacuoles, some of which appear to be continuous with one another. Fixed in osmium tetroxide and stained with phosphotungstic acid and potassium permanganate. × 34,000.

Fig. D. Section of fine filaments present in a follicle cell (diameter of oocyte 1.5 mm.). Fixed in osmium tetroxide and stained with phosphotungstic acid and potassium permanganate. × 72,000.

Fig. E. Section through part of a follicle cell to show the glycogen present in the cytoplasm. Diameter of oocyte 0.1 mm. Fixed in osmium tetroxide and stained with lead citrate. × 60,000.
Plate 3

Fig. F. Part of a follicle cell to demonstrate the microtubules (mt) extending out to the cell boundary (diameter of oocyte about 0.2 mm.). Note the large numbers of small vesicles present. Fixed in glutaraldehyde and post-fixed in osmium tetroxide. Stained with potassium permanganate. × 24,000.

Fig. G. Centriole from the centre of a complex mass in a follicle cell and some of the microtubules which appear to radiate from it. (Same specimen as Fig. J below). × 75,000.

Fig. H. Section through part of a follicle cell (diameter of oocyte 2 mm.). Notice the two sizes of mitochondrial profile present. Fixed in glutaraldehyde, post-fixed in osmium tetroxide. Stained with potassium permanganate. × 23,500.
Fig. 1. Junction of two follicle cells. The cell at the left has two lining bodies; the cell at the right has one. Note how the shape of the lining body follows that of the cell membrane. Fixed in osmium tetroxide. Stained with potassium permanganate. × 68,000.

Fig. J. Section through follicular-oocyte border (diameter of oocyte about 0.2 mm.). The 'complex mass' of the follicle cell lies between the nucleus and the oocyte border; its limits are not clearly defined but are indicated by arrows. Note, although microtubules are present in the complex mass, they are also numerous adjacent to the nucleus. The structure c' when examined at a higher magnification was found to be a centriole. Fixed in glutaraldehyde and post-fixed in osmium tetroxide. Stained with potassium permanganate. × 34,000.
PLATE 5

Fig. K. Section through the cortex of the oöcyte illustrated in Fig. A. Note the similarity between the vesicles containing dense patches (vdp) in the follicle cell, and the vesicles thought to be derived from lining bodies (lbd) in the oöcyte. Note the small granules and the beaded filaments in the oöcyte cytoplasm. × 24,000.
PLATE 6

Fig. L. Longitudinal section through a lining body that is indenting the cell membrane of the oocyte. Compare with Fig. M and with Text-fig. 2. Fixed in glutaraldehyde, post-fixed in osmium tetroxide and stained with potassium permanganate. × 200,000.

Fig. M. Transverse section through a lining body similar to the one shown in Fig. L. (Preparation as in L.) × 130,000.

Figs. N & O. Transverse sections of structures in the oocyte that appear to be derivatives of lining bodies. Compare with Figs. M and N and Text-fig. 2. Membranes c and d cannot be distinguished easily from membrane b. Fixed in osmium tetroxide and stained with potassium permanganate. × 130,000 and × 144,000 respectively.
PLATE 6

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Fig. P. Section through follicular–oocyte border (diameter of oocyte 0·3 mm.). The follicle cells lie at the top, the oocyte at the bottom of the picture. The oocyte cell membrane is indented in places and may be in process of forming pinocytic vesicles. Fixed in osmium tetroxide and stained with potassium permanganate. × 104,000.

Fig. Q. Section through follicular–oocyte border (diameter of oocyte 0·06 mm.) to show protuberances of oocyte cell membrane at the junction of two follicle cells (f′ and f″). Fixed in osmium tetroxide and stained with lead citrate. × 30,000.

Fig. R. Surface of the oocyte to show the villous processes of the zona radiata (zr) and the first signs of the vitelline membrane (vm) (diameter of oocyte 2·5 mm.). Fixed in glutaraldehyde and stained with lead citrate. × 50,000.

Fig. S. Enlargement of part of Fig. U to show the membrane (arrowed) surrounding one of the vacuoles at the surface of a large oocyte. × 22,500.
Fig. T. Section through follicular-oocyte border (diameter of oocyte 2.5 mm.). Fixed in potassium permanganate and stained with uranyl acetate. × 20,000.

Fig. U. Section through the surface of an oocyte (diameter 3 cm.) to show the large vacuoles beneath the surface (cf. Plate 7, Fig. S). Note that processes from the follicle cell extend down into the vitelline membrane. Fixed in glutaraldehyde and post-fixed in osmium tetroxide. Stained with lead citrate. × 9000.