The structure of the interstitial tissue of the active and resting avian testis

T.A. AIRE

Department of Veterinary Anatomy, Medical University of Southern Africa, Medunsa, 0204 South Africa

ABSTRACT

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The interstitial tissue of the testis was studied in gonadally active and gonadally inactive domestic fowl (*Gallus gallus domesticus*), guinea-fowl (*Numida meleagris*), duck (*Anas platyrhynchos*) and Japanese quail (*Coturnix coturnix japonica*). Gonadal inactivity in the fowl was induced by a single subcutaneous injection of 50 mg oestradiol benzoate.

The structure of this tissue was similar in all the birds studied. Lymphatic vessels were mostly thin and meandered between the peritubular tissue peripherally and the centrally located blood vessels, Leydig cells and macrophages. The basal lamina rested on a closely associated homogeneous microfibrillar layer free of collagen fibres. The myofibroblast layer was several cells thick, and quite compact. The basal lamina of gonadally resting birds was highly irregular, relatively electron-dense, contained electron-lucent globules, and sent numerous finger-like processes or plicae into the seminiferous epithelium, particularly into the Sertoli cells.

The Leydig cells were few but typical in structure. In gonadally inactive birds they accumulated lipid droplets, dense heterogeneous bodies, probably lysosomes, and appeared to degenerate.

The avian testicular interstitium is similar to that of the human and cat in possessing a multi-layered myofibroblast component, and to that of the rodent in possessing a small number of Leydig cells, as well as in the location of the lymphatic vessels. Thus the bird combines characteristics of the interstitium found variably in mammals.

Keywords: Active, avian testis, gonadal, interstitial tissue, resting, structure

INTRODUCTION

The interstitial tissue of the testis of several mammals has been studied, and its role in the morphophysiology of this organ, including that of mechanical support for the seminiferous tubules and testicular blood vessels, and participation in the blood-testis barrier as well as the regulation of Sertoli cell function is now largely understood (Christensen 1965; Fawcett, Heidger & Leak 1969; Dym 1973; Fawcett, Neaves & Flores 1973; Weaker 1977; Skinner, Norton, Mullaney, Rosselli, Whaley & Anthony 1991).

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Only a few reports on the structure of the testicular interstitium in birds are available (Marchand 1973; Rothwell & Tingari 1973; Rothwell 1975a). The present study is aimed at contributing to the knowledge of the structure of the testicular interstitium (including the peritubular tissue, interstitial cells of Leydig and lymphatic vessels) in several avian species during both the active and inactive phases of the reproductive cycle, in organs which were fixed by vascular perfusion.

MATERIALS AND METHODS

Adult, sexually active males of the domestic fowl (*Gallus gallus domesticus*) (n = 5), guinea-fowl

(*Numida meleagris galeata,* Pallas) (n = 5), duck (*Anas platyrhynchos*) (n = 3) and Japanese quail (*Coturnix coturnix japonica*) (n = 8) were used in this study. Adult guinea-fowl cocks (n = 5) and drakes (n = 5) in the resting phase of the reproductive cycle, and domestic fowl cocks (n = 3) that received a single dose of 50 mg of oestradiol benzoate subcutaneously and killed 5 d later, were also investigated.

All the birds were anaesthetized with chloroform or thiopentone sodium (May & Baker Limited) and prepared for vascular perfusion with 3% glutaraldehyde buffered with 0,067% cacodylate solution as previously described (Aire 1982). Tissue samples from the testes of the birds were prepared for transmission electron microscopy, and semi-thin and ultra-thin sections of Epon-embedded tissues were cut, stained and examined as previously described (Aire 1982).

OBSERVATIONS

The organization of the interstitium and peritubular tissue of sexually active birds

The seminiferous tubules in the birds studied were surrounded by a peritubular layer of contractile myofibroblast cells. The spaces or wedges formed by the peritubular layer of three adjacent tubules, often arranged in a triangular, and less frequently, quadrilateral shape (Fig. 1 and 2), contained Leydig cells, lymphatics and blood vessels, a few fibrocytes and a small amount of ground substance (Fig. 1, 2, 3 and 4). The interstitium was usually very compact in sexually active birds. Generally, only a small number of Leydig cells were present, especially in the gonadally active domestic fowl. The interstitium was well vascularized, with the blood vessels frequently centrally located within it (Fig. 1 and 4). The lymph drainage of the interstitium was by means of sparse lymphatic vessels that were usually located, and seen to meander, between the peritubular tissue peripherally and the Leydig cells and blood vessels centrally (Fig. 1, 2 and 3).

Ultrastructurally, the basal lamina of the seminiferous epithelium was homogeneously dense and more or less regular in width (Fig. 5 and 6). It was not laminated. In all birds, particularly the domestic fowl, drake and guinea-fowl, the basal lamina was occasionally seen to invaginate into the seminiferous epithelium by means of spike-like folds or plicae (Fig. 6). The basal lamina rested on a thin layer of microfibrils and amorphous, moderately electron-dense material (Fig. 5, 6 and 7). Adjacent to this layer were situated the peritubular contractile cells (myofibroblasts) containing microfibrils, a few ribosomes and oval or slightly elongate mitochondria (Fig. 2, 3, 4, 5 and 6). The nuclei of these cells were highly elongated, showed a slight margination of the chromatin and contained a single, eccentrically placed nucleolus (Fig. 4 and 5). The nucleoplasm showed a generally uniform granularity. Short profiles of fairly distended, rough endoplasmic reticulum (RER) were commonly seen. Only a few oval, mitochondria were encountered. Microfilaments associated with intracytoplasmic densities situated both within the cytoplasm and attached to the internal surface of the plasmalemma, were also present in moderate abundance (Fig. 5 and 7) and several vesicles and micropinocytotic vesicles were observed in the cytoplasm of these cells (Fig. 7 and 8). A few microtubules were also seen among the microfilaments. Several layers, up to five, and occasionally more, of these overlapping cells occurred (Fig. 8). They constituted the myofibroblast layer, and separated the lymphatic and blood vessels, Leydig cells and macrophages from the seminiferous tubules (Fig. 7 and 8). The intercellular spaces between myofibroblasts displayed no unusual features, and junctional complexes occurred between overlapping cells (Fig. 6).

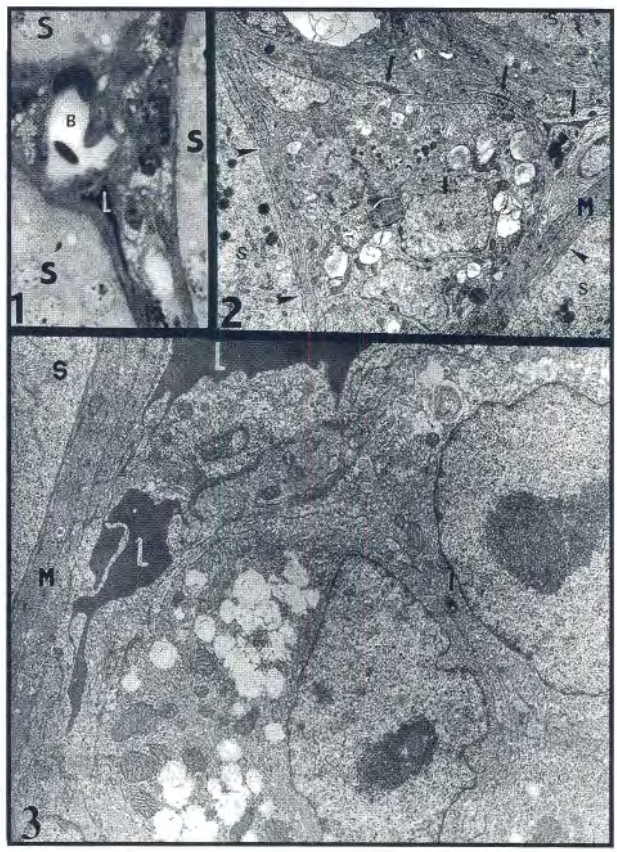
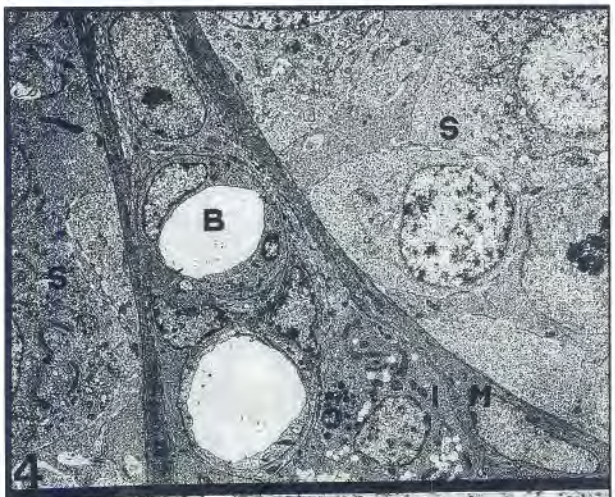
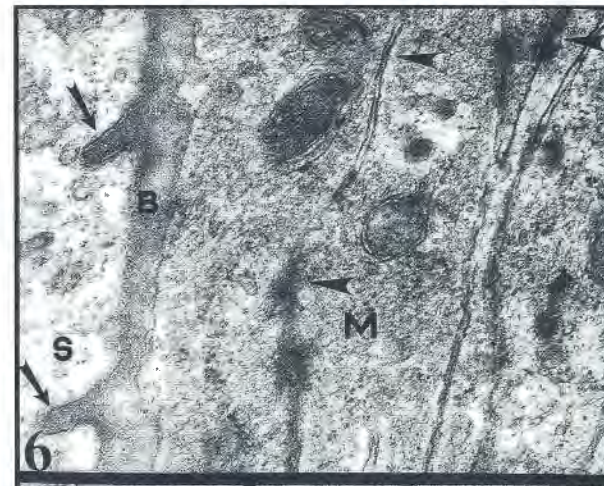


FIG. 1 A light micrograph of the interstitium of the testis of the domestic fowl. S = seminiferous tubule; B = blood vessel; L = lymphatic vessel; I = Leydig cell. Epon section, toluidine blue stain. X 640

FIG. 2 An electronmicrograph of the interstitium of the drake. S = seminiferous epithelium; arrowheads = basal lamina of the seminiferous epithelium; M = myofibroblast layer; arrows = lymphatic vessel; I = Leydig cell. X 7 000

FIG. 3 Part of the interstitium of the testis of the Japanese quail. S = seminiferous epithelium; M = myofibroblast layer; L = lymphatic vessel; I = Leydig cell. X 8 055





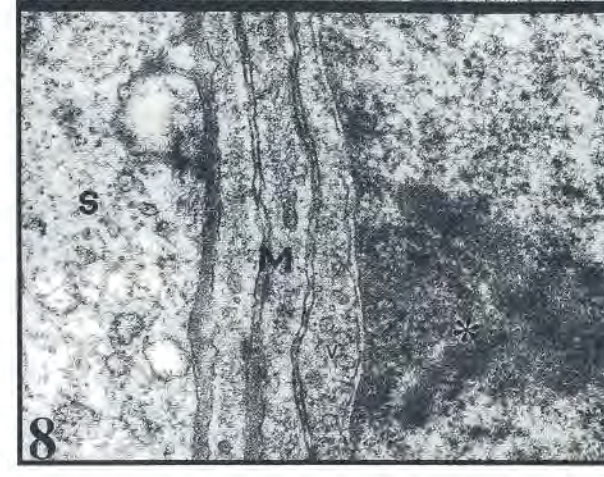


FIG. 4 A survey picture of the interstitium of the testis of the Japanese quail. S = seminiferous epithelium; M = myofibroblast layer; B = blood vessel; I = Leydig cell. X 17 700

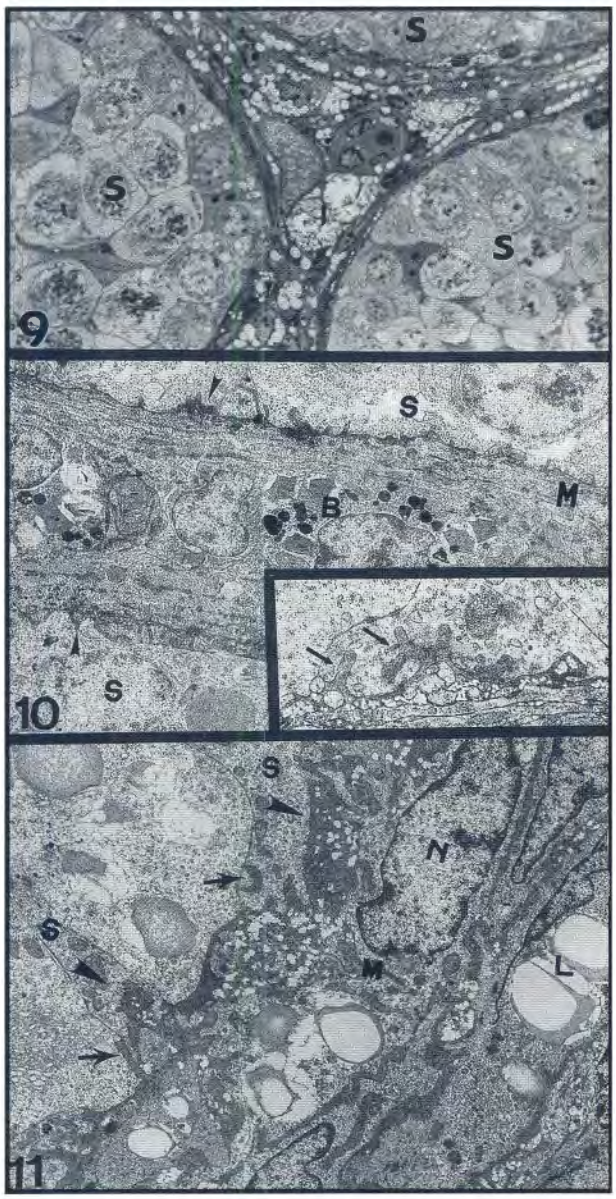
FIG. 5 The peritubular tissue of the Japanese quail showing the seminiferous epithelium (S), its basal lamina (arrowheads), myofibroblast (M) with its nucleus (N), microfibrils (F), and part of a Leydig cell (I). X 44 400

FIG. 6 The basal lamina (B) of the seminiferous tubule occasionally projects in a spike-like manner (arrow) into the seminiferous epithelium (S). M = myofibroblasts; Arrowheads = junctional complexes. Drake. X 54 857

FIG. 7 The peritubular tissue of the guinea-fowl showing the seminiferous epithelium (S); its basal lamina (B); myofibroblasts (M) with elongated nuclei and eccentrically located nucleolus (N); microfilaments (arrowheads); numerous vesicles (V). X 17 000.

Inset: arrowheads = intracytoplasmic densities; N = nucleus of myofibroblast; F = microfilaments. X 28 000

FIG. 8 The disposition of the myofibroblasts of the peritubular tissue of the drake. The myofibroblasts (M) overlap one another, are closely packed and show elongated, relatively euchromatic nuclei (N) with an eccentrically situated nucleolus (*). S = seminiferous epithelium; V = vesicles. X 53 333



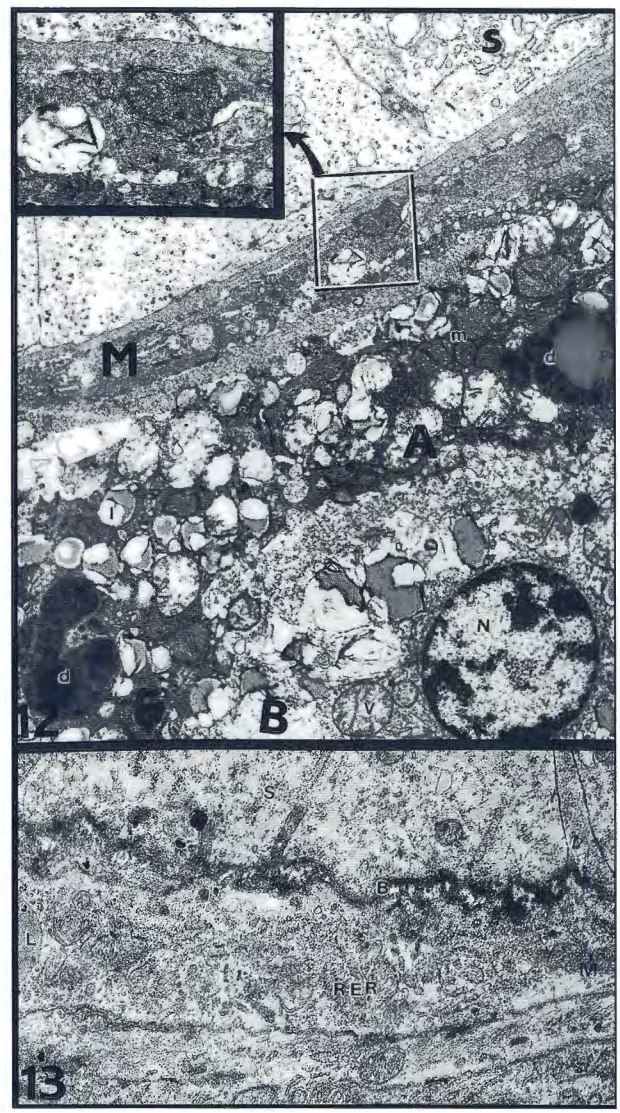


FIG. 9 A photomicrograph of the testis of the gonadally inactive guinea-fowl. S = seminiferous epithelium; I = Leydig cell giving a foamy appearance to the interstitium. Toluidine blue. X 1 400

FIG. 10 Non-wedge-shaped interstitium between two seminiferous tubules (S) of an inactive testis. Note the irregular outline of the basal lamina (arrowheads), Leydig cells showing numerous dense bodies (B), myofibroblasts (M)

Inset: note the spike-like protrusions of the basal lamina into the Sertoli cell of the seminiferous epithelium (arrows), and the electron-lucent globules associated with the basal lamina. Drake. X 4 666 (Inset = X 13 333)

FIG. 11 The interstitial tissue and seminiferous epithelium of the inactive gonad of the domestic fowl. The basal lamina (arrowheads) is highly irregular in outline, relatively more electron dense and shows numerous associated electron-lucent globules. Protrusions (arrows) of the basal lamina into the Sertoli cells (S) of the seminiferous epithelium occurs. The myofibroblasts (M) accumulate large lipid droplets (L), and their nuclei (N) are highly irregular in shape. Electron-lucent globules may also be seen in the intercellular boundaries of the myofibroblasts. X 21 454

FIG. 12 Part of the interstitium of the inactive testis of the guinea-fowl. S = seminiferous epithelium. Two Leydig cells of varying electron-density and mitochondrial structure are shown. Leydig cell A is electron-dense, contains numerous normal mitochondria (m), lipid droplets (l) and dense bodies (d). Leydig cell B is more electron lucent, shows a heterochromatic nucleus (N) and vesiculated mitochondria (v). The myofibroblasts (M) show lipid droplets and mitochondria with tubular cristae (enlarged, blocked area: X 27 500). X 13 767

FIG. 13 A myofibroblast displaying a large proportion of dilated RER, a large lipid droplet (L) and mitochondria with tubular cristae (M). Involuted drake testis. B = irregular basal lamina; S = seminiferous epithelium. X 12 000

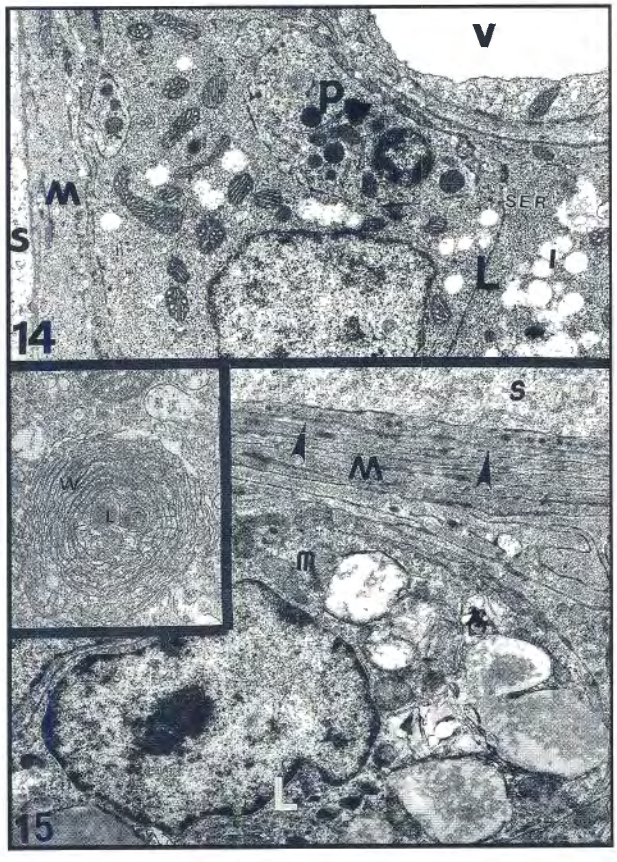


FIG. 14 Part of the interstitium of the Japanese quail testis, showing the seminiferous epithelium (S), myofibroblasts (M), a blood vessel (V), a macrophage (P) and Leydig cells (L). l = lipid droplets. The mitochondria and smooth endoplasmic reticulum (SER) are well developed in the Leydig cell. X 14462

FIG. 15 A Leydig cell (L) in the testicular interstitium of the gonadally active drake. The lipid droplets are large and partially extracted, and the SER is poorly developed. m = mitochondrion. Note the presence of several layers of myofibroblasts (M), and the basal lamina (arrowheads) of the seminiferous epithelium (S). X 14 878

Inset: a whorl of smooth endoplasmic reticulum (W) surrounding a lipid droplet (l) in a Leydig cell of the guinea-fowl. X 16578

Macrophages were few in the interstitium, and were usually located between blood vessels and Leydig cells with which they were closely associated. The organelle content of these cells was typical, and consisted of a heterochromatic nucleus, a few dense bodies, mitochondria and strands of RER (Fig. 14).

The organization of the interstitium and peritubular tissue of gonadally resting birds

The testis of the gonadally inactive bird displayed seminiferous tubules which were considerably involuted, and which revealed a cessation of spermatogenesis. The interstitium showed a relative increase in volume and the Leydig cells presented a foamy appearance (Fig. 9). The peritubular tissue layers appeared intact, and the basal lamina was thicker than in the gonadally active bird. It was highly irregular in form and varied remarkably in width, being highly folded (Fig. 10 and 11). A number of clear, globular spaces were seen in this lamina (Fig. 10 and 11). An unusually large number of spike-like folds invaginated into the seminiferous epithelium, particularly into the Sertoli cells (Fig. 10 and 11). The peritubular cells of the involuted guinea-fowl and drake testes showed a few lipid droplets, some oval vesicles containing a granular content, and mitochondria with well-developed tubular cristae (Fig. 12 and 13). Occasional myofibroblasts contained an unusually high content of RER and mitochondria with tubular cristae (Fig. 13). In the inactive testis of the oestrogen-treated domestic fowl, the peritubular cells displayed irregularly shaped nuclei with increased heterochromaticity (Fig. 11). Strands of RER were rarely seen, and mitochondria were few and inconspicuous. The intercellular spaces were thickened, and contained an amorphous and electron-dense substance that exhibited a few of the clear, globular structures observed in the basal lamina of the involuted testis (Fig. 11). The cytoplasm of the myofibroblasts showed large and often numerous, partially extracted lipid droplets that occupied a large proportion of the cell (Fig. 11). Lipid droplets were, therefore, more numerous in the myofibroblasts of oestrogen-treated domestic fowl than in that of the other birds that were in the normal physiological phase of involution in the reproductive cycle. In the involuted testis blood vessels were small and inconspicuous, and lymphatic vessels were only occasionally encountered or recognized in the interstitium.

The Leydig cells

The Leydig cells of the gonadally active birds possessed oval or polygonal nuclei which were generally euchromatic (Fig. 2, 3, 4, 14 and 15); with only slight margination of the chromatin occasionally being observed. The cytoplasm contained a number of prominent organelles. The Golgi apparatus was moderately developed, and the mitochondria were oval or elongate in shape and contained well-formed tubular cristae (Fig. 14 and 15). The mitochondrial matrix was electron dense in all of the birds studied. The smooth endoplasmic reticulum (SER) was by far better developed than the RER, but not nearly as well as in mammals (Neaves 1975). Whorls of smooth endoplasmic reticulum were seen only in the guinea-fowl Leydig cell (Fig. 15). The lipid droplets were partially extracted, fewer and much larger in the drake, guinea-fowl and domestic fowl than in the Japanese quail in which they were generally completely extracted.

The Leydig cells in the gonadally inactive birds present some noteworthy features, including an abundance of largely unextracted lipid droplets, swollen

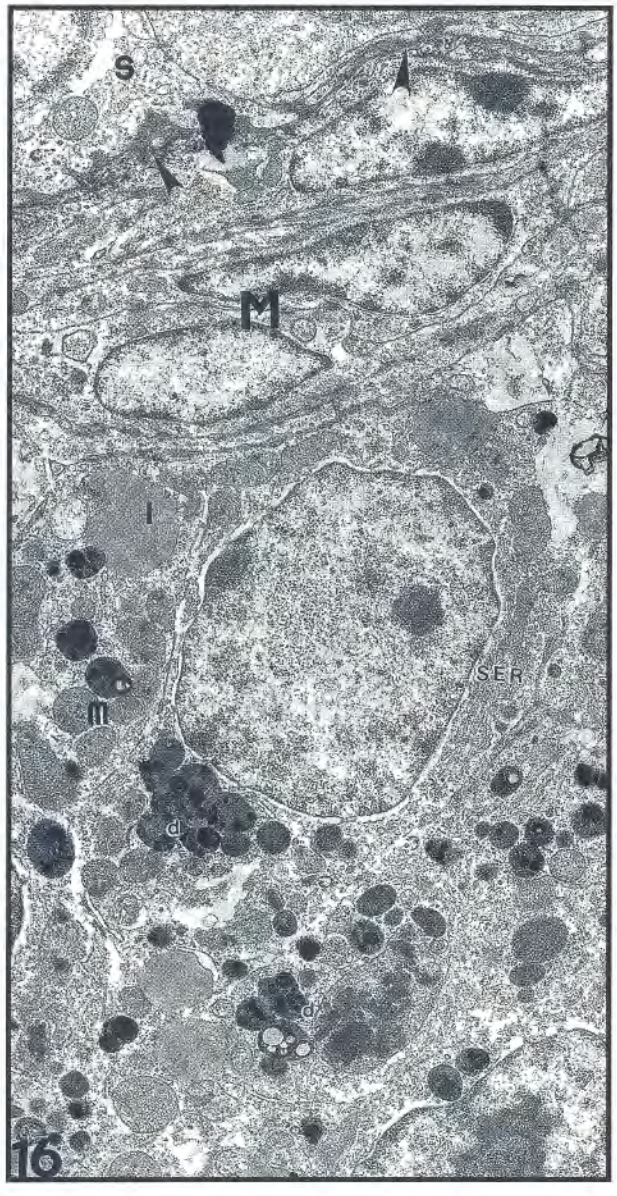


FIG. 16 The seminiferous epithelium (S); its irregular basal lamina (arrowheads), myofibroblast layer (M), and Leydig cells of the inactive testis of the drake. Numerous mitochondria (m) and dense bodies (d) occur in the cell. A few large lipid droplets (l) and smooth endoplasmic reticulum (SER) are also present in the cytoplasm of the cell. X 11 294

and vesiculated mitochondria, highly heterochromatic nuclei (Fig. 11 and 12) and an unusually large number of dense bodies, probably lysosomes (Fig. 16). Two types of Leydig cells, based on their electron density and mitochondrial characteristics, were encountered in the resting testis (Fig. 12). One type was less electron dense, showed a rarefied or reticular cytoplasm, large, partially extracted lipid droplets, a heterochromatic nucleus, and vesiculated, disorganized mitochondria (Fig. 12). The other type adjoined the peritubular tissue, was more electron dense and contained more ground substance, numerous lipid droplets and well-formed polymorphous mitochondria bearing tubular cristae within a highly electron-dense matrix (Fig. 12). Several disorganized mitochondria and oval, vesicular organelles containing granular or flocculent material were also seen, together with dense bodies, in this cell type (Fig. 12).

DISCUSSION

Unlike the situation in mammals (Fawcett *et al.* 1973), there is little or no variation in the structural organization of the interstitial tissue of the testis in the sexually active species of birds studied. The organization of the peritubular tissue is similar to that described by Rothwell and Tingari (1973) for the domestic fowl, except that in the present study there was not the clear region or internal (fibroreticular) lamellum containing loosely arranged collagen fibres immediately subjacent to the basal lamina. In contrast, the basal lamina seemed to rest on the myofibroblast layer, with only a thin non-obtrusive microfibrillar layer, about as thick as the basal lamina, intervening. Consistently, no collagen fibres were seen. The fixation of the tissues used in this study was by vascular perfusion as opposed to the immersion-fixation method used by Rothwell and Tingari (1973), but it is not clear how this difference in fixation procedure could have affected the relative obscurity of this layer and the absence of collagen fibres in it in all four species of birds.

The seminiferous tubules of gonadally inactive birds are physiologically atrophic. Their basal lamina was irregular in outline, commonly thickened, and sent plicae or folds into the seminiferous epithelium in all of the birds in the inactive gonadal phases, including the oestrogen-treated cocks. The basal lamina of the seminiferous tubule is thickened in deranged spermatogenesis in man (De Kretser, Kerr & Paulsen 1975; Cameron, Murrat & Drylie 1985), the rabbit (Bigazzi, Kosuda & Hsu 1976) and the bull (Veeramachaneni, Heath, Ott, McEntee & Hixon 1987). It is speculated that this thickening, as well as protrusions of the basal lamina into the seminiferous epithelium facilitates the flow of raw materials into or out of the seminiferous tubule (Chakraborty, Nelson & Jhunjhunwala 1976). In a physiological process, such as the periodic involution of the testes of seasonally breeding birds, it is even more likely that the protrusions of the basal lamina into the seminiferous epithelium and, in particular, into the Sertoli cells, could be involved in the exchange of materials between the seminiferous epithelium and the interstitium. The exact nature of the electron-lucent globules which are present in the basal lamina of such birds is unknown, but they could be involved in the exchange process in an organ soon to become recrudescent and enter another phase of the reproductive cycle.

In the involuted testes the peritubular tissue remained intact, and the increased number of RER, swollen with secretion in some of these cells, may indicate a change in their normal structure and function. Nicholls & Graham (1972), in their studies on the differentiation of the Leydig cell in the Japanese quail, observed that the membranes of the endoplasmic reticulum of the cell lost their attached ribosomes at maturity. The accumulation of lipid droplets and the change from lamellar to tubular cristae in the mitochondria of some of the myofibroblasts seem to support the observed morphological and functional cytodifferentiation. However, it is important to note that the reaction of the myofibroblasts to oestrogen treatment in the cocks varied in some respects from that of the cells of the birds which were in the inactive phase of their normal reproductive cycle. For example, the myofibroblasts of these cocks did not contain RER or mitochondria as much as in the inactive birds which were in their involutionary phase of the reproductive cycle. The presence of electron-dense, amorphous material between the myofibroblasts of oestrogen-treated cocks was also unique. The nature of this substance which is apparently produced by the cells is unknown, but it appears to be similar in consistency to the basal lamina of the seminiferous epithelium of these treated cocks. It is now known that the peritubular cells are involved not only in environmental interactions, that is structural interactions, but also regulatory interactions, in which they produce a paracrine or an autocrine agent to elicit a signal transduction event to influence cellular functions on a molecular level (Skinner 1987). In the inactive birds studied, the seminiferous epithelium lost only the later germ-cell series, such as the secondary spermatocyte and the various spermatid series. It seems, therefore, that some control of the seminiferous epithelium also occurs during the inactive phase of the testis. The various activities of the peritubular cells could play a role in this regard. The secretion of PModS by peritubular cells is known to influence Sertoli cell functions vital for the maintenance and control of spermatogenesis (Skinner *et al.* 1991). The peritubular tissue forms part of the blood-testis barrier, and in vasoligated cockerels this tissue was able to resist destruction despite the fact that in severely damaged seminiferous tubules, some macrophages apparently passed through the tissue into the tubular lumen (Aire & Heath 1979).

In this study, and for the first time, the relative position and size of the lymphatic vessels have been shown quite clearly in the interstitium of vascularly perfused testes. The lymphatic vessels are generally interposed between the Leydig cells and the blood vessels and the peritubular myofibroblast tissue. These lymphatics are, however, few in number and poorly developed in birds, especially in involuted testes where they are rarely identified under the microscope. This could indicate that the interstitial tissue of birds does not contain as much fluid as does that in mammals (Fawcett *et al.* 1973). However, the testes of birds have a very large fluid content, which is probably located in the seminiferous tubules (Lake 1971; Aire 1979), and serves to flush spermatozoa into the rete testis which itself has a large fluid content. The bird is similar to the rat, chinchilla, guinea-pig and mouse in possessing a relatively small volume of Leydig cells and in the location of the lymphatic vessels, but differs from these mammals in lacking an extensive peritubular lymphatic system (Fawcett *et al.* 1973). The arrangement of the myofibroblasts is similar to that of the human and cat (Burgos, Vitale-Calpe & Aoki 1970), in being multilayered. The bird therefore combines characteristics of the interstitium found variably in mammals.

The Leydig cells of birds appear in clusters of only a few cells in the intertubular space, especially where it is angular, and rarely so where the seminiferous tubules lie and run parallel to each other. The Leydig cells of birds generally show similar organelles to those of mammals and are similarly disposed (Neaves 1975). However, with the exception of the Japanese quail, the smooth endoplasmic reticulum is not as well developed in birds as it is in mammals. Nicholls & Graham (1972) considered that whorls of SER never occurred in the Leydig cells of the Japanese quail. They were not seen in the same species of bird in the present study, but they were encountered in the guinea-fowl .

Nicholls and Graham (1972) observe that lipid droplets do not appear to be numerous in mature Leydig cells of the Japanese quail, and Rothwell (1975b) considers them to be absent in the same species of bird. However, in the present study, numerous lipid droplets were observed in Leydig cells of this species (cf. Fig. 3). It is not clear why the lipid droplets in the Japanese quail were generally extracted, and only partially so in the other species of birds. In involuted testes of birds lipid droplets increase remarkably not only in number but also in size in the Leydig cells. There was also a considerable reduction in the content of the smooth endoplasmic reticulum, which has been considered to be an indication of reduced testosterone production by these cells (Mazzocchi, Robba, Rebuffat, Gotardo & Nusdorfer 1982). The presence of an unusually large number of dense granules, probably lysosomes, in the Leydig cells of gonadally inactive birds suggests that an increased lysosomal activity in these cells occurs during the endocrinological atrophy of the testis. Whether the lysosomal activity is heterophagic or autophagic is not understood, but it is quite likely that it is autophagic, and a prelude to cell death or remodelling. The presence of both the electron-dense and electron-lucent type of Leydig cell in the inactive but not the active testis, is not a fixation artefact. The electron-lucent cell exhibited relatively less organelle and

ground substance content, and was probably undergoing degeneration and subsequent removal from the interstitium.

Equally remarkable was the observed accumulation of large and numerous lipid droplets in the cytoplasm of some myofibroblasts, especially of the oestrogen-treated cockerel. Leydig cells differentiate *de novo* from certain mesenchymal cells in the immature testis (Black & Christensen 1969; Nicholls & Graham 1972; Nistal, Paniagua, Regadera & Santamaria 1986). It is now considered that cells of loose connective tissue lying close to the vascular system and peritubular tissues differentiate into new Leydig cells (Kerr, Bartlett, Donachie & Sharpe 1987; Hardy, Gelber, Zhou, Penning, Ricigliano, Ganjam, Nonneman & Ewing 1991). Peritubular and interstitial cells have been found to localize androgen receptors (Hardy *et al.* 1991). Lofts & Bern (1972) observe that the interstitial cells of birds, in the non-breeding season, disintegrate and are removed by macrophages, and that a new generation of Leydig cells is developed to replace the old. It seems plausible to consider that the myofibroblast cells which were seen here to accumulate lipid droplets and develop characteristic mitochondria, were in the process of being stimulated to cytological differentiation, apparently for the replacement of disintegrated Leydig cells resulting from the involution of the testis. As the breeding season approaches, Leydig cells of birds and reptiles are reported to build up lipid droplets which are cholesterol-positive (Lofts & Bern 1972). Perhaps lipid accumulation is one of the earlier observable cytodifferentiational processeses in certain mesenchymal cells in the interstitium of recrudescent testes.

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