J. Anat. (1973), **114**, 3, pp. 321–328
With 4 figures
Printed in Great Britain

The ultrastructure of the boundary tissue of the seminiferous tubule in the testis of the domestic fowl (*Gallus domesticus*)

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(*Accepted* 5 *January* 1973)

INTRODUCTION

The significance of the testicular peritubular boundary tissue as a mechanical support, a contractile aid to sperm transport, and a physiological barrier has been recognized by detailed studies of this tissue, particularly in mammals.

The first ultrastructural study was that of Clermont (1958, 1960) on the rat testis. Subsequent studies have been made on the rat (Lacy & Rotblat, 1960; Leeson & Leeson, 1963; Dym & Fawcett, 1970; Kormano, 1970; Kormano & Hovatta, 1972), on he mouse (Baillie, 1964; Ross, 1967), on the guinea-pig (Fawcett, Heidger & Leak, 1969) and on man (Ross & Long, 1966).

In a review of the ultrastructural characteristics of the boundary tissue or limiting membrane of the germinal epithelium, Burgos, Vitale-Calpe & Aoki, (1970) suggest that it involves the classic basement membrane or basal lamina, plus a framework of fibres and cells which gives support to the germinal epithelium and generally consists of filamentous glycoprotein, collagen fibres and elongated cells. In the mammalian species studied it is organized in such a way that three types can be recognized (Fig. 1). Dym & Fawcett (1970) consider that in the rat and guinea-pig the outer cellular component does not consist of fibroblasts, but of a layer of the endothelial cells of an extensive system of peritubular lymphatic sinusoids.

In view of the anatomical differences in the reproductive tract between mammals and the domestic fowl (Lake, 1957; Tingari, 1971), particularly the possession of abdominal as distinct from scrotal testes, a study of the structure of the peritubular boundary tissue in the fowl was undertaken.

MATERIAL AND METHODS

Adult cockerels of a lightweight laying strain (Shavers) were killed by a lethal dose of pentobarbitone sodium (Nembutal, Abbott Laboratories). Their testes were excised and pieces were fixed in a modified Karnovsky solution (1 % glutaraldehyde, 4 % paraformaldehyde) or 5 % glutaraldehyde buffered with 0·1 M Millonig phosphate followed by post-osmification. After dehydration the tissue was embedded in Araldite; sections were cut on an L.K.B. ultratome III, stained with uranyl acetate and lead citrate (Reynolds, 1963) and observed in a Phillips E.M. 300 microscope.

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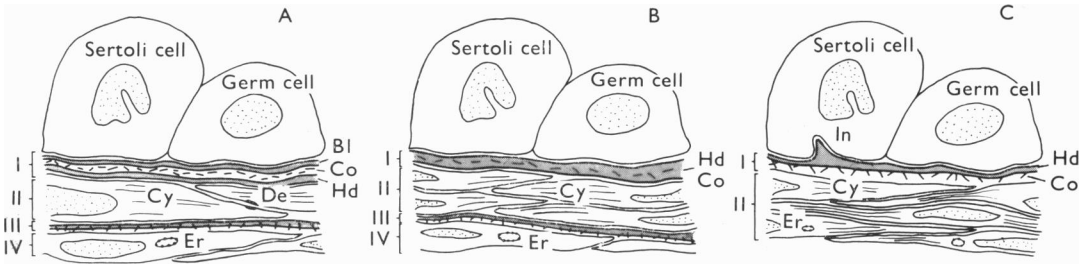

Fig. 1. The three types of boundary tissue (limiting membrane) organization identified in mammals. (Drawn and modified from Burgos, Vitale-Calpe & Aoki, 1970.) Type A, rat and mouse; type B, guinea-pig; type C, human and cat. I, inner lamella; II, interlamellar cells (peritubular contractile, fibroblast-like cells); III, outer lamella; IV, connective tissue cells. Bl, basal lamina; Co, collagen fibres; Cy, cytoplasmic filaments; Er, endoplasmic reticulum; Hd, Homogeneous dense material; In, infolding of inner lamella.

RESULTS

The boundary tissue of the seminiferous tubules of the testis of the fowl consists of an inner fibrous lamella and an outer multi-layered peritubular cellular component. Fibrous and collagenous tissue is found in varying amounts between the peritubular cells.

The inner fibrous lamella is made up of three components: a homogeneous basal lamina immediately adjacent to the seminiferous epithelium, a clear region containing loosely arranged collagenous fibres of no particular pattern or orientation along with non-striated fibrils which tend to form clusters, and, peripheral to this, a dense homogeneous layer similar to the basal lamina of the epithelium although generally not as well defined (Fig. 2). The two homogeneous layers stain with PAS in light microscopical preparations. The fibrous lamella is a constant feature in adult birds. Similar structural components are found to a varied extent associated with the peritubular cellular layers but here no clear pattern of organization is evident.

Two clearly identified cell types are found in the peritubular cellular layers. One is an elongated 'fibroblast-like' cell widest in the region of the nucleus and with long attenuated cytoplasmic processes (Fig. 3). These processes are frequently difficult to follow due to the plane of section. The elongated nucleus has a finely granular chromatin with some margination along the inner nuclear membrane: a single nucleolus is sometimes seen. The cell cytoplasm along the lateral and medial surfaces is reduced to a thin rim, and is rich in rough endoplasmic reticulum (RER), which often shows dilated profiles and contains a flocculent or filamentous dense material. A connexion between the RER cisternae and the perinuclear cisterna is observed. A well-developed Golgi complex, a number of oval or elongated mitochondria, some electron-dense bodies, and free ribosomes are found scattered throughout the peripheral cytoplasm, and some rough-coated vesicles are seen in relation to both cell surfaces. Patches of cytofilaments are frequently found in the attenuated processes of these cells. A degree of overlap, sometimes quite extensive, exists between the cellular processes and where there are regions of close apposition membrane modifications for adhesion are sometimes observed. Typical 'fibroblast-like' cells of the type described are

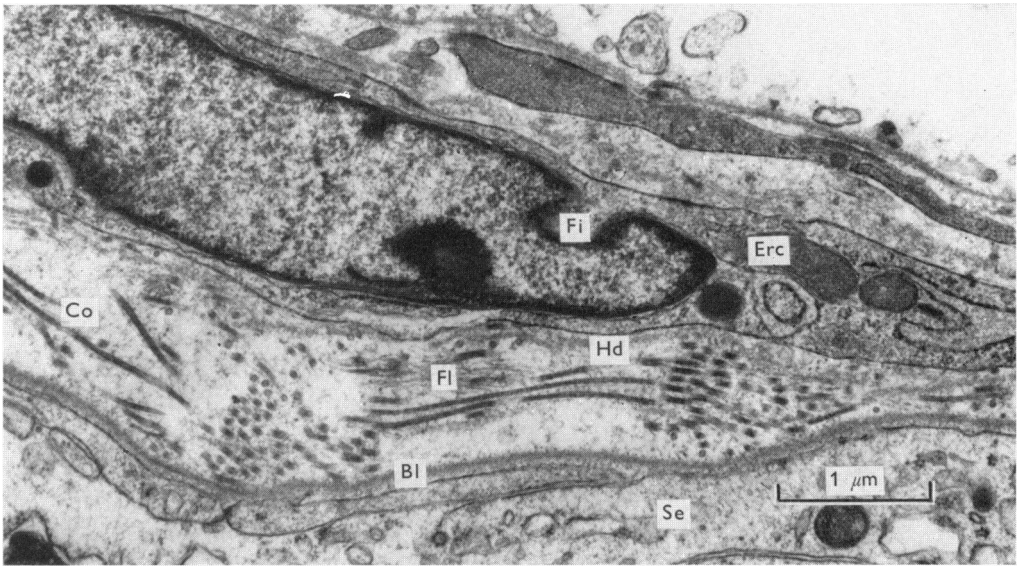

Fig. 2. Components of the inner fibrous lamella. A basal lamina (Bl) adjacent to the seminiferous epithelium (Se), a clear region containing collagen fibres (Co) and non-striated fibrils (Fl) and an indistinct band of dense homogeneous material (Hd) adjacent to a 'fibroblast-like' cell (Fi). Erc, confluence of cavity of endoplasmic reticulum with perinuclear cisterna.

characteristically found immediately adjacent to the fibrous lamella, although similar images are also found amongst the other peritubular cells.

Another cell type is also elongated but has cytoplasmic processes which are less attenuated than the 'fibroblast-like' cells and has a more regular outline. The nucleus of these cells is centrally placed and slender, with few or no surface invaginations. Its chromatin is evenly distributed, except for some clumping along the inner surface of the nuclear membrane and at the site of the eccentrically located nucleolus. The characteristic feature of this cell is the abundance of cytoplasmic filaments, together with small interfilamentous dense bands (Fig. 4). The pattern of distribution of both the filaments and the dense bands is parallel to the long axis of the cell. The dense bands are not confined to any particular location; they are observed in relation to the cell surface as well as elsewhere in the cytoplasm.

Cytoplasmic organelles in these cells are located mainly in the juxtanuclear region and in the wide middle part of the cell. A Golgi complex, mitochondria, profiles of RER, and free ribosomes are present in the cytoplasm, as well as a centriole and electron-dense bodies. Numerous micropinocytotic vesicles are associated with both the medial and the lateral surfaces of the cell membrane.

This second cell type retains a significant cytoplasmic feature of the fibroblastic cell, in that dilated elements of RER are seen and a continuity of the cavity of the RER with that of the perinuclear cisterna is also found. Where the overlapping cell processes are apposed, membrane modifications for adhesion are seen.

In some images peritubular cells of both types are seen which possess essentially the same basic ultrastructural features as in the foregoing account but have an

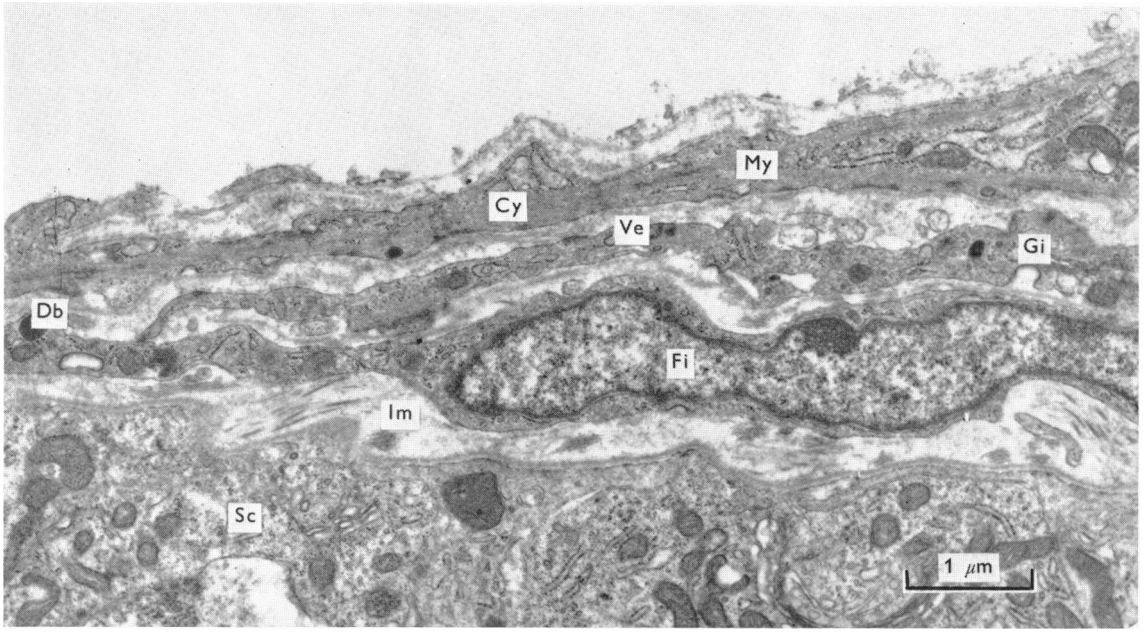

Fig. 3. Overlapping processes of elongated peritubular cells. Inner 'fibroblast-like' cells and outer contractile cell (My) with cytoplasmic filaments (Cy). Db, dense cytoplasmic body; Gi, Golgi apparatus; Im, inner fibrous lamella; My, myoid cell; Ve, rough-coated vesicle.

irregular surface outline and a correspondingly irregular nuclear shape. Where such images are found the fibrous lamella is also contorted, giving the basal plasma membrane of the seminiferous epithelium numerous infoldings in which the whole fibrous lamella, and not just one part of it, is involved.

As well as the image variation associated with the contracted state of the boundary tissue there are darker and lighter images of the basic cell types described. These are considered to be a fixation artefact associated with immersion fixation in glutaraldehyde (Christensen & Gillim, 1969).

As the diameter of the seminiferous tubule increases with age and activity, the processes of the peritubular cells become more attenuated, and the whole boundary tissue becomes thinner. Where blood and lymphatic capillary elements are found interstitially, their endothelium forms the peripheral limit to the boundary tissue, as in the rat and guinea-pig (Dym & Fawcett, 1970). Where there are no interstitial elements intervening, the peritubular boundary layers run parallel and allow a clear observation of the constituent processes.

DISCUSSION

The boundary tissue of the testis of the domestic fowl, like that of other vertebrates, has three basic components; homogeneous electron-dense material, collagenous fibres and elongated peritubular cells. The non-cellular elements form a

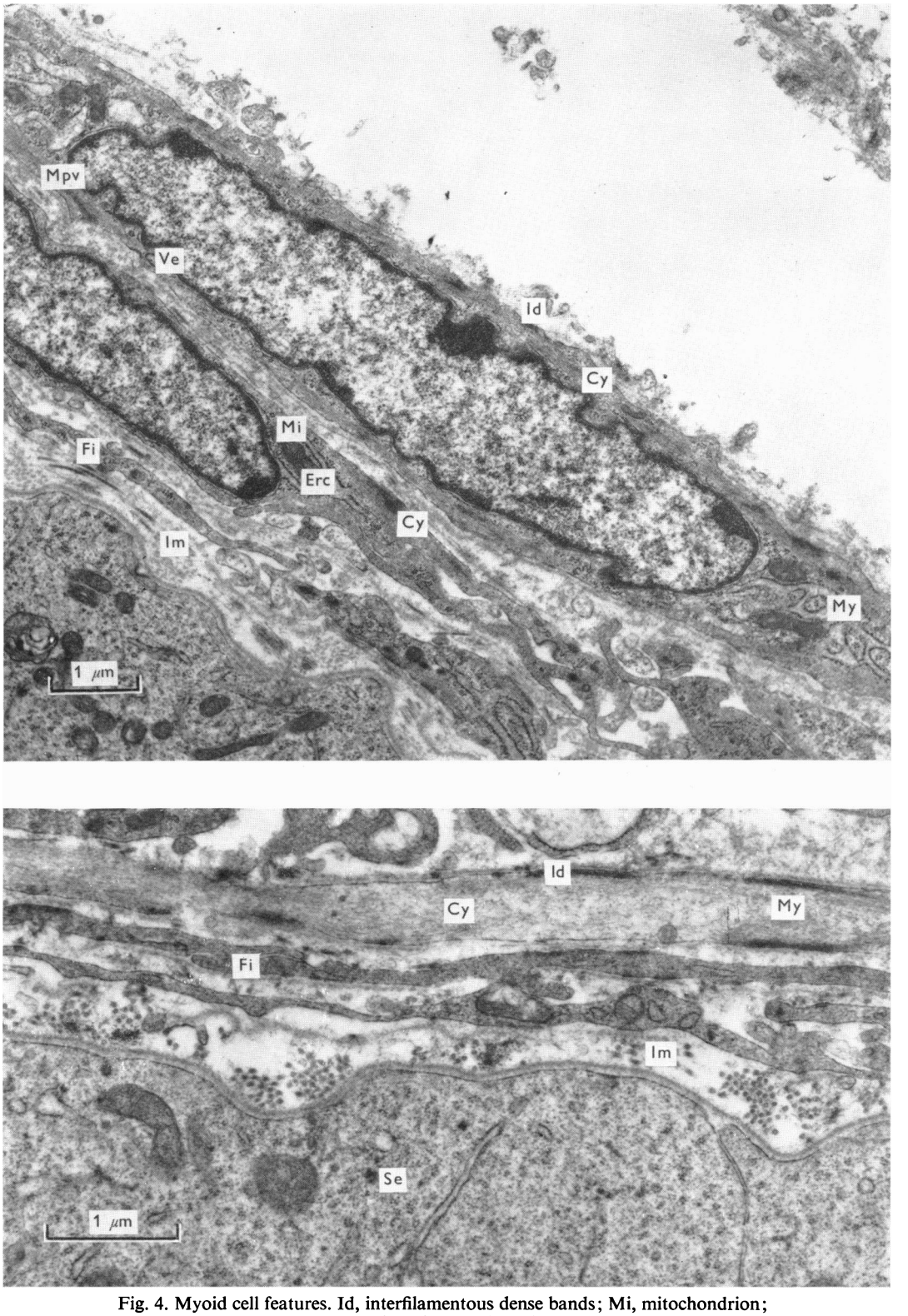

Fig. 4. Myoid cell features. Id, interfilamentous dense bands; Mi, mitochondrion; Mpv, micropinocytotic vesicles.

clearly defined inner fibrous lamella and less ordered associations with the peritubular cells.

The inner 'fibroblast-like' cells are indeed ultrastructurally similar to fibroblasts (Ross, 1968). The shape of the cell, the profiles of RER and their connexion with the perinuclear cisterna, the clusters of ribosomes, and the fine cytoplasmic fibres support such a suggestion. Among the functions assigned to this cell is the production of collagen, with which it is clearly associated. The fine non-striated fibres are probably tropocollagen, a precursor of collagen. The collagen and other fibrous elements between the layers of the peritubular cells may be produced by the fibroblasts in these more peripheral layers. The production and maintenance of collagen by the fibroblasts fulfils the support requirements of the seminiferous tubules and provides for their integrity (Baillie, 1964; Ross, 1967).

The second cell type found in the concentric peritubular layers retains an RER component connected with the perinuclear cisterna, but has cytoplasmic processes broader than those of fibroblasts and numerous cytoplasmic filaments arranged in a parallel array with interfilamentous dense bands; numerous micropinocytotic vesicles are found along their surfaces.

Although the ultrastructure of these cells leads one to classify them as myoepithelial, their shape, arrangement and association with a connective tissue compartment make them quite different from such elements (Clermont, 1958; Ross, 1967). Their cytological features resemble those of contractile cells described for a number of mammalian testes and variously designated. In the rat they were described as 'interlamellar cells' (Clermont, 1958, 1960) and 'cells of the inner cellular layer' (Lacy & Rotblat, 1960; Leeson & Leeson, 1963); in man (Ross & Long, 1966) and mouse (Ross, 1967) they were described as 'peritubular cells'. Fawcett *et al.* (1969) described them as 'myoid cells' and more recent authors (Soranto & Kormano, 1970; Kormano, 1970; Hovatta, 1972; Kormano & Hovatta, 1972) have continued this terminology. In view of the ultrastructural similarity between mammalian myoid cells and the peripheral peritubular cells in the fowl, these too are best designated myoid cells. An indication of the possible contractility of these cells is provided by the images in which they present a scalloped outline similar to that of the mammalian cells described by Ross & Long (1966).

The whole pattern of arrangement of the peritubular boundary tissue in the fowl most closely resembles that shown in Fig. 1(*c*) and described for man (Ross & Long, 1966), cat (Burgos *et al.* 1970), ram, and boar (Dym & Fawcett, 1970). As in these mammalian species, the peritubular contractile component in the fowl is multilayered. However, in direct contrast to any of the mammalian species studied, a typical fibroblast layer is found immediately adjacent to the inner fibrous lamella, any contractile elements therefore being entirely peripheral.

The contractility of the seminiferous tubules of rat, guinea-pig, rabbit, dog, ram and bull is well documented in the literature (Roosen-Runge, 1951; Niemi & Kormano, 1965; Wojcik, 1967; Soranto & Kormano, 1970; Kormano & Hovatta, 1972). It was first observed in the seminiferous tubules of the rat and dog and has been attributed to the Sertoli cells (Roosen-Runge, 1951). However, Clermont (1958) showed that through the application of light pressure it was possible to displace the cells of the seminiferous epithelium from short portions of the tubules, so that the

limiting membrane or boundary tissue became free from association with Sertoli cells. In such areas the boundary tissue showed contractions comparable to those of the intact tubules, thereby demonstrating that the contractile elements are located in the boundary tissue and not the Sertoli cells.

The significance of these observations is in their association with the movement of spermatozoa from the seminiferous tubules into the excurrent duct system, a mechanism which is generally regarded as a passive one. Among the factors affecting it are the diffusion of fluids which are subsequently resorbed in the ductuli efferentes and the proximal part of the ductus epididymidis (Mason & Shaver, 1952; Crabo & Gustafsson, 1964; Setchell, 1970; Tingari & Lake, 1972), and the contractility of the seminiferous tubules (Clermont, 1958; Lacy & Rotblat, 1960; Niemi & Kormano, 1965; Ross & Long, 1966; Ross, 1967; Wojcik, 1967; Kormano & Hovatta, 1972). Wojcik (1967) concluded both mechanisms are involved in the release of spermatozoa from the epithelium and their transportation into the excurrent ducts. It seems reasonable to assume, therefore, that the myoid cells in the fowl testis are both morphologically and functionally similar to the mammalian cells and play a role in the release of spermatozoa and their subsequent movement along the initial parts of the ducts. The distinct separation of the components of the fibrous lamella in the fowl and the staining of the dense homogeneous layers with PAS, which suggests a glycoprotein nature, resembles the situation in the rat and mouse (type A, Fig. 1).

In mammals there are clear species differences in the degree and type of fibrous components around the seminiferous epithelium and between the peritubular cells. There is also variation in the presence or absence and possibility type of attachment that exists between peritubular cells. These features may have physiological significance. Dym & Fawcett (1970) found that the peritubular cells of the rat constituted a significant permeability barrier and that the degree of patency of the cell-to-cell junctions was important in this respect.

A functional morphological study is now planned for this tissue in the fowl to elucidate its true significance.

SUMMARY

The ultrastructure of the boundary tissue of the seminiferous tubules of the domestic fowl consists of a fibrous lamella of homogeneous dense material and collagen fibrils, and peripheral to this a multilayered peritubular cellular component. Two distinct cellular forms are observed – an inner fibroblast cell and an outer myoid cell containing many cytoplasmic filaments and interfilamentous dense bands indicative of a contractile function. The significance of these observations in relation to sperm transport, mechanical support, and physiological impedance is discussed.

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