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Structure–Function Relationships in the Wall of the Ovarian Follicle*

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Abstract

This paper reviews current knowledge of the light and electron microscopic structure of the three layers of the mammalian follicular wall—follicular epithelium (membrana granulosa), theca interna and theca externa—and discusses correlations between structure and function. The ultrastructure of follicular epithelial cells in growing follicles emphasizes their protein synthetic and secretory functions; features suggestive of a major steroidogenic function appear only at later stages. Regional differences in follicular epithelial cell function are probably important, although structurally these cells are relatively homogeneous. Structural diversity is more marked in the thecal layers: differentiation in the theca interna is towards fibrocytic and steroidogenic cell types, while that in the theca externa is towards fibrocytic and myoid types. Adherens and gap junctions are present between cells in all layers; however, tight (occludens) junctions have not been convincingly demonstrated between the cells in any of the three layers. Blood and lymph vessels are confined to the thecal layers. However, follicles possess no structural barrier comparable to that associated with the 'blood–testis barrier', and show a correspondingly greater permeability to large molecules than seminiferous tubules. Interactions between the layers of the follicular wall have not yet been intensively investigated, but are likely to play an important role in follicular function. To date, the best-documented interaction between layers is that described in the 'two-cell hypothesis' of oestrogen production. Some potentially useful directions for future research are proposed.

Introduction

The main features of the composition and structural organization of the mammalian ovarian follicle, and of the ways in which the structure of the follicle changes during its development and growth, have been well known for many years. Early literature on follicular structure, particularly at the light microscope level, and on comparative features of folliculogenesis, has been reviewed in detail by Brambell (1956), Harrison (1962), Mossman and Duke (1973) and Harrison and Weir (1977).

The purpose of the present review is to summarize some recent observations on mammalian follicular morphology, primarily at the ultrastructural level, and to consider ways in which structural observations have contributed to current understanding of follicular function. Attention will be concentrated on the tissues of the three layers of the follicular wall—follicular epithelium (membrana granulosa), theca interna and theca externa—and their interrelationships with one another. Other areas in which important contributions have been made in recent years, including

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oocyte structure, the origin of the follicular epithelium, the changes which occur in atresia, follicular dynamics, and the events of luteinization, will be largely or wholly omitted.

Cytology of the Three Layers of the Follicular Wall

Follicular Epithelium

The innermost, avascular, layer of the follicular wall maintains exclusive contact with the oocyte. It is physically separated from the surrounding stroma or thecal layers, at all stages from the primordial follicle until close to ovulation, by a complete basal lamina.

In primordial follicles the follicular epithelium consists of a single layer of flattened cells with irregularly shaped nuclei (Bjorkman 1962—rat; Hope 1965—Rhesus monkey; Gondos 1969—rabbit). At later stages of development, and particularly in antral follicles, the cells of the follicular epithelium show more structural homogeneity than those of the thecae. However, there are several lines of evidence that regional differences may be significant.

The major subdivision is between the cumulus cells, many of which retain close cytoplasmic contact with the oocyte until after the preovulatory luteinizing hormone (LH) surge (Anderson and Albertini 1976; Gillua *et al.* 1978), and the cells lining the remainder of the follicular antrum. The latter cells can be further subdivided into those which contact the basal lamina, the peripheral cells, and those which do not, the para-antral cells (Zoller and Weisz 1980). Cells in the deeper layers of the epithelium are closely apposed to their neighbours, while those near to the antrum are more loosely packed (Hay and Moor 1975*a*). Functional differences between the two populations are suggested by observations that the peripheral cells contain greater numbers of lipid droplets (Bjorkman 1962) and specific binding sites for human chorionic gonadotrophin (hCG) (Amsterdam *et al.* 1975), and a higher content of cytochrome P-450 (Zoller and Weisz 1978), glucose-6-phosphate dehydrogenase and Δ5-3β-hydroxysteroid dehydrogenase (3β-HSD) (Zoller and Weisz 1979). Greater lysosomal fragility, which may possibly be related to steroidogenic activity, has also been reported in the peripheral cells by Zoller and Weisz (1980).

The major ultrastructural features of follicular epithelial cells at various stages of follicular growth have now been documented in several species including the rat (Bjorkman 1962), cow (Priedkalns and Weber 1968), sheep (Hay and Moor 1975*a*; Cran *et al.* 1979), Rhesus monkey (Amin *et al.* 1976) and man (Mestwerdt *et al.* 1977). In preantral and antral follicles these cells have a relatively high nuclear:cytoplasmic ratio, and contain a wide range of cytoplasmic organelles. Consistently observed features include a predominantly granular endoplasmic reticulum on which ribosomes are rather unevenly distributed, abundant free ribosomes, well-developed Golgi complexes, and many mitochondria whose cristae are mainly lamellar. Lysosomes (Zoller and Weisz 1980—rat), coated vesicles (Mestwerdt *et al.* 1977—man), microtubules and microfilaments (Motta and Didio 1974—rabbit; Albertini and Anderson 1977—rat, rabbit) have also been reported.

The presence of granular endoplasmic reticulum and numerous free ribosomes is consistent with a protein-secretory function in a mitotically active population of cells, and there is now substantial evidence that the follicular epithelium secretes a variety of proteins (reviews by Edwards 1974; Richards 1980). Structural evidence in support

of a steroidogenic function is less consistent, and generally lacking in small follicles. The appearance of features suggestive of a substantial level of steroidogenic activity appears to depend on both species and stage of follicular development. Ultrastructural studies have shown the development of a more tubular form of endoplasmic reticulum, with fewer associated ribosomes, and of some tubular mitochondrial cristae and cytoplasmic lipid droplets, in large follicles of the rat (Bjorkman 1962), cow (Priedkalns and Weber 1968) and horse (Bjersing and Younglai 1972). These features are consistent with a steroidogenic capacity (Christensen and Gillim 1969). However, such features are less evident in large antral follicles in some other species, including man (Amin *et al.* 1976) and the sheep (Cran *et al.* 1979).

Functional studies of follicular epithelium *in vitro* have generally shown that these cells possess an aromatase system capable of converting androgen to oestrogen, but, with the exception of human follicles (McNatty *et al.* 1979), little or no ability to synthesize androgens. However, they are capable of synthesizing progesterone *in vitro*, a capacity which is greater in preovulatory follicles, particularly after the stimulus of an LH surge or in response to LH added to the medium (see Richards 1980). Species variation in the timing of appearance of ultrastructural features suggestive of steroidogenesis may thus relate primarily to differences in the time of commencement of luteinization and the development of a progesterone synthetic capacity. The corresponding appearance of 3β-HSD detected histochemically in follicular epithelium in large antral follicles of the rat (Pupkin *et al.* 1966), cow (Al-Dahash and David 1977) and horse (Bjersing and Younglai 1972), but not the sheep (Hay and Moor 1975*b*), would be consistent with this interpretation.

Coated vesicles and lysosomes have now been shown to be involved in the process of receptor-mediated endocytosis and the subsequent degradation of internalized proteins, including hormones, in several cell types (review by Goldstein *et al.* 1979). Studies on follicular epithelial cells *in vitro* (Amsterdam *et al.* 1979) make it appear likely that these observations apply to gonadotrophin–receptor interactions in this epithelium. Microtubules and microfilaments have also been implicated in the endocytosis of receptor-bound hormones in follicular epithelial cells (Albertini and Anderson 1977). However, the full significance of internalization of receptor-bound hormones is not understood, and it is unclear whether the gonadotrophins, or fractions thereof, exercize any of their biological functions after internalization.

Data from ovarian and other tissues suggest a number of additional functions for these organelles in follicular epithelium, including involvement in steroidogenesis, protein secretion, motility, and phagocytosis. Thus, there are data to suggest the involvement of coated vesicles and microtubules in steroid secretion (Bassett and Pollard 1980), of microtubules and microfilaments in modulation of the adenylate cyclase system (Zor *et al.* 1978), and of microfilaments in intracellular transport of cholesterol (Mrotek and Hall 1977). Microtubules also play a role in the exocytosis of protein secretions (Lacy *et al.* 1968). The importance of microtubules (Porter *et al.* 1974) and microfilaments (Lazarides and Revel 1979) in cell movement and cell shape is now well established, and there is evidence that follicular epithelial cells *in vitro* possess motile properties (Nicosia and Tojo 1979) and the ability to undergo active changes in shape in response to follicle stimulating hormone (FSH) (Lawrence *et al.* 1979). However, apart from observations suggesting a role in the rearrangement of follicular epithelial cells around the time of ovulation, discussed below, there are no available data on the extent or significance of follicular cell movements *in vivo*.

Finally, lysosomes are likely to be involved in the process of phagocytosis, which is demonstrated by the follicular epithelium during atresia (Hay *et al.* 1976) and in the early developmental period when there is substantial oocyte death (Gondos 1969).

Theca Interna

Primordial follicles are embedded in undifferentiated stroma, and lack any distinct thecal investment. The theca is first recognizable as a concentric stromal sheath around growing preantral follicles. By the time of antrum formation, a distinct theca interna, whose cells are less flattened than those of the surrounding theca externa, is distinguishable (see Brambell 1956).

Details of the ultrastructure of the theca interna, and its changes during the later stages of follicular growth, have now been reported in the rat (Belt 1962), cow (Priedkalns *et al.* 1968), horse (Bjersing and Younglai 1972), mouse (Hiura and Fujita 1977), pig (Krzysztofowicz and Stoklosowa 1977), man (Mestwerdt *et al.* 1977) and sheep (O'Shea *et al.* 1978*a*). A reasonably consistent picture emerges. Two major forms of cellular differentiation are recognizable, namely fibrocytic and 'steroidogenic', the latter identification being based on the possession of the characteristic features referred to above. Maximal differentiation in the theca interna is attained late in follicular development, and structural dichotomy between the two cell types is greatest in large antral follicles at around the time of onset of oestrus, at which time oestrogen and androgen production are maximal (Baird *et al.* 1976). At this time the steroidogenic cells enlarge and take on an 'epithelioid' appearance (Priedkalns *et al.* 1968). At earlier stages, many thecal cells appear intermediate in structure between the two major forms, and transformations appear possible. Furthermore the widespread distribution of 3β-HSD (Hay and Moor 1975*b*) and binding sites for hCG (Amsterdam *et al.* 1975) in this layer make it likely that a steroidogenic function does not reside exclusively in large, 'epithelioid' cells.

The steroidogenic capacity of the theca interna has been established by many studies *in vitro* (see Richards 1980), and there is much evidence that this layer produces androgens. Whether any significant oestrogen production occurs in the theca is less certain, although there are data in the pig (Rodway *et al.* 1975; Stoklosowa *et al.* 1978), Rhesus monkey (Channing and Coudert 1976) and man (McNatty *et al.* 1979) to suggest that this may be so in some species.

Having attained a large size and structural features consistent with active steroidogenesis, the 'epithelioid' thecal cells show marked structural changes in the period between the LH surge and ovulation. During this period, when oestrogen and androgen secretion falls sharply (Bahr 1978), these cells acquire many cytoplasmic lipid droplets (Bjersing *et al.* 1972). This has been interpreted as a morphological correlate of the decline in steroidogenesis (O'Shea *et al.* 1978*a*).

Lysosomes (Krzysztofowicz and Stoklosowa 1977), coated vesicles (O'Shea *et al.* 1978*a*), microtubules and microfilaments (Mestwerdt *et al.* 1977) have also been observed in theca interna cells, and comments on these structures in relation to the follicular epithelium are generally applicable to the theca.

In many species, including certain insectivores, bats, rodents, carnivores and lagomorphs, the cells of the theca interna hypertrophy during atresia of antral follicles, giving rise to steroidogenic interstitial cells (Harrison and Weir 1977). However, in some groups, including artiodactyls, interstitial cells are rare, (Harrison

and Weir 1977), and during atresia the theca interna shows degenerative changes (O'Shea *et al.* 1978*b*). Under these conditions the thecal cells show a capacity for phagocytosis in the destruction of their dead and fragmenting neighbours.

Theca Externa

Before the advent of electron microscopy there was a protracted and unresolved debate as to whether or not smooth muscle cells were a significant component of the theca externa. The earliest electron microscopic studies of this layer (Espey 1967; Priedkalns and Weber 1968) endorsed the view that the theca externa cells were fibrocytes.

Subsequent and more detailed studies, initially in the rat and Rhesus monkey (O'Shea 1970*a*, 1970*b*; Osvaldo-Decima 1970), have now shown that the theca externa in many mammalian species contains not only fibrocytes but also many cells whose cytoplasm shows varying degrees of smooth muscle-like (myoid) differentiation (review by Espey 1978). Relevant ultrastructural features include parallel bundles of microfilaments about 6 nm in diameter associated with characteristic dense bodies (fusiform densities), some degree of basal lamina formation, and foci of specific cell-to-cell attachment. Further evidence of the myoid nature of many theca externa cells derives from the demonstration by immunofluorescence of thecal binding of antibodies to actin and smooth muscle myosin (Kapinus and Rukosuev 1975; Amsterdam *et al.* 1977; Walles *et al.* 1978). Species in which myoid theca externa cells have now been demonstrated include the rat, Rhesus monkey, sheep, rabbit, cat, mouse, guinea pig, man, gerbil, cow (see Espey 1978), and more recently the hamster (Pendergrass and Talbot 1979) and pig (Patterson and O'Shea 1980).

These structural studies have provided the stimulus for a renewed and vigorous interest in follicular contractility and its significance in follicular function, particularly in relation to ovulation. Evidence of follicular contractility, as distinct from ovarian contractility which probably depends largely on non-follicular smooth muscle which is well developed in the ovarian hilus and medulla (O'Shea 1970*a*; Osvaldo-Decima 1970), has now been demonstrated in the sheep (O'Shea and Phillips 1974), man (Owman *et al.* 1975; Gimeno *et al.* 1976), cow (Walles *et al.* 1975*a*, 1975*b*) and pig (Patterson and O'Shea 1980).

The precise role of follicular contractility in reproductive function is uncertain. It is now widely accepted that contraction is not likely to be the primary cause of follicular rupture at ovulation (see Espey 1978). However, a role in extrusion of the follicular contents, including the oocyte and cumulus, and in the collapse and infolding of the follicular wall, which aid in the genesis of the corpus luteum, seem possible. The development of methods for effectively blocking follicular contractility *in vivo* could contribute to evaluating these possibilities. Whether the thecal myoid cells have any role in the function of growing follicles is not known, although they presumably influence the physical properties of the follicular wall. Their presence could perhaps in part explain the high pressures which can develop within the follicular antrum *in vivo*, in which levels greatly in excess of capillary hydrostatic pressure have been reported in the pig by Bronson *et al.* (1979).

Although both adrenergic and cholinergic nerves have been shown to occur in the theca (Owman *et al.* 1975; Walles *et al.* 1975*a*), and follicular tissue can be induced to contract by both α-adrenergic and cholinergic transmitters (O'Shea and Phillips

1974), the role of nerves in the physiological control of follicular contractile tissue remains unknown. Nor has any other natural control mechanism for this tissue yet been clearly defined.

Intercellular Junctions in the Follicle

The three best-known forms of specific intercellular junction in mammalian cells are those commonly termed occludens, adherens and gap junctions. These are illustrated in diagrammatic form in Fig. 1.

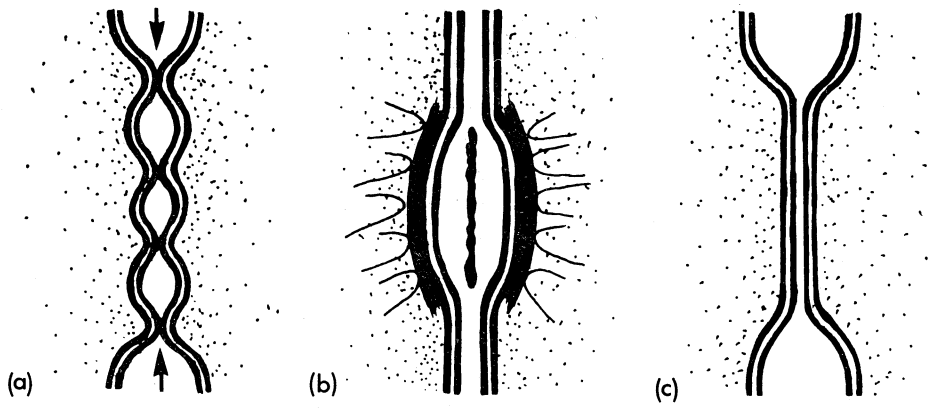

Fig. 1. Diagrammatic representation of the three main types of specialized intercellular junctions of mammalian cells, as seen by transmission electron microscopy. (*a*) Occludens (tight) junction. Outer surfaces of the apposed cell membranes are in direct contact at several focal points, occluding the intercellular space and preventing the movement of materials between the cells in the directions shown by the arrows. (*b*) Adherens junction. Diagram shows a highly developed (desmosome) form of adherens junction. Cell membranes are not in direct contact, but specializations are present in the subjacent cytoplasm and intercellular space. Junctions of this type are associated with physical adhesion between the apposed membranes. (*c*) Gap junction (nexus). Membranes are separated by a narrow ($\simeq$ 2 nm) gap which is, in life, bridged by many narrow connecting units (connexons). Connexons are believed to act as channels permitting the movement of small molecules and ions between cells.

Follicular Epithelium

It is now well established that adherens-type and gap junctions are characteristic of follicular epithelium. Both types of junction also occur between cumulus cells and the oocyte (Albertini and Anderson 1974; Anderson and Albertini 1976). Although zonula occludens-type junctions were reported in the follicular epithelium of the rabbit by Espey and Stutts (1972), this has not been substantiated, and the structures illustrated resemble gap junctions.

Junctions of the adherens type, variably described as desmosomes, maculae adherentes, zonulae adherentes and intermediate junctions, have been reported at many stages of follicular development in the rat (Bjorkman 1962), rabbit (Espey and Stutts 1972), Rhesus monkey (Zamboni 1974), sheep (McClellan *et al.* 1975), man (Fukushima 1977) and mouse (Nicosia and Tojo 1979). These junctions, which appear even in primordial follicles (Zamboni 1974), presumably serve to hold neighbouring cells together.

Gap junctions, whose role in intercellular communication is now well established (Evans 1980), are also present in many species including the rat (Merk *et al.* 1973), rabbit (Albertini and Anderson 1974), Rhesus monkey (Zamboni 1974), sheep (Hay and Moor 1975*a*), man (Fukushima 1977) and mouse (Burghardt and Anderson 1978). Morphologically these junctions take two major forms, termed abutment and annular nexuses (Merk *et al.* 1973). At least some of the annular forms are wholly internalized in the cells in which they are present, and their contents often appear degenerate. Merk *et al.* (1973) have suggested that internalization may represent a means of removing gap junctions from the surface. In this event an increase in annular nexuses, as occurs in the preovulatory period (Espey and Stutts 1972; Bjersing and Cajander 1974*a*), could provide indirect morphological evidence of a reduction in gap junctional coupling.

Quantitative variations in gap junctions have been demonstrated at different stages of follicular development. Thus Albertini and Anderson (1974) in the rabbit, and Cran *et al.* (1979) in sheep, reported that these junctions were absent in preantral follicles, while Merk *et al.* (1972) noted that they were more numerous after the theca interna had become well developed in the rat. In rabbits, their number increases as the follicle grows (Coons and Espey 1977). Gap junctional contact is progressively reduced during atresia (Hay *et al.* 1976), although there is no evidence that this reduction represents a primary change in atresia.

Changes in the extent of gap junctional linkage during follicular development are probably under hormonal control, as Merk *et al.* (1972) and Burghardt and Anderson (1978) have shown that hypophysectomy reduces the numbers of these junctions in rats and mice, while administration of oestrogen causes an increase. This fact could be relevant to the observed effects of oestrogen on ovarian responsiveness to gonadotrophins (see Richards 1980).

The high incidence of gap junctions between follicular epithelial cells would seem to imply that cell-to-cell communication is important in the function of this layer of the follicular wall, which may be regarded in many respects as a "functional syncytium' (Merk *et al.* 1972). Since only a proportion of the cells are in direct contact with the basal lamina, and these peripheral cells possess higher numbers of LH receptors, Lindner *et al.* (1977) have suggested that gap junctions may play an important role in propagating the effects of hormonal stimulation from the peripheral cells to those of the cumulus and para-antral regions. To the extent that this is so, the numbers of gap junctions could be significant in determining the degree of responsiveness to this hormone, and in minimizing the problems associated with obtaining rapid, generalized responses to hormones in an avascular, stratified epithelium. Evidence that the effects of hormonal stimulation can spread from cell to cell, possibly via the mediation of cAMP, has now been provided by the ingenious experiments of Lawrence *et al.* (1978) who produced gap junctional linkage between follicular epithelial and myocardial cells *in vitro*. It would clearly be of interest to know whether any other hormone receptors in the follicular epithelium are preferentially localized on the peripheral cells.

Theca Interna

Gap junctions have been reported between cells of the theca interna in several species including the rabbit (Bjersing and Cajander 1974*a*), man (Fukushima 1977),

sheep (O'Shea *et al.* 1978*a*) and the rat and mouse (Burghardt and Anderson 1978). In sheep these junctions are preferentially distributed between cells of the steroidogenic type (O'Shea *et al.* 1978*a*). Their earliest appearance in rats and mice was at 12 days post partum, at the time of initial differentiation of the thecal layers (Burghardt and Anderson 1978).

Burghardt and Anderson (1978) observed that thecal gap junctions persisted for as long as 90 days after hypophysectomy. Although their report did not make it clear whether hypophysectomy caused any quantitative change in these junctions, the subsequent administration of hCG caused their 'growth'. Gap junctions in the theca interna may thus be, at least in part, under the control of LH, for which receptors are present in this layer (see Richards 1980). Oestradiol was without effect on the numbers of gap junctions in the theca (Burghardt and Anderson 1978). The functional significance of gap junctions in the theca interna has not been investigated, but, as in the follicular epithelium, presumably reflects the need for some form of metabolic cooperation.

Other junctions observed in the theca interna of the sheep (O'Shea *et al.* 1978*a*) were of the adherens type. However, an additional form of attachment, the septate-like junction, has been reported in the human theca interna by Fukushima (1977). This junction, which was originally described in some detail in the adrenal cortex, testicular interstitial cells and corpus luteum by Friend and Gilula (1972), resembles in many ways the septate junctions of invertebrates. Septate junctions (Wood 1959) are regarded as specializations for cell-to-cell adhesion, and for restriction of permeability through the intercellular spaces. Little is known of the properties of vertebrate septate-like junctions, but Friend and Gilula (1972) have suggested that they may be typical of steroid hormone secreting tissues.

Theca Externa

There are no detailed published reports of the nature of junctions between theca externa cells. However, gap junctions, which were originally mistakenly identified as tight junctions, occur between myoid cells in the sheep (O'Shea 1971), and adherens-type junctions have been observed between myoid cells in the cow (Walles *et al.* 1975*a*) and pig (Patterson and O'Shea 1980). Electrical linkage across gap junctions could be significant in coordination of contractile activity in the theca externa, as occurs in smooth muscle, but there is as yet no direct evidence on this point.

Follicular Blood Vessels and Lymphatics

Primordial follicles lack any distinct, independent vasculature, and a discrete follicular capillary bed, confined to the thecal layers, develops in association with the formation of the theca (Andersen 1926). In antral follicles, the thecal capillaries are concentrated in a dense network, the 'inner wreath', close to the basal lamina of the follicular epithelium (Andersen 1926; Antonucci 1972). This arrangement is presumably advantageous in meeting the metabolic requirements of the avascular follicular epithelium and the oocyte. The capillary bed is less dense in other parts of the theca, and the larger vessels supplying and draining the thecal capillaries are more peripherally situated in and around the theca externa. Follicular blood flow is high, reaching levels comparable to those of functional luteal tissue when calculated per unit weight of tissue in the sheep (Bruce and Moor 1976).

Ultrastructurally, the thecal capillaries in the mouse (Payer 1975) and sheep (O'Shea *et al.* 1978*a*) have a continuous endothelium, whose cell junctions lack any zonula occludens, and a continuous basal lamina. However, fenestrations have been reported in the rabbit (Bjersing and Cajander 1974*b*).

An extensive lymphatic drainage of the follicular wall has also been reported (Andersen 1926). Present ultrastructural criteria for the identification of lymphatic capillaries are somewhat equivocal, and only Bjersing and Cajander (1974*b*) have identified lymphatics by this method. Unfortunately, their criteria were not defined, and no illustration of vessels identified as lymphatics was provided.

Follicular Permeability and the 'Blood–Follicle Barrier'

The idea of a blood–follicle barrier limiting the free movement of solutes between the blood plasma and the follicular fluid dates back to Zachariae (1958), who demonstrated that follicular permeability increased in the preovulatory period in rabbits.

Structural data identify potential barrier sites in the capillary endothelium and its basal lamina, and in the follicular epithelium and its basal lamina. However, neither of these two cellular layers possesses zonula occludens-type junctions comparable to those of the testicular Sertoli cells (Dym 1973), and structural data would not support predictions of a functional barrier equivalent to that of the seminiferous tubules.

Ultrastructural tracer studies in the mouse (Payer 1975), sheep (Cran *et al.* 1976) and rabbit, sow and cow (Szollosi *et al.* 1978) have shown that antral follicles are permeable to tracers of molecular weight less than about 500 000, including lanthanum, horseradish peroxidase and ferritin, but not to colloidal gold (mol. wt $\simeq$ 1 000 000). All tracers were able to escape from the thecal capillaries, but colloidal gold appeared unable to penetrate the basal lamina of the follicular epithelium (Cran *et al.* 1976). These findings are consistent with the data of Shalgi *et al.* (1973) which indicated that the blood–follicle barrier behaves like a molecular sieve, allowing the passage of proteins in inverse proportion to their molecular weight, and being impermeable to proteins of molecular weight greater than 850 000. This is in marked contrast to the 'blood–testis barrier' (Fawcett *et al.* 1970), which excludes plasma proteins almost entirely from the lumen of the seminiferous tubules (Tuck *et al.* 1970).

Although the follicular wall presents little of a barrier to the entry of most blood molecules, due to the metabolic and secretory activities of the follicular epithelium, and perhaps other mechanisms, the contents of the follicular fluid do differ in many important ways from those of the blood plasma. Thus, for example, steroid hormones frequently reach levels in follicular fluid far higher than those of the plasma (see Edwards 1974), whereas gonadotrophin levels in follicular fluid can be significantly lower than those of the plasma (McNatty *et al.* 1975).

Relationships between the Layers of the Follicular Wall, and Changes Following the LH Surge

As described above, the follicular epithelium is consistently separated from the surrounding stromal or thecal layers by a continuous basal lamina which becomes discontinuous only at around the time of ovulation. Thus, follicular epithelial cells and thecal cells are never in direct contact in the growing follicle, and separation is increased along much of the interface by the capillaries of the inner wreath.

In spite of these physical barriers, close functional interaction between layers appears likely. The precise spatial disposition of the thecal layers relative to the follicular epithelium is strongly suggestive of the possibility, originally proposed by Dubreuil (1957), that a process of 'morphogenetic induction' may be involved in the development of the former. However, there is no experimental evidence for this.

The best-documented case for cooperative interaction between the follicular epithelium and the theca is found in the data supporting the 'two-cell hypothesis' of follicular oestrogen production (see Richards 1980). This hypothesis, for which there is now extensive experimental support in some species, requires that androgen produced by the theca is aromatized to oestrogen in the follicular epithelium. Although the evidence for this interaction is persuasive, it is puzzling that the structural design of the follicle does not seem to reflect the need for a substantial transfer of androgen from the theca interna to the follicular epithelium (O'Shea *et al.* 1978*a*). While it is unlikely that the barriers between the theca and the follicular epithelium would present any serious impediment to the diffusion of androgens, it might be predicted that this would be a very 'leaky' route of transfer, with substantial diversion into the blood. There are, in fact, several lines of evidence to support the suggestion that appreciable amounts of theca-derived steroids do enter the blood stream directly (McNatty *et al.* 1979).

Now that techniques are available for the independent study of thecal and follicular epithelial cells *in vitro* (Moor 1977; Fortune and Armstrong 1978), prospects for obtaining a better understanding of the functional interactions between these layers appear promising.

A wide range of structural and functional changes in the follicular wall occurs in the hours between the preovulatory LH surge and ovulation, involving both the individual layers of the follicular wall and their relationships with one another. Structural changes in the follicular epithelium include dissociation and separation of the cells, which is accompanied by a reduction in the extent of gap junctional contact (Coons and Espey 1977). This reduction may be brought about by increasing internalization of these junctions, as an increase in annular forms of junction has been reported (Bjersing and Cajander 1974*a*; Cran *et al.* 1979). Whether the reduction in gap junctional contact is functionally advantageous in itself, or is merely a necessary corollary of cell separation and reorganization, is not clear. However, the corpus luteum, unlike the follicular epithelium, is highly vascular, and would be unlikely to need gap junctional linkage to propagate the effects of hormonal stimulation. In fact, gap junctions between luteal cells are numerous in some species, for example the rat, mouse (Albertini and Anderson, 1975) and guinea pig (Paavola 1977), but rare in the sheep (O'Shea *et al.* 1979).

The presence of pseudopodial processes on follicular epithelial cells at this time (Byskov 1969—mouse; Bjersing and Cajander 1974*a*—rabbit; O'Shea *et al.* 1978*a*—sheep), which penetrate the disintegrating basal lamina and bring these cells into their first direct contact with the cells of the theca interna, is strongly suggestive of active motility. There is also evidence that follicular epithelial cell mitosis ceases in the periovulatory period (McClellan *et al.* 1975; Meyer and Bruce 1980), presumably as a response to LH (Rao *et al.* 1978). Gospodarowicz and Gospodarowicz (1975) have demonstrated the antimitotic effect of LH *in vitro*, and suggested that the resultant cessation of mitosis represents an example of the general principle that growth and differentiation (in this case luteinization) are as a rule mutually exclusive.

Ultrastructural studies have also suggested active motility by cells of the theca interna in the periovulatory period (Byskov 1969—mouse; Nunez-Duran 1977—rabbit; O'Shea *et al.* 1978*a*—sheep). Although there have been some differences in interpretation of the fate of the 'epithelioid' cells of the theca interna, recent ultrastructural and histochemical evidence confirms that the theca interna in sheep is incorporated, apparently completely, into the developing corpus luteum (O'Shea *et al.* 1980). Motility on the part of cells of the follicular epithelium and theca interna thus probably plays a role in the rearrangement which brings them into extensive and direct contact in the formation of the corpus luteum.

Degradative changes have been reported in the theca externa in the period preceding ovulation, and it has been proposed by Espey (1971) that small, multivesicular blebs appearing on the surface of many thecal fibroblasts at this time in the rabbit may be the source of a substance concerned in degradation of the follicular connective tissue. However, there is no evidence that these 'multivesicular structures' actually contain or release collagenolytic or other relevant enzymes, and the possibility that they represent a degenerative process, or even an artifact, has not been eliminated.

A marked dilatation of the thecal capillaries also follows the LH surge, accompanied by an increase in capillary permeability which leads to substantial thecal oedema (Bjersing and Cajander 1974*b*) and may assist in the rapid influx of fluid to the follicular antrum. At this time, open gaps appear in the capillary endothelium (Byskov 1969—mouse; Bjersing and Cajander 1974*b*—rabbit; Szollosi *et al.* 1978—pig). These gaps may be large enough even to permit the escape of erythrocytes. While the evidence indicates that these vascular changes are initiated by LH, the mechanism of action of LH on the follicular vasculature has not been established. However, the possibility of an indirect mechanism involving prostaglandin synthesis is suggested by the observation of Lee and Novy (1978) that indomethacin can block the increase in ovarian blood flow which follows LH administration in rabbits.

Conclusion

The main ultrastructural features of the cells in each of the three layers of the follicular wall have now been documented in several mammalian species, and the past decade has seen major advances in the interpretation of follicular ultrastructure in functional terms. These advances have resulted from the application of a wide range of techniques, including electron microscopy, histochemistry, antibody labelling, autoradiography, molecular tracer administration, and tissue culture. By the use of these techniques, frequently in combinations, it has been possible to link structural observations more directly with function at the level of organelles and even molecules.

However, many questions remain unanswered, and among areas in which further progress in understanding would be particularly welcome might be mentioned (i) the sequence of events following the binding of gonadotrophic hormones to their receptors, (ii) the nature and purpose of the signals passed between follicular cells via gap junctions, and (iii) the physiological function and control of the thecal myoid cells.

In contrast to the rapid advances in knowledge of the biology of individual cell types in the follicle, progress in understanding the interrelationships between different layers of the follicular wall has lagged. The notable exception has been in relation to thecal/follicular epithelial cooperation in oestrogen synthesis. Hence questions as to why the follicle is constructed as it is, and why its components are so drastically rearranged following ovulation, continue to pose a major challenge.

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