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The structure of the germinal disc region of the hen's ovarian follicle during the rapid growth phase

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INTRODUCTION

The third and final growth phase of the hen's ovarian follicle is characterized by a rapid increase in size resulting mainly from the incorporation by the oocyte of yellow yolk precursors from the circulation. By contrast, in the preceding growth phase incorporation is restricted to white yolk precursors. This pigmentation reflects a change in the predominant composition of the yolk from protein to lipid (see Gilbert, 1971*a*, *b*).

The germinal disc, which attains a diameter of 2–3 mm in the mature ovum, is visible as a white plaque at the surface of the rapidly growing oocyte. This region of the cell includes the germinal vesicle and the surrounding mass of dense cytoplasm (Romanoff, 1960; Jordanov, 1969). It lies on top of a column of white yolk which extends from the latebra, a core of white yolk at the centre of the cell. This suggests that the germinal disc is unable to incorporate yellow yolk precursors. Furthermore, when the dyes Sudan III and Evans blue are administered they are taken into the oocyte but leave the germinal disc unstained (Warren & Conrad, 1939; Smith, 1959). It therefore seems to provide a convenient control system for investigations on the transport of yellow yolk into the cell.

Observations with the electron microscope have shown that follicles in the third growth phase (Wyburn, Aitken & Johnston, 1965*a*; Wyburn, Johnston & Aitken, 1965*b*) differ from those in the second growth phase (Press, 1964; Bellairs, 1965; Wyburn *et al.* 1965*a*, *b*; Greenfield, 1966; Schjeide *et al.* 1970; Paulson & Rosenberg, 1972) in two main respects. The cells of the granulosa layer become separated, facilitating the movement of intercellular material across this layer. In addition the oocyte surface is modified for the extensive absorption of macromolecules by the formation of deep pouches associated with large, pinocytotic coated vesicles in place of the smaller coated vesicles and microvilli of the preceding phase (Perry, Gilbert & Evans, 1978). In this report the structure of the germinal disc, comprising nuclear and cytoplasmic regions, is described and compared with the major part, i.e. the non-disc region, of the follicle, particularly in relation to yolk transport mechanisms.

MATERIALS AND METHODS

The stocks of birds, methods of collection of follicles of between 15 and 30 mm diameter, and the procedures for processing them for electron microscopy were similar to those described in the preceding paper (Perry *et al.* 1978). It was found that the individual follicular layers required particular methods of fixation. The following methods were employed:

Method 1. The loose outer covering of connective tissue was removed manually and the follicles immersed in a fixative containing either 5% acrolein, 2% glutaraldehyde and 2 mM-$CaCl_2$ in 0·1 M sodium cacodylate buffer at pH 7·2, or 1% acrolein, 1·5% paraformaldehyde and 2·5% glutaraldehyde in the same buffer, for 24 hours at room temperature. They were transferred to a buffer of 0·075 M sodium cacodylate containing 0·2 M sucrose. The germinal disc with attached theca was excised from the follicle and some of the adherent yolk removed to reveal the position of the germinal vesicle and also to facilitate penetration of the processing fluids. Strips of the non-disc follicle wall were also taken for comparison.

Method 2. The theca was stripped off (Gilbert, Evans, Perry & Davidson, 1977) to allow rapid penetration of the fixative into the granulosa and oocyte. This manipulation pulled the granulosa cells apart. The stripped follicles were fixed in dilute Karnovsky's solution containing 2% paraformaldehyde, 2·5% glutaraldehyde and 2 mM-$CaCl_2$ in 0·1 M sodium cacodylate buffer, for 2 hours at room temperature. The disc was then removed. The material from Methods 1 and 2 was post-fixed in 1% osmium tetroxide in veronal acetate buffer, pH 7·2, containing 0·2 M sucrose, for 2 hours at 4 °C, rinsed briefly in the buffer and then stained *en bloc* in a solution of 0·5% uranyl acetate in veronal acetate buffer, pH 5·0, for 1·5 hour at room temperature.

Method 3. The stripped follicles were kept intact until transferred to the lower alcohols to obtain suitable preservation of the germinal disc cytoplasm. In the example illustrated in Figure 13 the material was fixed in 2·5% glutaraldehyde in 0·1 M sodium cacodylate buffer, rinsed briefly, and post-fixed in 1% osmium tetroxide in the same buffer.

The tissue blocks from all methods were dehydrated in a graded series of alcohols, infiltrated with epoxy propane, and embedded in an Epon/Araldite mixture. Thin sections were mounted on Formvar/carbon coated grids, stained sequentially with uranyl acetate and lead citrate, then examined in an electron microscope.

To obtain an estimate of the number of dividing cells in the granulosa, this layer was stripped off the oocyte in saline and the area above the germinal disc was separated from the rest of the granulosa. The cell sheets were stained in orcein–HCl, dehydrated and mounted. An interval of 0·5–1 hour elapsed between the time of death and fixation of the material. Counts were made of the number of mitoses in fields outlined by the frame graticule of the focusing telescope of a light microscope. In each follicle, ten randomly selected fields were scored in the non-disc granulosa and four fields in the disc granulosa. The number of cells/field was estimated from counts on photomicrographs of 1 field/region/follicle.

RESULTS

In the hen's ovarian follicle the oocyte is encased in a relatively thin but complex sheath of tissue forming the follicle wall. The theca externa, constituting the bulk of the follicle wall, is a tough supporting layer traversed by blood vessels. The narrower theca interna is highly vascularized and contains groups of interstitial cells. Beneath the theca is a single layer of granulosa (follicle) cells, bounded distally by a basal lamina and proximally by the perivitelline layer which circumscribes the oocyte (see Perry *et al.* 1978).

The theca

Observations on the theca above the germinal disc showed that it was essentially similar in structure to the rest of the theca. In so far as the vascular supply was concerned, the endothelial cells lining the capillaries in the theca interna were perforated by pores; the capillaries were not enclosed in a basal lamina. Erythrocytes were frequently packed together in the capillaries; some were also found lying freely in the wide spaces around the capillaries and the granulosa basal lamina.

The basal lamina

This layer was, on average, 0·66 μm thick, as compared with 1·0 μm elsewhere in the follicle. In many cases, especially in follicles fixed by Method 1 (5% acrolein, etc.), tangentially orientated striations were evident. At higher magnifications the striations were less distinct and merged with the granular matrix, which constituted the bulk of the lamina. Poorly defined particles could also be discerned in the matrix. As in the non-disc region, these particles were clearly revealed (Fig. 1) in preparations stripped of their thecal coverings before fixation (Method 2). They were of low electron density and measured 20–30 nm in diameter.

The granulosa layer

Over short sections the cells were uniformly arranged as a row of closely packed cuboidal cells; more commonly they had a staggered arrangement, with nuclei located either apically or basally, and the cells were less closely packed (Fig. 2). In many cells the nucleolar material was dispersed. A few mitotic figures were noted in follicles of all sizes, contrary to observations on the non-disc region where dividing cells have seldom been observed except in small follicles of less than 15 mm diameter. The irregular cellular arrangement may therefore be explained by the fact that above the germinal disc the granulosa consists of a growing cell population. To test this proposal large areas of granulosa were examined by light microscopy for the presence of dividing cells. The results are given in Table 1 and Figure 2 (inset).

The location and dimensions of the intercellular spaces showed considerable variation across the layer (Fig. 2). Broad gaps were often present where neighbouring cell margins diverged at intersections with the basal lamina. Cells in the process of dividing tended to become round and separated from adjacent cells. Proximally the intercellular spaces were in general narrower, but close association was prevented by the presence of microvilli which extended from the lateral cell margins. In areas where the cells were uniform their lateral margins were smooth and closely apposed (Fig. 3), being separated by gaps of about 13 nm in the apical regions. Here specialized cell junctions in the form of maculae adherentes were fairly common. They consisted of dense cytoplasmic plaques on either side of the parallel plasma membranes. In rare instances adjacent plasma membranes in the basal regions appeared to fuse, indicating gap or tight junctions. Thus, in contrast to the rest of the granulosa, wide intercellular spaces above the disc were discontinuous. They contained mere traces of the granular material which normally fills the wide intercellular channels. At all stages of growth some clumps of electron-dense material were seen in the basal spaces; such clumps have been noted only in the non-disc region of mature follicles, where there is a reduction in the rate of yolk uptake.

A description of cytoplasmic structures will be confined to cells which spanned the width of the layer and may be assumed to be in the resting stage of the cell cycle.

Table 1. *Cell division in the granulosa of ovarian follicles in the final growth stage*

Follicle size (mm)	No. follicles	Percentage mitoses	
		Germinal* disc region	Non-disc region
15–20	4	1·0	0·5
20–25	4	0·9	0·1
25–30	4	0·3	0·03

* No mitoses were observed above the germinal vesicle.

In the apical cytoplasm were Golgi bodies, numerous small vesicles, several dense granules and, occasionally, paired centrioles (Fig. 4). The heterogeneous granules which are prominent in non-disc cells were seldom observed. Rough endoplasmic reticulum occurred as scattered, small cisternae and as stacks of flattened cisternae, especially in the basal cytoplasm (Fig. 5). Typical mitochondria were randomly distributed throughout the cell. Many free ribosomes and polysomes were embedded in the dense cytoplasmic matrix.

The perivitelline layer

This layer consisted of a meshwork of branched fibres of high electron density (Fig. 2). In follicles about 15 mm in diameter it was less well developed in the region peripheral to the vesicle than it was directly above the vesicle and in non-disc regions (cf. Figs. 6–8). It exhibited a sudden growth in follicles about 25 mm in diameter, increasing to a uniform thickness of 1·5 μm over the entire disc area. The component fibres also showed an increase in diameter, from 0·05–0·1 to 0·1–0·2 μm, with follicular growth. They were more numerous and thinner than the fibres in the non-disc region. These differences would be expected if, as proposed for insect follicles (Beams & Kessel, 1969), the vitelline layer is a product of the granulosa cells, and if these cells are proliferating above the germinal disc.

The surface layer of the oocyte

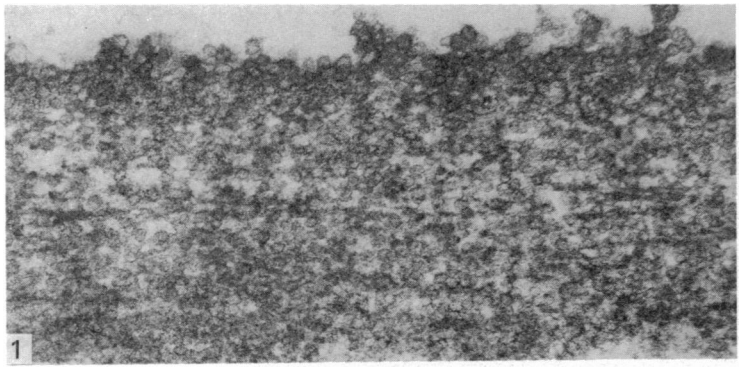
The oocyte periphery showed distinct structural differences in its disc and non-disc regions. Differences within the disc also occurred with increasing follicle size and with proximity to the germinal vesicle. In follicles about 15 mm in diameter (Fig. 9) the surface was covered with numerous microvilli which persisted throughout further stages of growth except in the area over the germinal vesicle (cf. Figs. 10 and 11). Bundles of aligned microfilaments extended deep into the cytoplasm from the micro-

The granulosa layer above the germinal disc

Fig. 1. The basal lamina contains numerous particles of low electron density. Faint linear elements lying in the plane of the lamina are evident. Fixation Method 2; > 25 mm follicle. × 70000.

Fig. 2. The granulosa cells are irregularly arranged with spaces of variable width between them. *BL*, basal lamina; *PVL*, perivitelline layer. Fixation Method 1; 28 mm follicle. × 6000. Inset: mitoses in a whole mount preparation of the granulosa. × 800.

Fig. 3. To show a region of close contact between the cells. Distally microvilli are compressed between the cell margins, proximally the lateral margins are closely apposed and attached at a cell junction (*CJ*). Fixation Method 1; 25 mm follicle. × 15000.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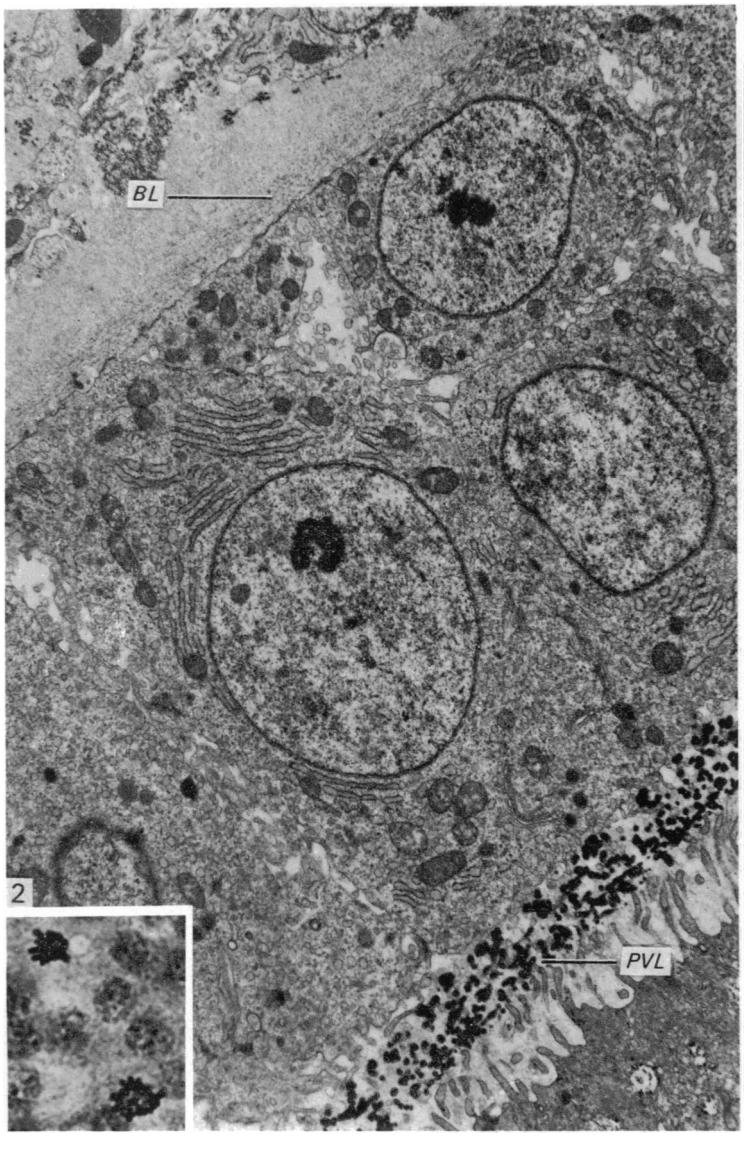
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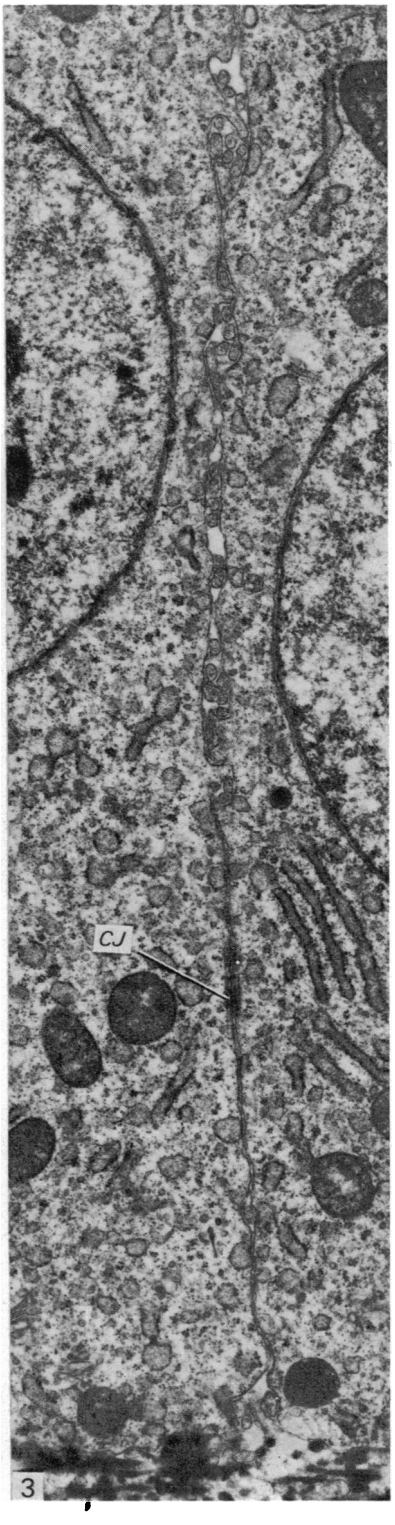
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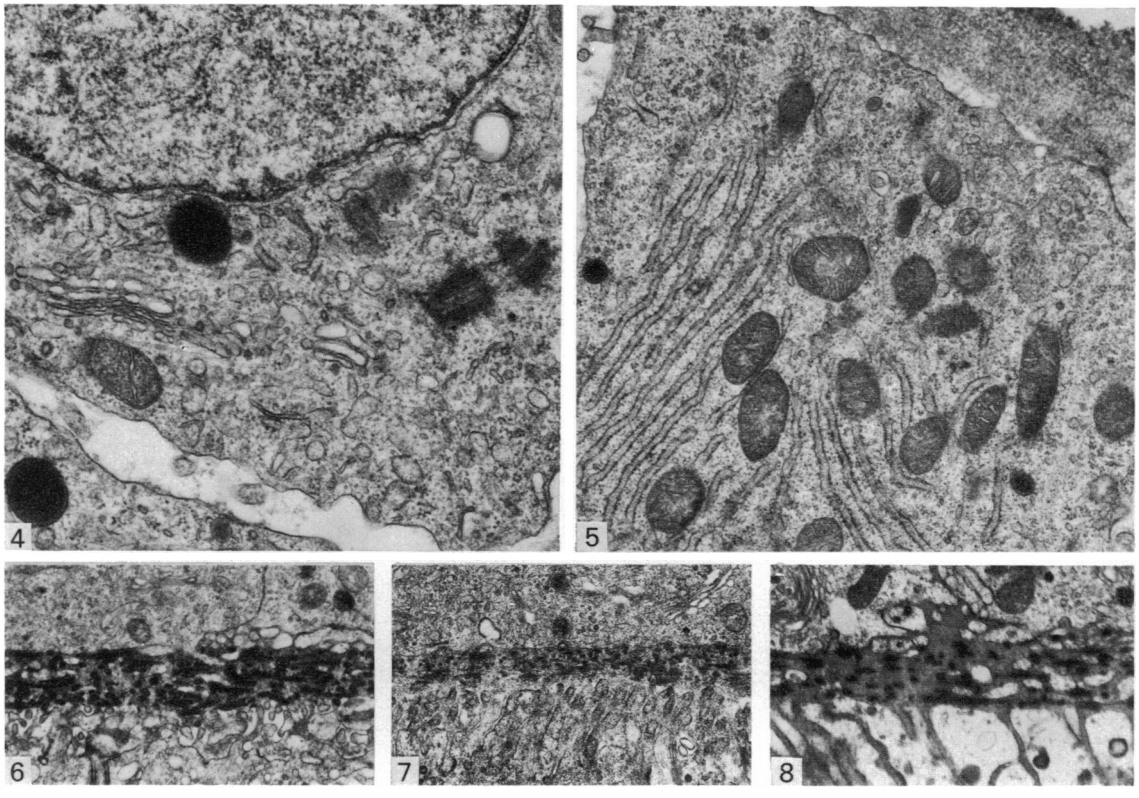


The granulosa layer above the germinal disc

Fig. 4. In the apical cytoplasm of the granulosa cells are Golgi elements, dense granules and centrioles. Fixation Method 2; > 25 mm follicle. × 22000.

Fig. 5. In the basal cytoplasm of the granulosa cells are stacks of rough endoplasmic reticulum embedded in a dense matrix containing abundant ribosomes. Fixation Method 2; > 25 mm follicle. × 14000.

Figs. 6–8. The perivitelline layer exhibits regional differences in thickness in a 16 mm follicle. Fig. 6: above the germinal vesicle. Fig. 7: the germinal disc peripheral to the vesicle (cf. the larger follicle in Fig. 2). Fig. 8: the non-disc region. Fixation Method 1; × 7500.

villi (Fig. 13). In the non-disc region microvilli were comparatively sparse. At irregular intervals macrovilli protruding from the granulosa cells extended into narrow indentations in the oocyte surface. Small invaginations in the form of coated pits were present along the sides of the indentations and at the bases of the microvilli. In the superficial cytoplasm were coated vesicles, some of which were of similar dimensions to the coated pits, ranging from 110 to 130 nm in diameter; other, more numerous coated vesicles, were 70–80 nm in diameter. The coats appeared as arrays of dense bristles on the cytoplasmic aspect of the bounding membrane or, in grazing sections of the vesicles, as a lattice of polygonal subunits (Fig. 15).

In follicles about 25 mm in diameter, the surface above the germinal vesicle was fairly smooth (Fig. 10), and few of the features described above were evident. In the surrounding area (Fig. 11) macrovilli and 110–130 nm coated vesicles were uncommon, whereas at the periphery of the disc (Fig. 13) they had a similar distribution to that seen at earlier stages. These appearances were in marked contrast to the structure

of the bulk of the surface layer of the oocyte. Throughout the third growth stage it was pitted with deep pouches, contained numerous 250–350 nm coated pits and vesicles (Fig. 12), and macrovilli featured prominently. Another point of difference was the absence from the germinal disc of a diffuse coating of material on the external leaflet of the oolemma (cf. Figs. 14 and 15). In the perivitelline space above the disc were traces of the granular material which normally is seen to fill the intercellular compartment around the rest of the oocyte, but is mostly extracted in stripped preparations (Method 2).

Other components embedded in the dense cytoplasmic matrix of the disc were many smooth membranes in the form of stacked cisternae, scattered vesicles and, near the yolk mass, definitive Golgi bodies. Small mitochondria and accumulations of dense granules, probably glycogen, were also noted. The structural composition of the disc cytoplasm is not unlike that of the early developing blastoderm described by Gipson (1974).

DISCUSSION

The observations show several differences, not only between the germinal disc and non-disc regions of the ovarian follicle, but also within the disc itself, subdividing it into a zone above the germinal vesicle and a zone peripheral to the vesicle. The distinguishing features relate to the granulosa layer and the superficial layer of the oocyte; they are illustrated diagrammatically in Figure 16.

The role of plasma proteins and lipoproteins in the growth of the oocytes of oviparous species has been discussed by Schjeide *et al.* (1970). In the ovarian follicle of the domestic fowl, Perry *et al.* (1978) have related the following features to the transport of material from the circulation in the third growth phase of yellow yolk deposition: (1) fenestrated thecal capillaries and free erythrocytes in the theca interna, indicating that many macromolecular components of the plasma have free access from the capillaries to the granulosa basal lamina, (2) numerous 20–30 nm particles in the basal lamina, identified as very low density lipoprotein (VLDL) (Evans, Perry & Gilbert, unpublished), (3) wide spaces between the granulosa cells and in the meshwork of the perivitelline layer, forming continuous intercellular channels for the passage of the basal lamina filtrate to the oocyte, (4) numerous 250–350 nm coated pits and vesicles in the highly convoluted surface layer of the oocyte, indicative of pinocytosis. By contrast, in the second growth phase of white yolk deposition, the granulosa cells are more closely packed, many microvilli project from the surface of the oocyte, and the coated vesicles range from 70–120 nm in diameter. In addition there is evidence of direct transfer to cytoplasmic components from the granulosa cells via specialized structures, the transosomes or lining bodies (Press, 1964; Bellairs, 1965; Wyburn *et al.* 1965*a*, *b*; Greenfield, 1966; Schjeide *et al.* 1970; Paulson & Rosenberg, 1972).

The present observations on the germinal disc region show that here too the plasma components, including the VLDL particles, can gain access to the basal lamina. However, it appears that the movement of the basal lamina filtrate across the granulosa layer is impeded by the lack of wide intercellular channels; indeed, the smallest distances of separation between the apices of some cells are not of sufficient width to permit the passage of some macromolecular entities. It is in the oocyte that the most striking differences between disc and non-disc regions occur. In general the surface layer of the germinal disc bears a resemblance to that of the second growth phase oocyte in possessing microvilli and 70–120 nm coated vesicles,

i.e. it retains characteristics associated with white yolk incorporation. Towards the end of oocyte growth, around 24–48 hours before ovulation, pinocytosis virtually ceases, but a continued absorption of small molecules is indicated by the presence of microvilli. This trend towards a decrease in pinocytotic activity in the disc suggests that the germinal vesicle is exerting a controlling influence on the action of extraneous factors such as hormones which have been reported to promote yolk uptake in amphibian (Follett, Nicholls & Redshaw, 1968) and insect oocytes (Bell & Barth, 1971). Hormonal stimulation of pinocytosis has been demonstrated in the urinary bladder of the toad (Masur, Holtzman, Schwartz & Walter, 1971). The inhibition of yolk transport is clearly required to prevent the cell's nucleus from being embedded in a mass of yolk which would adversely effect the nuclear events of fertilization.

The variations in the size of the coated vesicles in the third growth phase oocyte may have some functional significance. In general these organelles fall into two categories on the basis of their diameter, namely around 70 nm and around 120 nm. The former have been implicated in transport between intracellular compartments (Friend & Farquhar, 1967) and the latter in the transport of protein from the extracellular milieu (Roth & Porter, 1964; Friend & Farquhar, 1967; Wallace & Dumont, 1968; Rodewald, 1973; Moxon, Wild & Slade, 1976). The involvement of the small vesicles in the recycling of the plasma membrane of secretory cells has also been proposed (Heuser & Reese, 1973). The bristle coating, which is formed of a lattice of polyhedral subunits, has been isolated from both small and large vesicles in a variety of cells and shown to consist of a single protein species (Pearse, 1976). The vesicles in the germinal disc correspond in size with those found in other cell types. The 120 nm vesicles are presumably involved in the absorption of white yolk precursors, whilst the 70 nm vesicles may be related to activities of the perinuclear region concerned with the synthesis of material required for oocyte growth and the formation of the blastodisc cytoplasm. The largest vesicles, on the other hand, seem to be unique to the hen's oocyte and as such may be a modification for the absorption of yellow yolk in the non-disc region. Whether their size is related to the quality or quantity of material absorbed remains to be elucidated. The selective uptake of proteins (immunoglobulins) into coated vesicles has been demonstrated in the intestine of the suckling rat (Rodewald, 1973) and rabbit yolk sac endoderm (Moxon *et al.* 1976). Furthermore, the latter authors have concluded that the coated vesicles participate in the transport of intact proteins across the cell layer, whereas the

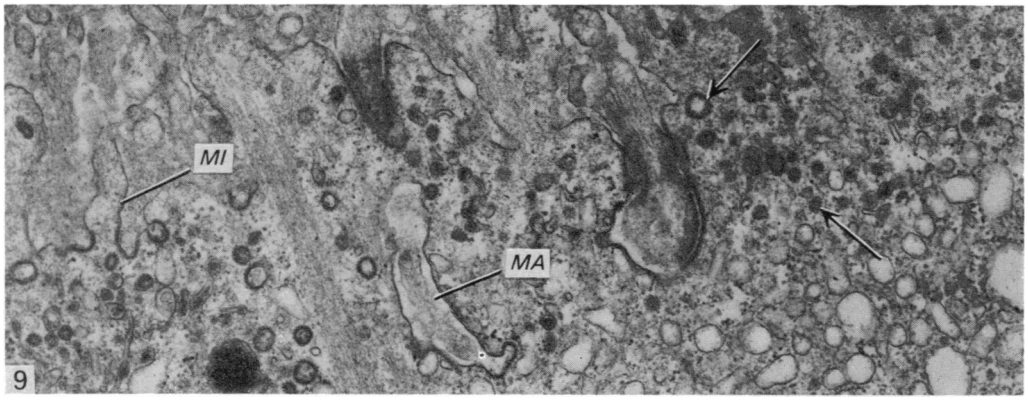
The surface layer of the oocyte

Fig. 9. Coated vesicles of two sizes, 70 and 120 nm (arrowed), are present in the cytoplasm of the germinal disc. The oocyte surface is thrown into microvilli (*MI*) and deep indentations containing macrovilli (*MA*) from the granulosa cells. Fixation Method 1; 19 mm follicle. ×22000.

Fig. 10. In a larger follicle the surface of the germinal disc directly above the germinal vesicle (*GV*) is irregular. In the perivitelline space there are a few microvilli (*MI*) together with traces of granular material. Fixation Method 2; > 25 mm follicle. ×22000.

Fig. 11. Peripheral to the germinal vesicle numerous microvilli extend from the surface. Some 70 nm coated vesicles (arrowed) are scattered in the cytoplasm; the 120 nm coated vesicles are absent (cf. Fig. 9). Fixation Method 2; > 25 mm follicle. ×22000.

Fig. 12. The non-disc region of the same follicle as in Fig. 11 has a highly irregular surface with few microvilli. Numerous coated pits and vesicles, about 300 nm in diameter, are evident. There are some traces of the granular material which fills the perivitelline space in intact follicles fixed by immersion. ×22000.



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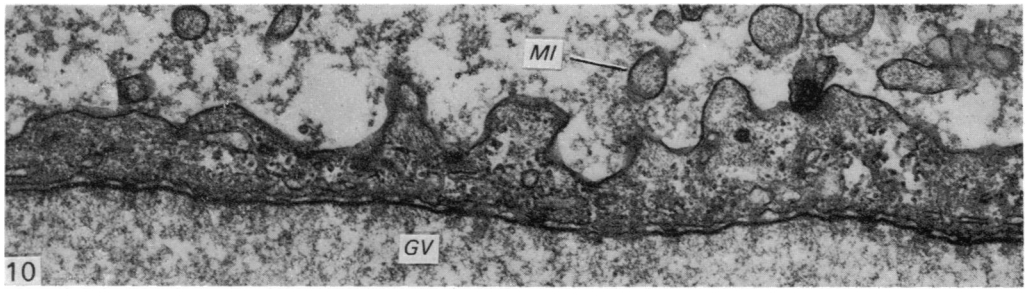

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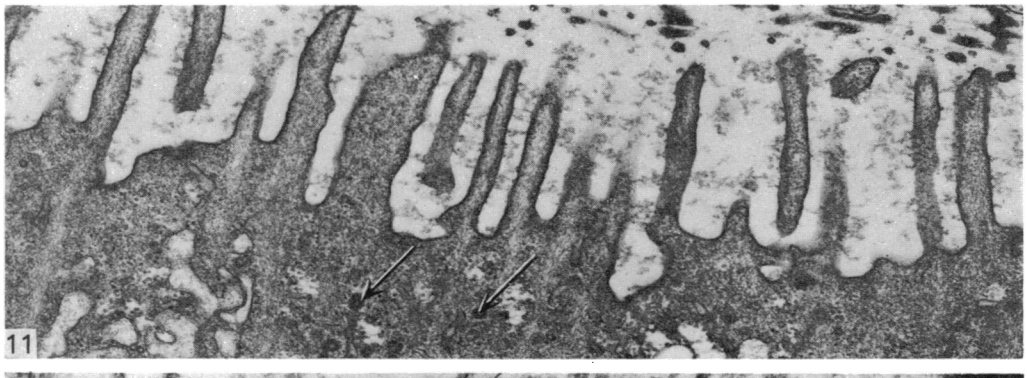

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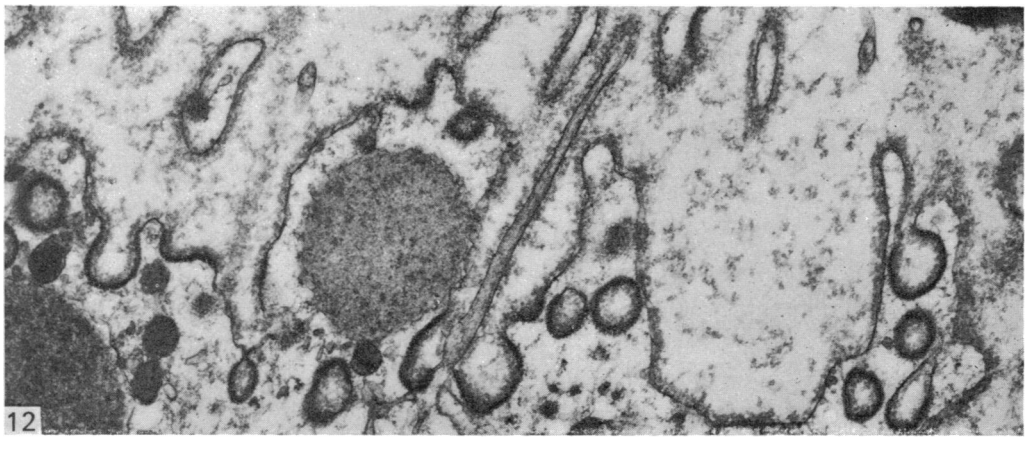

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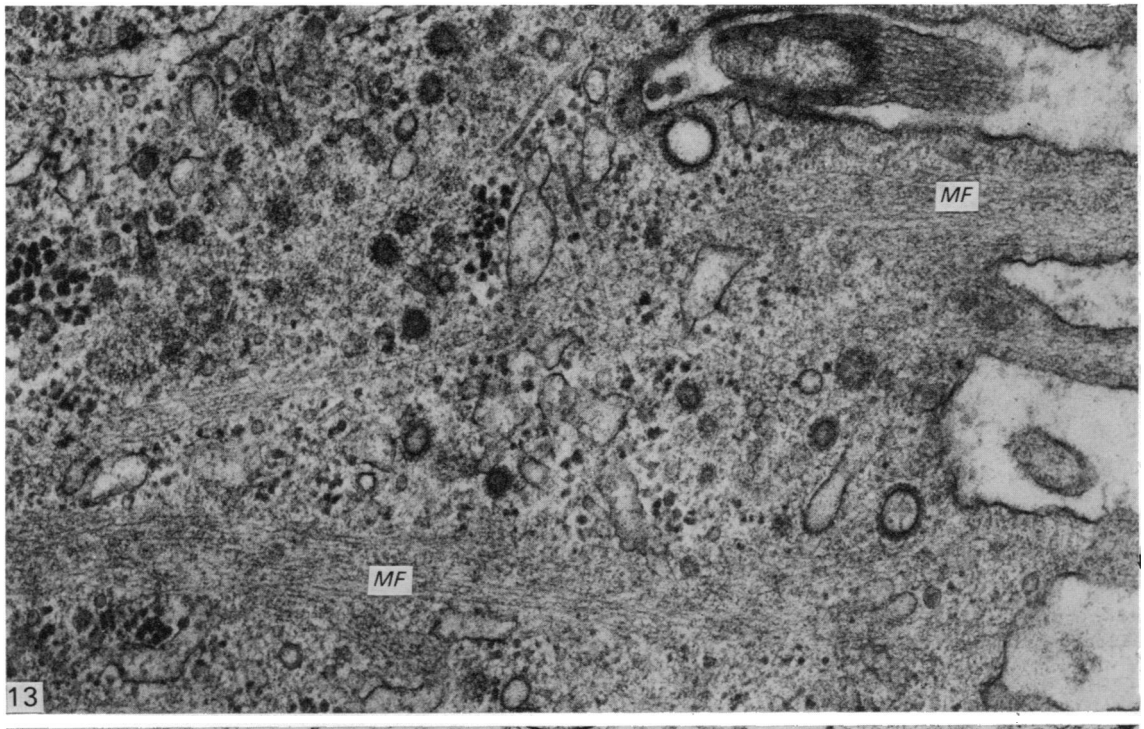

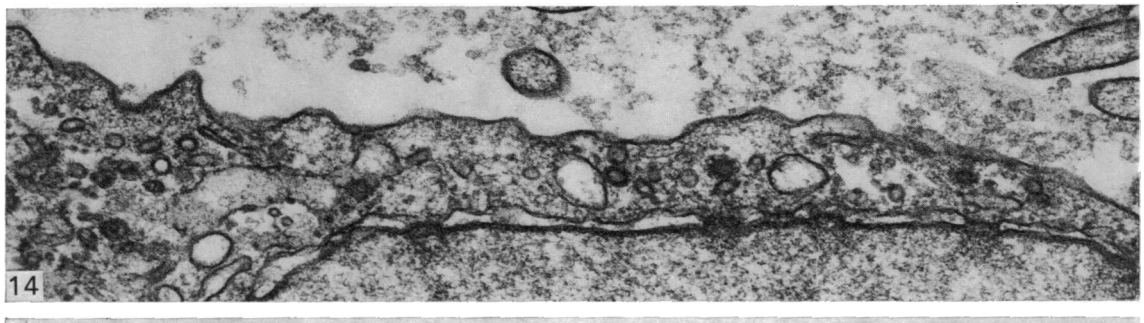

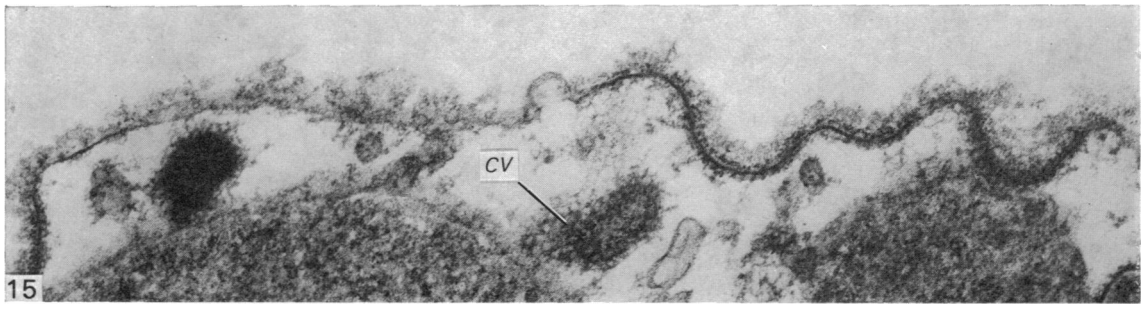

The surface layer of the oocyte

Fig. 13. The peripheral region of the germinal disc in large follicles retains the characteristics of the earlier disc in possessing coated vesicles of two sizes (cf. Fig. 11). Bundles of cytoplasmic microfilaments (*MF*) extend into the microvilli. Also present are smooth-surfaced vesicles and scattered glycogen granules. Fixation Method 3; > 25 mm follicle. × 52 000.

Fig. 14. The oolemma above the germinal disc has no external coating. Fixation Method 2; > 25 mm follicle. × 52 000.

Fig. 15. The oolemma of the non-disc region is uniformly covered with a layer of fuzzy material. A grazing section shows the polygonal arrangement of subunits on the cytoplasmic aspect of a coated vesicle (*CV*). Fixation Method 2; > 25 mm follicle. × 52 000.

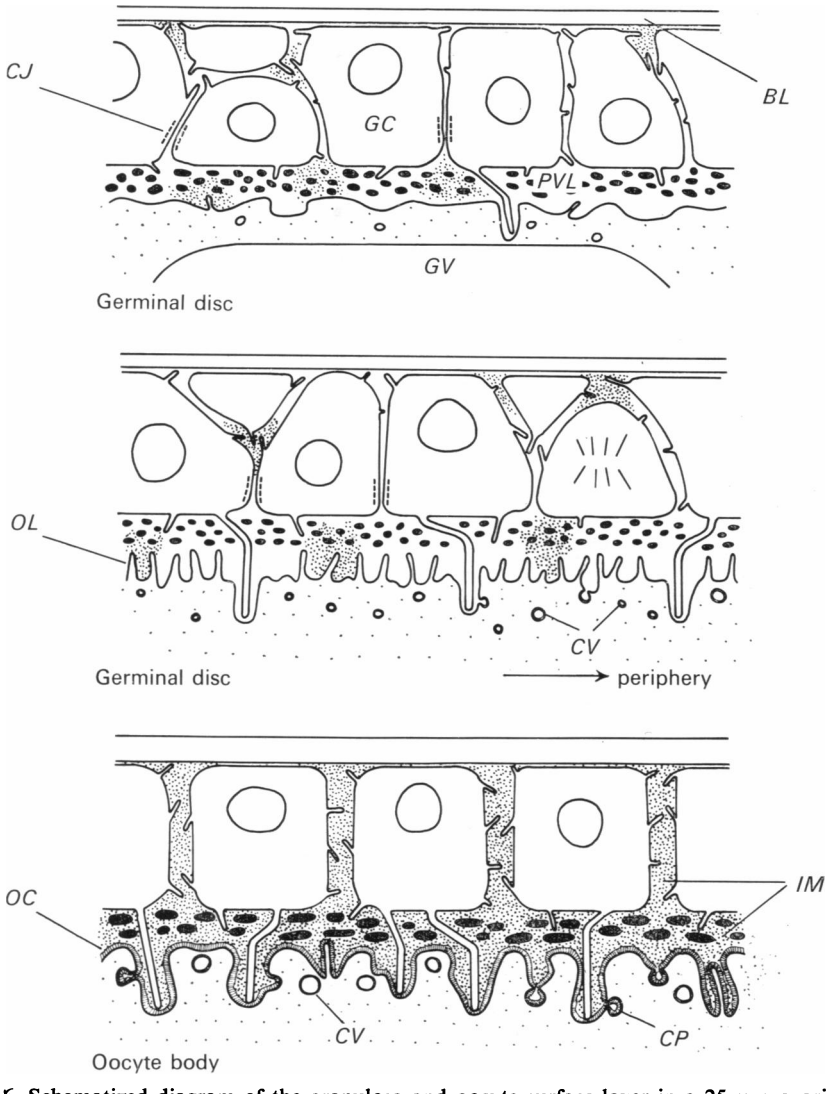

Fig. 16. Schematized diagram of the granulosa and oocyte surface layer in a 25 mm ovarian follicle to show regional differences in structure. The germinal disc, comprising the germinal vesicle and surrounding cytoplasm, overlies a column of white yolk. The non-disc region overlies the yellow yolk mass of the oocyte body. *BL*, basal lamina; *CJ*, cell junction; *CP*, coated pit; *CV*, coated vesicle; *GC*, granulosa cell; *GV*, germinal vesicle; *IM*, intercellular material; *OL*, oolemma; *OC*, oolemmal coat; *PVL*, perivitelline layer. The drawing is not to scale.

contents of the smooth-surfaced, macropinocytotic vesicles are destined for degradation within the lysosomal complex. A useful parallel can perhaps be drawn with the coated vesicle system in insect (Roth & Porter, 1974), amphibian (Wallace & Dumont, 1968) and hen oocytes, in which the absorbed material is stored until utilized for embryonic development.

The absence of the layer of diffuse material from the external surface of the germinal disc oolemma raises the question of the surface properties of the two regions. As a rule surface coats of cells can only be demonstrated with the use of certain electron-dense stains (Luft, 1976). The fuzzy layer may therefore represent material

adsorbed to the oolemma of the non-disc region. Since extracellular material is present throughout the perivitelline space it is likely that the adsorptive capacity of the plasma membrane differs in the two regions, by virtue perhaps of a differential distribution of surface receptors. Surface adsorption has been proposed as a mechanism for selective incorporation, as opposed to the non-selective incorporation of substances contained in the fluid phase of the pinocytotic vesicles (Jacques, 1969; Williams, Kidston, Beck & Lloyd, 1975).

In regard to the growth and differentiation of the granulosa and its attendant acellular layers, the regional differences suggest that the disc region is in a comparatively immature state. Apart from the presence of mitoses, the differences in the granulosa cells comprise the dense cytoplasmic matrix containing populations of polysomes, the absence of heterogeneous granules, and fewer smooth cytomembranes. The macrovilli linking the cell layer to the oocyte are also less abundant. The perivitelline layer and its component fibres are thinner, particularly in smaller follicles, indicating that they are of more recent origin than those in non-disc regions. This may also be the case for the basal lamina. The germinal disc region may well be considered as the growth centre for the granulosa in follicles larger than 15 mm, contributing to the lateral expansion of the layers adjacent to the oocyte. However, the flattening of the granulosa cells in non-disc regions is probably a major factor in this process. The gradual decline in the number of mitoses is a prerequisite for the onset of functional differentiation of the granulosa and seems to occur once the cells are removed from the sphere of influence of the germinal disc. Although the effects of hormones on the growth of the hen's follicle are well established (see Gilbert, 1971*a*), the extent to which they act at the cellular level is a matter for investigation. The opposing roles of growth factors and gonadotropins respectively in controlling proliferation of cultured mammalian granulosa cells has been discussed recently by Gospodarowicz, Ill & Birdwell (1977).

SUMMARY

The structure of the ovarian follicle in the region of the germinal disc, which appears as a white plaque at the surface of the oocyte, was examined by electron microscopy and compared with the non-disc region which overlies the yellow yolk mass of the oocyte in the final growth phase. The main differences concerned the granulosa cell layer and the surface layer of the oocyte.

In the disc the granulosa cells were less regularly arranged and the spaces beween them varied in width. Their mitotic rate was higher than that in the non-disc region, where cell division was seldom observed at maturity. The perivitelline layer was comparatively poorly developed at the periphery of the germinal vesicle in 15 mm follicles, but eventually attained a uniform thickness throughout the follicle. In the intercellular and perivitelline spaces there were smaller amounts of granular material.

Marked differences were observed in the oocyte surface layer. In 15 mm follicles the surface of the germinal disc was thrown into numerous microvilli and some narrow indentations containing macrovilli from the granulosa cells. Coated vesicles, 120 nm diameter, appeared to be invaginating from the oolemma, whereas 70 nm coated vesicles were present in the deeper cytoplasm. In follicles of more than 25 nm diameter these structural conformations were evident only at the periphery of the disc; for the most part the 120 nm coated vesicles were absent, and over the germinal vesicle microvilli were of rare occurrence. On the other hand, the bulk of the oocyte

surface was highly convoluted throughout this period of growth, numerous granulosa cell macrovilli extended into deep pouches associated with 300 nm coated vesicles, and the oolemma possessed a coating of fuzzy material.

These observations suggest that there is a restricted passage of yolk precursors to the surface of the germinal disc, and that the inability to transport yellow yolk into the disc is related to differences in the oolemmal surface coat and the population of coated vesicles. The surface modifications, as well as the proliferation of the granulosa cells, are likely to be influenced by the presence of the germinal vesicle.

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