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On the structure of the epididymal region and ductus deferens of the domestic fowl (*Gallus domesticus*)

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INTRODUCTION

There are differences between the fowl and common domestic mammals in the biochemistry and physiology of seminal plasma and spermatozoa (Lake, 1966). Glover & Sale (1969) and Glover & Nicander (1971) emphasize the need to reappraise the anatomy of, and the nomenclature applied to, apparently similar parts of the reproductive tract in different classes of animals, and also in mammals with either scrotal or abdominal testes.

Aspects of gross anatomy of the reproductive tract of the male fowl have been described by Kaupp (1915), Burrows & Quinn (1937), Gray (1937), Parker, McKenzie & Kempster (1942), Bradley & Grahame (1960) and Marvan (1969). A histological study by Lake (1957) was made to localize the possible sources of seminal cellular and fluid products in the fowl. Stoll & Maraud (1955) and Maraud (1963) reconstructed and described the development of the epididymis from the mesonephros.

The present observations are intended to serve as a reference for further investigations on the fine structure and histochemistry of the male reproductive tract of the domestic fowl, the maturation of spermatozoa therein, and their relationship to special fertility phenomena in the bird.

MATERIALS AND METHODS

Thirty-two mature Leghorn cocks were used in this study, together with a few immature 2-month-old cockerels. Both good and poor producers of semen were included amongst the mature birds.

Tissues for histology were obtained from the epididymal region, the cranial, middle and caudal segments of the ductus deferens and the dilated portion of the ductus deferens. Bouin's fixative was found to be more satisfactory than either Susa, Zenker's or 10 % buffered formalin. Tissues were embedded in paraffin and sectioned at 5–7 μm. Sections were stained with haematoxylin and eosin and Masson's trichrome.

Transverse serial, 10 μm sections of the epididymal region of a mature bird were used for making a graphical reconstruction. Retrograde injection methods were also used to establish the architecture of the epididymal region. For this purpose the lateral half of the testis was removed to assist the penetration of the injection media into the ducts. The seminal contents of the ductus deferens were milked out, some-

times followed by a saline wash, and each injection was made at a controlled pressure of 150–200 mmHg from the distal end of the ductus deferens. Injection media included micropaque (Damancy and Co., Ltd.) for X-ray examination and neoprene latex followed by corrosion in concentrated hydrochloric acid or clearing (Williams, 1943; Siller & Hindle, 1969).

RESULTS

Gross features

An active testis is large, white and soft (Lorenz, 1959; Lake & El Jack, 1966); in an inactive bird it is much smaller, greyer and harder. The epididymal region is about 2 cm long in a sexually active bird (Figs. 1, 2). The ductus epididymidis appears as a short coiled white tubule in the juxtatesticular tissue (epididymal region) applied to the dorsomedial surface of the testis (Figs. 2, 3). The ductus deferens extends for about 14 cm from the caudal end of the epididymal region to the base of the ejaculatory duct (Figs. 1, 2). It is white and turgid when full with semen, and when inactive contains a watery secretion. In the latter condition it is small and flaccid and difficult to dissect. The apparent coils of the ductus deferens are impossible to pull out into a straight tube; in a longitudinal section the lumen appears to consist of a series of pockets.

Histological features

The seminiferous tubules of the testis pass directly into the channels of the rete testis (Figs. 3–6) which in turn connect with the ductuli efferentes. Initially the latter structures are wide and they gradually taper before forming connecting ductules. The ductules follow a tortuous course towards the medial aspect of the epididymal region where they interconnect and become wider; they eventually open into the ductus epididymidis (Figs. 2–4).

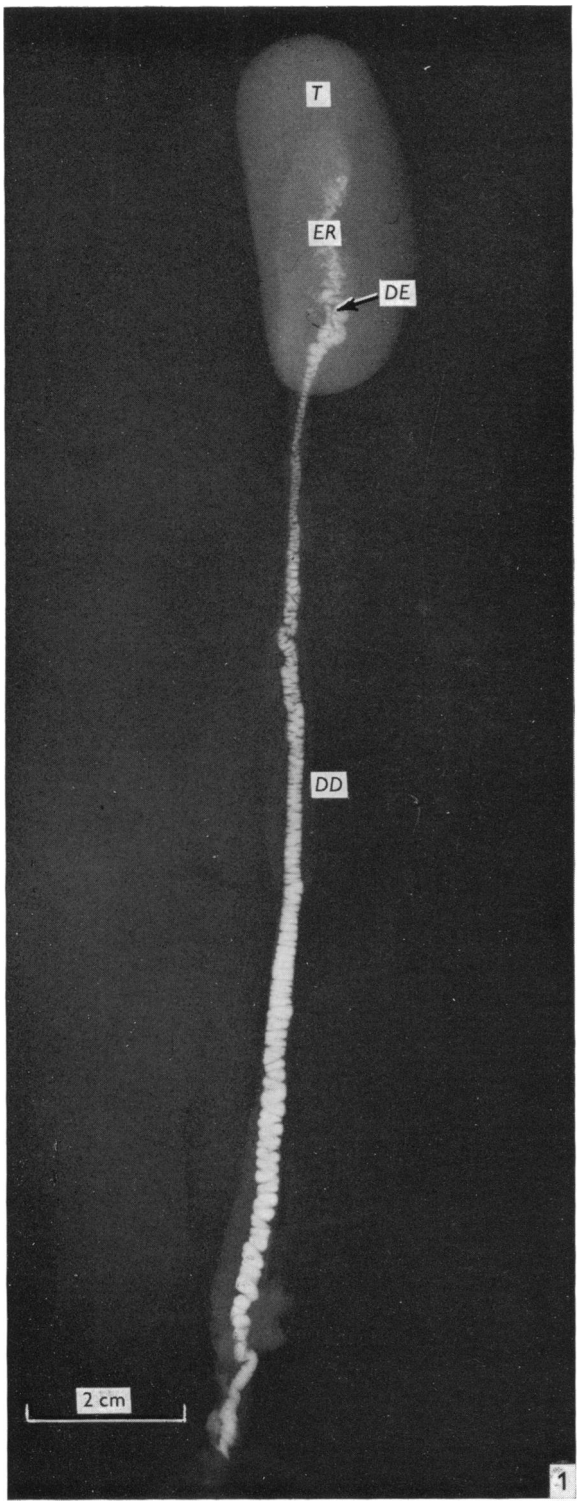
The whole duct complex immediately adjacent to the testis is embedded in a fibrous stroma containing a few smooth muscle fibres (Fig. 7). In inactive (Fig. 8) and immature birds, there is a relative increase in the amount of connective tissue as the tubules become smaller and more folded. These changes resemble those seen in lactating and non-lactating mammary glands.

Lymphocytes and macrophages are present in the intertubular connective tissue. Large blood vessels supplying the testis are numerous, passing through the lateral aspect of the region (Nishida, 1964). The blood supply is poor within the epididymal region itself.

Fig. 1. An X-ray after micropaque injection into the dilated region of the ductus deferens. *T*, testis; *ER*, epididymal region; *DE*, ductus epididymidis; *DD*, ductus deferens.

Fig. 2. Neoprene cast of the epididymal region (*ER*) and cranial segment of the ductus deferens (*DD*). The flexuous course of the ductus epididymidis (*DE*) is continued into the ductus deferens. The connexions between the epididymal region and testis (*T*) are at certain interspaced points (arrows).

Fig. 3. A diagrammatic representation of the epididymal region showing an arbitrary number of ductuli efferentes (*DEF*), *T*, testis; *CD*, connecting ductules; *DE*, ductus epididymidis; *AE*, appendix epididymidis; *P*, paradidymis; *RT*, rete testis; *DD*, ductus deferens.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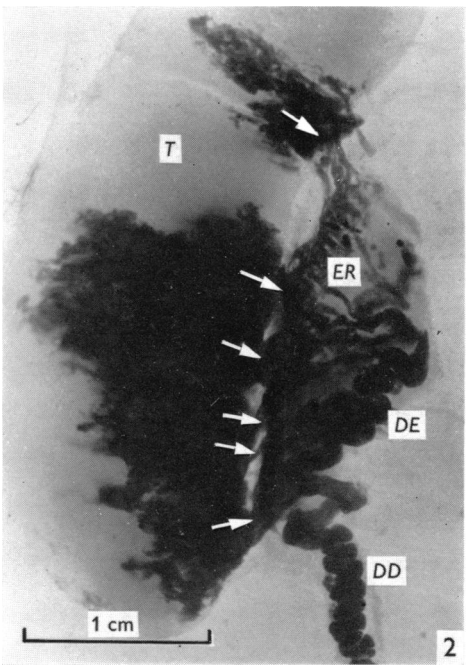
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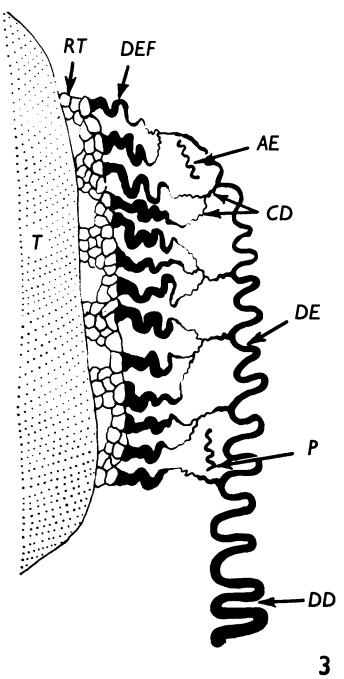
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Rete testis

The rete (Figs. 5, 6) is a network of thin-walled spaces embedded in connective tissue and located entirely outside the testis, mainly in the lateral edge of the epididymal region, but also spreading over its ventral and dorsal aspects. The confluence of the seminiferous tubules with the rete is not along the entire length of the opposed surfaces of the testis and epididymal region but occurs at certain interspaced points forming definite bridges between them; six such points are illustrated in Figs. 2 and 3.

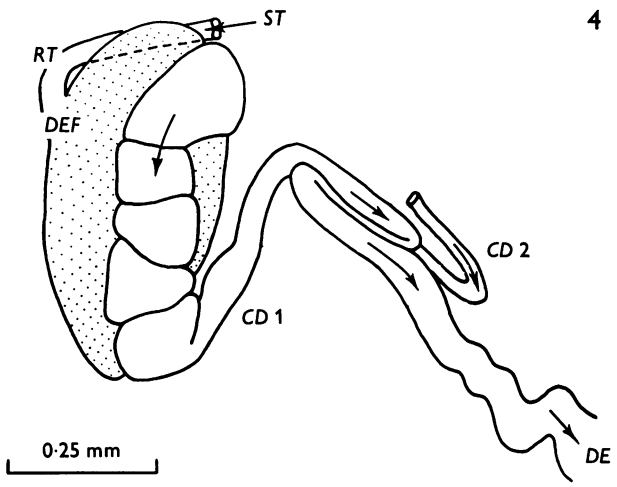

Fig. 4. A drawing from a graphical reconstruction of a connexion between the seminiferous tubules (*ST*), and the ductus epididymidis (*DE*). The rete testis (*RT*) joins the efferent ductules (*DEF*) which in turn narrow to become a connecting ductule (*CD*1). It is joined by another connecting ductule (*CD*2).

The epithelial lining is simple squamous to low cuboidal (4–7 μm). Gray (1937) suggested that this resulted from the pressure of the luminal contents but this seems unlikely as the lining is not affected by variations in sexual activity. There are abrupt changes of the epithelial types at the connexions of the rete with the ductuli efferentes.

Ductuli efferentes

These are the commonest and largest of the tubules in the epididymal region. The average luminal diameter in the initial wide part is 500 μm (300–700 μm), reducing to about 100 μm distally. The epithelial walls are folded, the folds involving the basal lamina and the luminal surface. The latter is the more extensively folded because of the presence of alternating groups of tall and low cells (Fig. 9). In the thin parts of the epithelium the height of the cells is about 26 μm. There are tall dark slender cells with an expanded ciliated luminal border which connect to the basal lamina by narrow stalks (Fig. 9). Their nuclei are elongated, occcupying the luminal half of the cytoplasm, and have one or two nucleoli. There are also light and intermediately stained cells of variable heights which may reach the lumen; these possess

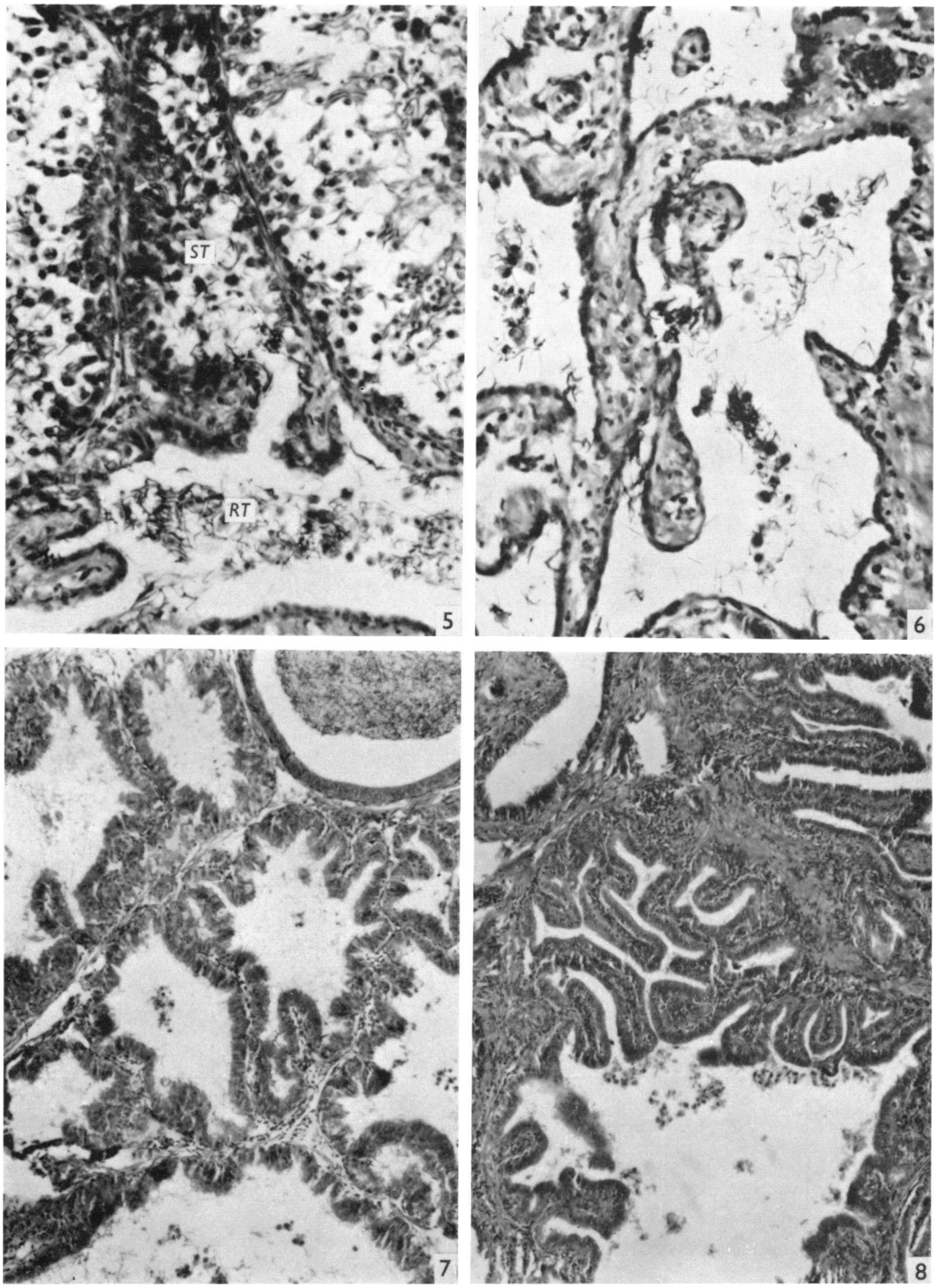

Fig. 5. Seminiferous tubule (*ST*) opening directly into rete testis (*RT*). ×275.

Fig. 6. Rete testis lined by simple squamous or low cuboidal cells. Spermatozoa and spermatids at various stages of development are dispersed in the lumen. ×275.

Figs. 7, 8. Epididymal regions of sexually active and inactive birds respectively. In the active state the ducts are wider and intertubular tissue is scanty. In the inactive region the tubules are more folded, have narrower lumina and comparatively more interlobular tissue. Luminal contents are reduced. ×275.

spherical nuclei in the basal cytoplasm. Occasional short angular cells are wedged among the bases of the tall cells. Thus the general nuclear distribution produces pseudostratification.

When sexual activity is minimal the ductuli efferentes are narrower and more folded (Fig. 8), and the epithelial height reduces to about 15 μm.

Connecting ductules

The initial parts of the connecting ductules resemble the ductuli efferentes except for their narrower lumina (60 μm) and more regular and smoother outline (Fig. 10). A compact mass of spermatozoa is characteristically seen in the lumen. The connecting ductules are lined by pseudostratified ciliated columnar epithelium, 15 μm in height. The tufts of cilia of the dark staining cells are more prominent than those of the similar cells in the ductuli efferentes. Light staining cells and angular basal cells are present as in the ductuli efferentes.

The ductules of sexually poor birds are narrower (30 μm) and their epithelial lining is thinner (10 μm).

The ductules gradually widen as they join each other towards their confluence with the ductus epididymidis. In these parts the ciliated cells disappear and the ductules become indistinguishable from the ductus epididymidis (Fig. 11).

Ductus epididymidis and ductus deferens

The ductus epididymidis is a very tortuous tube which starts at the cranial end of the epididymal region and courses along its medial border to continue past the caudal end as the ductus deferens (Figs. 1–3, 11). The ductus epididymidis is confined within the connective tissue of the juxtatesticular region, but the ductus deferens is free. Otherwise, they appear macroscopically to be different parts of the same duct, and are also histologically similar. The epithelial folds, which start in the wide connecting ductules, are continued into the ductus epididymidis and the cranial part of the ductus deferens (Figs. 11, 12).

The initial part of the ductus epididymidis is very narrow (300 μm), but a gradual increase in diameter occurs caudally and continues into the ensuing ductus deferens. The average epithelial height of the ductus epididymidis is 30 μm. With poor sexual activity the diameter of the lumen and the height of the epithelium are halved.

The ductus deferens has an average luminal diameter of 400 μm at the cranial end which gradually increases to 550 μm in the middle and 900 μm in its caudal parts. At the extreme caudal end there is a dilatation, 2 mm in diameter, which opens into the ejaculatory duct. The epithelium of the ductus deferens at the beginning is as high

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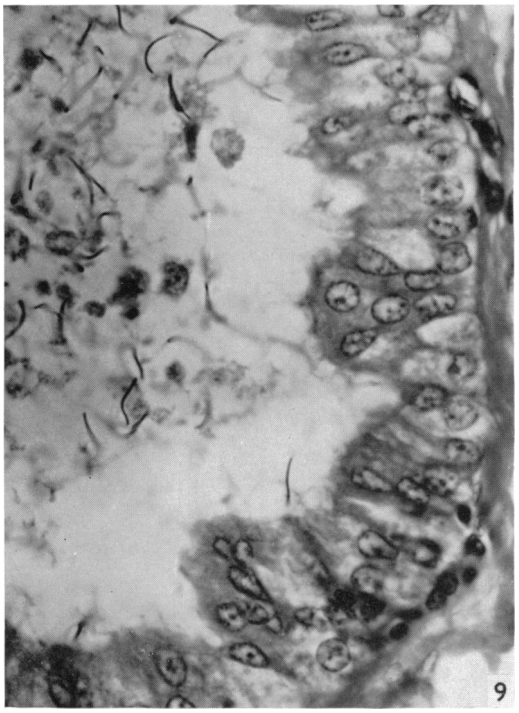
Fig. 9. Ductuli efferentes. Among the lining cells are the dark staining ciliated cells, clear cells and some intermediate ones. Luminal content is similar to that of the rete. × 690.

Fig. 10. Initial part of a connecting ductule. Note the high concentration of spermatozoa in the lumen compared with that in the ductuli efferentes. Most of the lining cells are ciliated and basal cells are occasionally present. × 690.

Fig. 11. Connecting ductules on the left joining the caudal end of the ductus epididymidis, which is sectioned many times on the right. Note the epithelial folds in both ducts. In the centre is the paradidymis. × 46.



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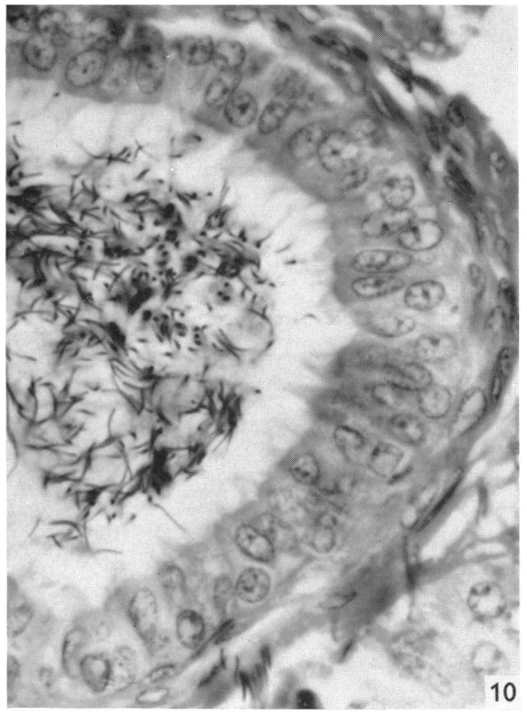

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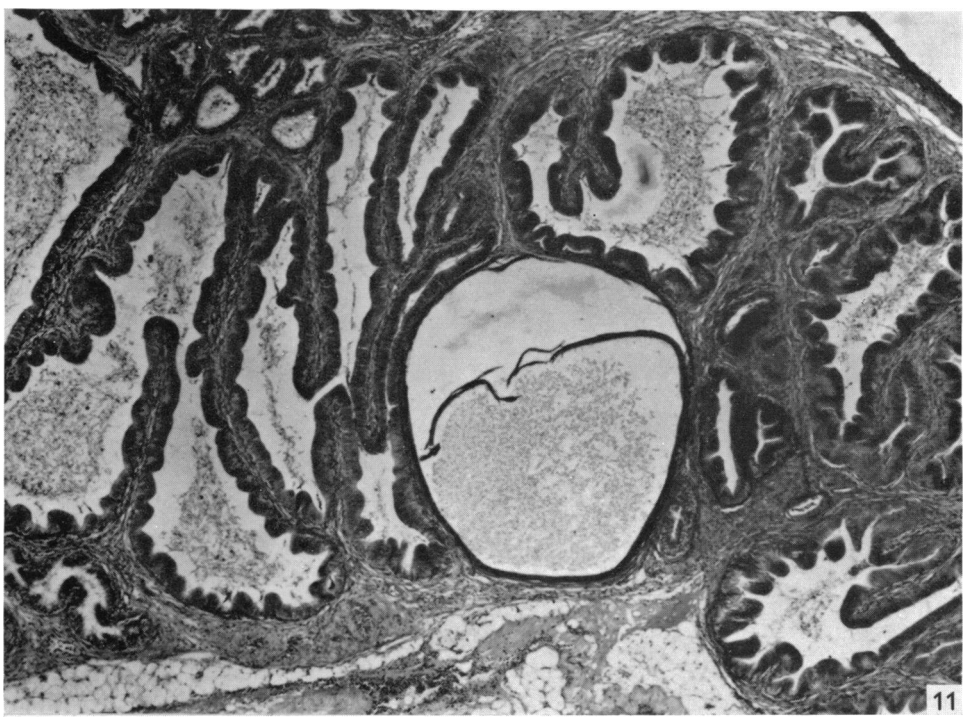

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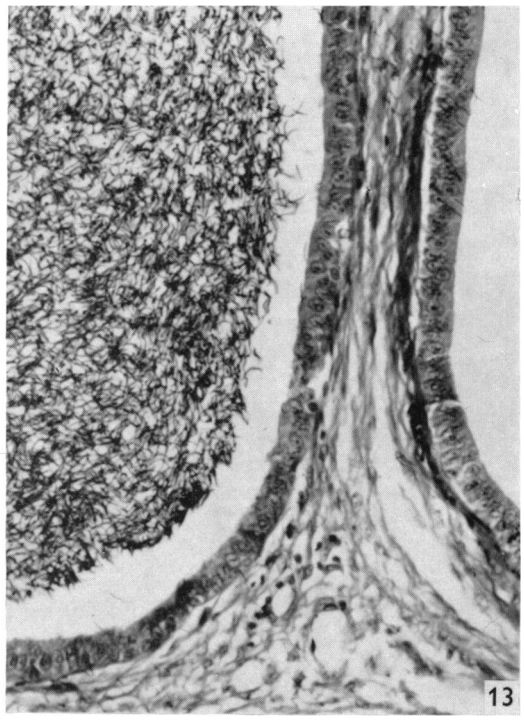
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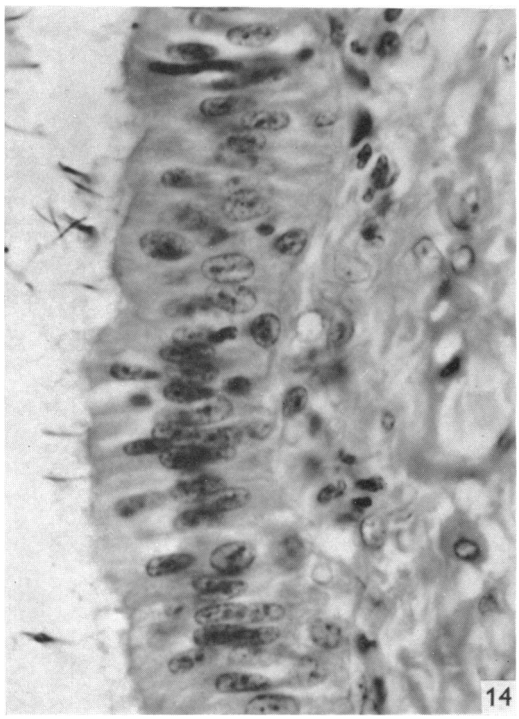
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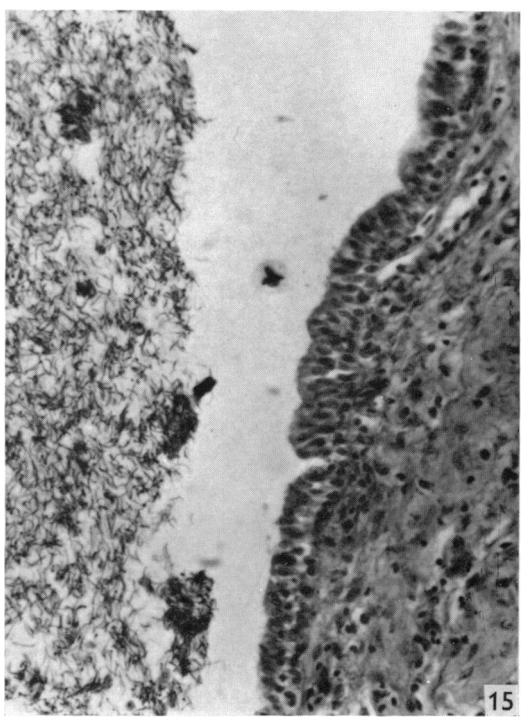
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as that of the ductus epididymidis (30 μm), but steadily falls to 20 μm in the middle and 15 μm in the caudal parts; the height again rises in the dilated portion to 25 μm (Figs. 12–15).

The lumen is lined by pseudostratified, non-ciliated columnar epithelium consisting of light and dark staining cells (Fig. 14). The darker cells have more slender nuclei with more densely packed chromatin and they occupy the luminal portion of the cytoplasm. The other cells have centrally placed oval, vesicular nuclei, with one or two nucleoli. Some smaller angular cells next to the basal lamina are wedged among the bases of the tall cells. They have spherical nuclei, somewhat smaller than any of the others (Fig. 14). Bleb-like projections on the free surface of the epithelium are seen in the cranial segment of the ductus deferens.

In the distal dilated portion of the ductus deferens the epithelial lining is similar to the remainder, but the basal cells constitute a more definite layer (Fig. 15).

The ductus deferens has a relatively thin, 100 μm, fibromuscular coat closely applied to the epithelium. In the distal dilated portion it is 275 μm thick. The whole ductus deferens is poorly vascularized.

In sexually inactive males the walls of the duct are collapsed and the cavity shrinks to half its usual diameter. The fibromuscular coat is contracted and thus appears thickened (Fig. 16). The dilated part is only 1 mm in diameter and a similar halving occurs in the height of the lining epithelium.

Luminal contents

During normal sexual activity the luminal contents vary in different parts of the duct system. The density of spermatozoa is lowest in the rete testis and in the ductuli efferentes (Figs. 6, 9), while a conspicuous dense mass of interwoven spermatozoa lies in the initial narrow part of the connecting ductules (Fig. 10). In the remainder of the tract the spermatozoa appear to be distributed at a fairly uniform density, occupying almost the entire lumen, but leaving a narrow sperm-free zone separating them from the epithelial lining (Figs. 12–15). This probably represents a shrinkage artefact.

Spermatids in different stages of maturation are mixed with the spermatozoa. Large numbers occur in the rete, a few in the ductuli efferentes, and very few in the initial segments of the connecting ductules. Only occasional ones appear elsewhere.

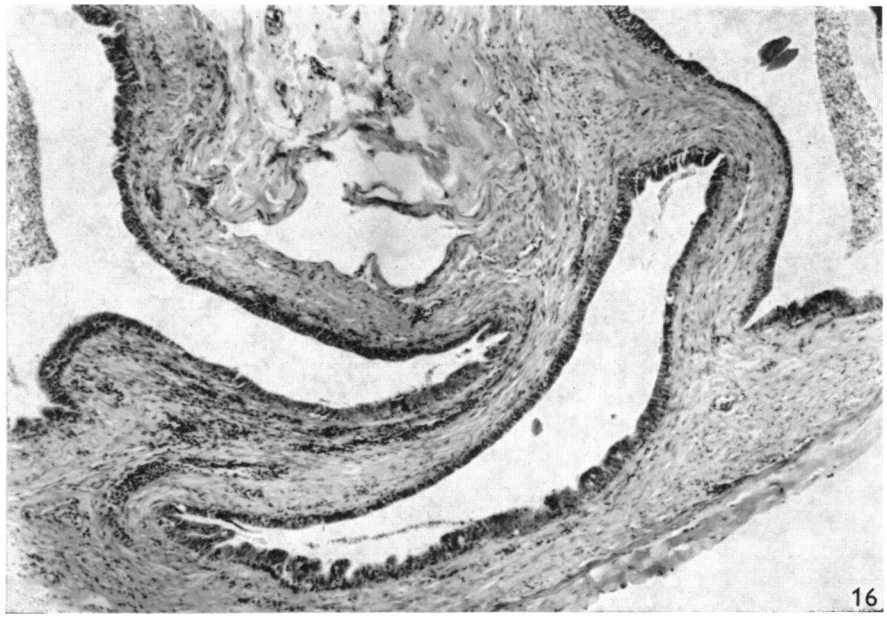
Entire epithelial cells, as a third component of the luminal contents, are often seen in the ductuli efferentes.

In immature males and in those of poor sexual activity all these contents are reduced or absent (Fig. 8).

Figs. 12, 13. Cranial and caudal segments of the ductus deferens respectively. The epithelial folding of the connecting ductules and ductus epididymidis is continued in the cranial segment and disappears caudally. ×275.

Fig. 14. Cranial segment of ductus deferens showing tall columnar cells, some of which stain darker than others. Basal cells and bleb-like luminal projections are present. ×690.

Fig. 15. Dilated region of ductus deferens. Epithelial lining is similar to other parts of ductus deferens, but the basal cells are numerous and seem to constitute a definite layer. ×275.



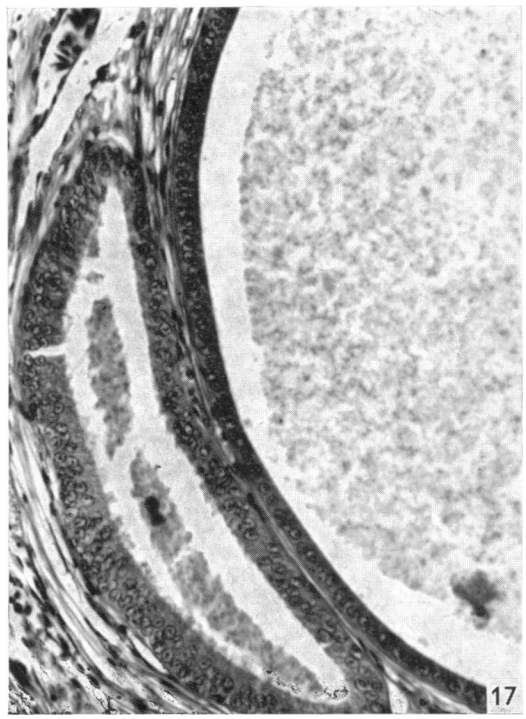


Fig. 16. Middle segment of ductus deferens from a poor semen producer. The walls are collapsed and there is hardly any luminal content. × 107.

Fig. 17. Appendix epididymidis with homogeneous luminal contents is lined by simple cuboidal or low columnar epithelium. × 275.

Vestiges of embryonic mesonephros

Two similar types of tubules are found in the cranial (Fig. 11) and caudal (Fig. 17) parts of the epididymal region. They have smooth regular outlines with an average diameter of 280 μm for the cranial and 440 μm for the caudal tubule. They are lined by simple cuboidal or low columnar cells, 10 μm high, with no surface specialization. The luminal content is a homogeneous material devoid of spermatozoa or any cellular constituents. These cyst-like ducts are probably derived from the cranial and caudal mesonephric tubules and hence may be considered analogous to the appendix of epididymis and paradidymis of mammals (Bloom & Fawcett, 1968).

DISCUSSION

The juxtatesticular duct system of the avian genital tract, unlike that of most mammals, starts with the rete testis, since no distinct tubuli recti are present. There is no distinct mediastinum testis. The rete network lies outside the testis – a situation similar to that which is found in the rat (Reid & Cleland, 1957).

The rete testis, ductuli efferentes, connecting ductules, and ductus epididymidis of the fowl are embedded in connective tissue which partly serves to anchor the testis to the dorsal body wall, and is evident as a tissue mass adjacent to the dorsomedial surface of the testis. Kaupp (1915), Gray (1937), Parker, McKenzie & Kempster (1942) and Bradley & Grahame (1960) referred to the collection of these structures as the epididymis, and Lake (1957) later referred to it as the epididymal region.

The sequence of the different ducts constituting the juxtatesticular region reported by Stoll & Maraud (1955), Maraud (1963), and Marvan (1969) is confirmed by the present study. Stoll & Maraud (1955) reconstructed an epididymal region of a 2-month-old cockerel and counted 51 ductuli efferentes. They also reported a number of aberrant ducts and fragmentary canaliculi constituting the paradidymis.

Anatomically, and by etymological derivation, the avian epididymis has been regarded as confined to the structures of the juxtatesticular region. From the present work there are cogent reasons for regarding the ductus deferens of the fowl as equivalent to the corpus and cauda of a mammalian epididymis, and most of the juxtatesticular region as corresponding to the caput. This suggestion is supported by the similar tortuous course of both ducts, the identical epithelium with a falling height, and the continual widening of the lumen from the cranial end of the attached 'ductus epididymidis' to the caudal end of the free 'ductus deferens'. These sequential changes in the avian 'ductus epididymidis' and 'ductus deferens' follow closely those described only for the epididymis in the rat (Reid & Cleland, 1957). Also, the epithelial types of these avian ducts are similar to those of the epididymides of the rat (Martan & Risley, 1963; Kreth, 1965) and mouse (Martan & Allen, 1964). Glover & Sale (1969) studied the genital tract of the male hyrax, a mammal with intra-abdominal testes. They considered that the part of the mesonephric duct lying on the surface of the testis and its caudal continuation were both parts of the epididymis. Classically, these parts had always been referred to as the epididymis and ductus deferens respectively.

In the present investigation, clear and ciliated cells are demonstrable in the

epithelia of both the ductuli efferentes and the initial segments of connecting ductules. Basal cells are present mainly in the ductus deferens. There is no evidence of the presence of the halo or apical cells which are associated with holocrine secretory activity in the epididymis of the rat (Reid & Cleland, 1957; Martan & Risley, 1963) and that of the mouse (Martin & Allen, 1964).

The significance of epithelial cells in the lumina of ductuli efferentes is not clear. There is no evidence that these are part of the holocrine secretion suggested by Lake (1957). Nevertheless, they may be exfoliated cells or fixation artefacts. Bleb-like projections from cells are found in the epithelium, particularly in the cranial segment of the ductus deferens. This has been interpreted as a sign of apocrine secretory activity, but again fixation artefacts cannot be excluded (Bloom & Fawcett, 1968).

The presence of spermatogenic cells in various stages of maturation in the rete testis, and to a lesser extent in the ductuli efferentes, could be attributed to mechanical handling prior to fixation, but they may represent a normal occurrence. The high density of spermatozoa in the lumen of the initial segments of the connecting ductules could be due to fluid vehicle being absorbed in the ductuli efferentes or to a mechanical accumulation of spermatozoa, since they are moving from the wide ductuli into the much narrower connecting ductules.

SUMMARY

The genital tract of the male fowl was investigated by dissection, injection, micro-radiography and conventional histological methods.

The fowl testis does not have a mediastinum and tubuli recti are absent. The seminiferous tubules therefore lead directly into the rete testis, which is drained by the ductuli efferentes. The latter are continued by connecting ductules which in turn open into the ductus epididymis.

Particular emphasis was given to the structure of the ductus epididymidis and ductus deferens, and they were considered to be merely different segments of the same tube. This tube gradually increases in diameter from its beginning at the cranial end of the epididymal region to its caudal end. Thus, the avian epididymal region might be regarded as equivalent to the caput epididymidis of scrotal mammals. Different regions of the ductus deferens are considered analogous to the corpus and cauda epididymidis.

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