THE MALE REPRODUCTIVE TRACT OF THE FOWL

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The male reproductive organs of the fowl have been studied by Kaupp (1915), Gray (1937), Burrows & Quinn (1937), Parker, McKenzie & Kempster (1942), and Bradley (1950), mainly by histological methods of fixation and staining; little attention has been paid to details from the point of view of the origin of semen constituents. Hence, in the course of investigations on the development of artificial insemination methods in the fowl, it was thought appropriate to examine the male tract and associated organs, using gross dissection, cytochemical and histological methods in an attempt to locate sites of secretory activity, as a necessary preliminary to studies of ejaculation phenomena and of chemical analyses of fowl semen in relation to the *in vitro* activity of spermatozoa. Fowl spermatozoa have been shown to be particularly difficult to store *in vitro*. Apart from their inherent structural peculiarities, this difficulty might be due to constituents of the accessory fluids collected by the massage method; collectively, these have been taken to represent the seminal plasma. It is therefore desirable to locate in the male tract and associated organs all possible sources of secretions which could gain access to semen collecting vessels, so that a subsequent assessment can be made of the efficiency of semen collection by massage (Burrows & Quinn, 1935, 1937).

The organs in the male cloaca are interesting and need special attention when discussing massage collection. Nishiyama (1950*a*) has described variations of phallus form in the fowl; during sexual excitation the phallus swells by engorgement with lymph from the internal pudendal artery, and this process, together with relaxation of the posterior retractor penis muscle and the engorgement of the lymph folds in the cloaca (Nishiyama, 1950*b*, *c*) contribute to the protrusion of the phallus. Lymph folds and vascular bodies are linked with lymph channels of the phallus and are considered by Nishiyama (1954) as analogues of accessory reproductive organs of mammals, since they showed a positive growth response to male hormone administration. It is generally held that there is an analogy between the enlarged, distal portions of the vasa deferentia of the bird and the seminal vesicles of the mammal (Riddle, 1927; Bailey, 1953). On the other hand, as Grecka (1933) and Munro (1938) have stated that there appear to be no glands corresponding to those of the mammalian seminal vesicles in the fowl there is confusion as to the existence of homologous or analogous accessory glands in the two classes of vertebrates. During sexual excitation the lymph folds and vascular bodies are erected, together with the ejaculatory ducts. Nishiyama (1951, 1952*a*, 1955) observed a lymph-like fluid that flowed through their surface epithelium during massage collection of semen. Together with a little fluid secreted by the epithelial cells, this lymph constituted the so-called 'transparent fluid' of fowl semen and was considered by Nishiyama as true seminal plasma. Lake (1956) did not wholly agree with this

interpretation and so, in view of the importance of assessing the true nature of fowl seminal fluid, it was decided to investigate further the lymph folds and vascular bodies.

MATERIAL AND METHODS

Birds

All birds used were from the Brown Leghorn flock maintained at the Poultry Research Centre, Edinburgh.

(1) *Gross dissections.* Seven-month-old cockerels were used for observations on the anatomy of the male organs at the height of the reproductive season in March and April. The arrangement of seminiferous tubules in the testis was examined by the method used by Bailey (1953) to study testes of Fringillid birds. Immediately after killing, a solution of 2 g. Nigrosin in 20 ml. 0·85 % sodium chloride was injected into the tubules through a large bore needle inserted into the epididymal region. The testes were then taken out and kept in 40 % nitric acid at −2° C. for 24 hr., the tunica albuginea being punctured at several places to facilitate entry of acid. This treatment hardened and stained the tubules, and enabled the gross internal structure of the testis to be teased out and examined under a binocular dissection microscope.

(2) *Histochemical analysis of various parts of the urinogenital tract.* One-year-old males were used for histochemical examination of the epithelia lining the reproductive and urinary tracts. Immediately after killing, by dislocation of the neck vertebrae, pieces of testis, epididymal region, upper and lower vas deferens, ureters, phallus, lymph folds and vascular bodies were fixed in appropriate solutions for 24 hr. Frozen sections of each region of the tract were also prepared after formol-calcium fixation (Baker, 1946). After fixation in Carnoy-Lebrun fluid (McClung, 1950) saturated with mercuric chloride, Masson's trichrome and Ehrlich's haematoxylin and eosin staining methods were used to examine the distribution of muscle, connective tissue and fibrous tissue. In addition, the following staining procedures were used.

Acid and alkaline phosphatase. In the mammalian reproductive tract, phosphatases are secreted mainly by cells in specific accessory glands. Acid phosphatases are secreted by the prostate gland, and alkaline phosphatases by the seminal vesicles (Mann, 1954). The presence or absence of phosphatases in the lymph folds and vascular bodies of the fowl cloaca would give some indication as to whether or not these organs are analogous to the seminal vesicles, prostate glands or other accessory glands of mammals. For alkaline phosphatases the method of Gomori (Pearse, 1953) on paraffin embedded sections was used; fresh tissue pieces, not exceeding 2 or 3 mm.^{3}, were fixed in acetone at 2° C. On similar sections Gomori's method (Pearse, 1953) for acid phosphatases was employed. For both enzymes the sections were incubated in the substrate media for 24 hr. to get maximal reaction (Rollinson, 1954). Control sections were incubated for the same length of time in the buffer medium without substrate.

Lipids. By the term 'lipids' is meant all material (*a*) reacting positively with Sudan black B in frozen sections and extractable by acetone, ether, xylene, benzene or pyridine; and (*b*) all 'bound lipid' material not easily soluble in orgainic solvents

after formalin fixation and reacting in a manner characteristic of glycolipids and phospholipids.

Frozen sections of tissue after formol-calcium fixation were used for (1) Schultz test for unsaturated steroids, principally cholesterol (Glick, 1949), and (2) examination with the polarizing microscope. It has been shown by Cain (1950) that many lipids become crystalline in fixed preparations, and spherocrystals showing the black cross of polarization are formed by steroid esters and phosphatides. To look for the presence of such compounds, frozen sections of tissue fixed in formol-calcium were mounted in glycerine jelly and examined with polarized light.

Previous experience had shown that, in certain regions of the male reproductive tract, some lipid existed which was not easily soluble in fat solvents; pieces of each organ were therefore fixed in formol-calcium for 24 hr., washed in chilled acetone for two periods of 1 hr. each, placed in two or three changes of tertiary-butyl alcohol and then embedded in paraffin wax. Sections were subsequently stained with Sudan black B; it was considered that a positive reaction demonstrated the presence of a glycolipid, or a similar complex lipid. Pearse (1953) has shown that phosphatides and cerebrosides (glycolopids) give positive reactions to Sudan black in formol-fixed paraffin sections. Baker's acid haematin and pyridine extraction test (Baker, 1946; Cain, 1947) was used to examine the presence of phospholipid and glycolipid as both are extracted with pyridine treatment, but only the former gives a positive reaction with acid haematin.

Staining for general lipid material was done on fixed, frozen sections using Nile blue and Sudan black B (Pearse, 1953).

Muco-substances. It is generally recognized that mucopolysaccharides (Leblond, 1950), muco-proteins and lipo-protein-polysaccharides form the basis of some mucoid secretions. The function of these substances is little understood. Mucopolysaccharides are present in the pre-sperm fraction of bull ejaculates (Lutwak-Mann & Rowson, 1953). To identify the sites of production of these types of secretion in the male fowl tract, pending a more detailed biochemical analysis, the following histological tests were applied: (*a*) Lison's method (Glick, 1949) using toluidine blue metachromatic staining after fixation in equal volumes of basic lead acetate and 15 % neutral formalin. This method is considered to reveal mucopolysaccharide-sulphate compounds and possibly other acid mucopolysaccharides (Wislocki & Singer, 1950; Pearse, 1953); (*b*) after fixation in Orth's fluid, periodic acid treatment followed by fuchsin-sulphurous acid (FSA) (Hotchkiss, 1948; McManus, 1946), modified by Leblond & Clermont (1952), was used for detecting suitably reacting mucopolysaccharide which was not principally basophilic or metachromatic. Saliva, as a source of α-amylase, was used on control sections to determine how far glycogen was the basis of FSA-reactive material. It is known that certain monosaccharides, polysaccharides, mucoproteins, phosphorylated sugars, cerebrosides and inositol-containing lipids (Gersh, 1949) can give a positive FSA-reaction, and so with due consideration of the extent of solubility of these compounds in the fixation and staining fluids used, the colour reaction also served as a general indication of the presence of these types of compounds in the genital tract.

RESULTS

Gross morphological features of the male reproductive tract in the cock

Pl. 1, fig. 1, is a drawing from a dissection of a 7-month-old Brown Leghorn male weighing 2200 g. at the height of the reproductive season in Edinburgh in March. The paired testes (4·5 cm. long and 2·5 cm. broad) are internal and attached by connective tissue to the dorsal body-wall slightly medial to the anterior ends of the kidneys. The left testis (14 g.) was slightly smaller than the right (14·75 g.). The anterior portions were adjacent to the abdominal air sacs. Internally they resembled those of the Fringillid birds (Bailey, 1953) and differed from the mammalian testes. The membranous tunica albuginea covering the mass of seminiferous tubules is extremely thin. The tubules form an anastomosing network, are not discrete as in the mammal and are impossible to tease out individually (Pl. 1, fig. 2). Under the binocular dissecting microscope, portions of the tubules appeared swollen, possibly indicating sites of active multiplication of germ cells. They discharge their contents into the cavernous spaces of the rete testis and then into the extra-testicular vasa efferentia. The latter, with some exceptions (Stoll & Maraud, 1955), connect the seminiferous tubules with the ductus epididymis. Capillaries, arterioles and venules of the testicular artery and vein ramify throughout the intertubular tissue of the testis, and also in the tunica albuginea. Unlike that of the mammal, the active fowl testis is not firm to the touch, and when cut with a scalpel extrudes an abundance of milky fluid composed of lipoprotein material and spermatozoa from the seminiferous tubules. In contrast to the mammals, the ductus epididymis is extremely short and is not divided into caput, corpus or tail regions (Pl. 1, fig. 2).

The vas deferens receives the contents of the epididymis and is the main storage organ for spermatozoa in the cock. In the dissected bird it was a long, coiled tube running for about 9·5 cm. along the ventral aspect of the kidney. From midway along its length it ran parallel with the main ureter to the cloacal region (Pl. 1, fig. 1). The distal end was greatly thickened owing to an increase in musculature and connective tissue which will be described later. Contrary to the mammals, there was no region to compare anatomically with the ampulla. Each vas deferens connected directly with an erectile ejaculatory duct which protruded into the urodaeum of the cloacal chamber. The ducts were conical in shape, and those illustrated in Pl. 1, fig. 1, were 2·5 mm. long in the flaccid state.

The ureteral openings to the urodaeum were in close proximity, and medial to, the base of the ejaculatory ducts. The rectal opening into the coprodaeum lay posteriorly in the midline of the cloacal chamber. The phallus of the cock is composed of a central 'white body' and lateral 'round folds', and is situated in the anterio-ventral part of the cloacal chamber (Pl. 1, fig. 1). Discrete lymph folds lie posteriorly to the round folds and in front of the ejaculatory ducts. Vascular bodies forming a ring of erectile tissue lay behind the ejaculatory ducts and encircled the posterior aspect of the urodaeum. Nishiyama (1955) described vascular bodies as being two discrete bodies surrounding the base of each ejaculatory duct only.

Histological and histochemical characteristics of testis, epididymis, vas deferens and certain other parts of the reproductive tract

Testis. As already mentioned, the fowl testis is softer than that of mammals, and for this softness several reasons may be suggested. First, contrary to the condition in mammals, the tunica albuginea is extremely thin and does not appear to give off any connective tissue septa dividing the tubules into lobules (Pl. 1, fig. 3). In the inactive testis, where intertubular connective tissue is more prominent, there is also no sign of these septa and so, it is concluded, their absence in active testes is not just apparent owing to great distension of the tubules. Secondly, the seminiferous tubules of the fowl testis are proportionately larger in diameter than those of some mammals. Thirdly, one gets the impression by comparing the fowl seminiferous tubule (Pl. 2, fig. 4) with that of a mammal having a comparable testis size, that the spermatocytes of the bird are smaller, and a more rapid process of germ cell division is evident from the number of cells seen per unit area within the tubules.

In the tubules of the active testis there was no material showing birefringence under the polarizing microscope, or which stained positively with acid haematin, thus indicating the absence of cholesterol and phospholipids. Similar observations with regard to cholesterol were made by Marshall & Coombs (1952) on wild bird testes, and Mancini, Nolazco & de la Balze (1952) on human testes. Traces of such material, were, however, detected in the intertubular tissue. The Sertoli cells appeared to contribute some type of lipid to the contents leaving the seminiferous tubules; their cytoplasm was very extensive and appeared to ramify between all the cells of the germinal epithelium. With phase-contrast microscopy large lipid droplets were seen in preparations from seminiferous tubules of fresh testis material. After fixation of the testis in formol-calcium, frozen sections stained with Sudan black B showed lipid chiefly at the sites surrounding the transforming spermatozoa in the lumen and interspersed between the later spermatocytes. Tissue fixed in formol-calcium, and treated with chilled acetone prior to paraffin embedding, still showed in these places a positive, but slightly reduced, reaction to Sudan black. In the same areas there was also a positive FSA reaction indicating the presence of mucopolysaccharide or aldehyde groups produced from complex lipids (Pearse, 1953). It was difficult to decide whether the FSA-positive material was in the cytoplasm being shed by the spermatozoa in the latter stages of their formation or whether it was in the Sertoli cell cytoplasm.

After salivary digestion the FSA-picture in the testis was altered only very slightly from that given by the control sections. If this indicates solely loss of glycogen, then the amount of this substance present is small. Metachromasia, indicating acid mucopolysaccharide, was confined to the basement membrane of seminiferous tubules, and to chromosomes in the spermatocytes during the metaphase stage of division. The results of staining with Nile blue showed that there was a slight increase of acidic lipid material in cells towards the lumen of tubules.

Alkaline phosphatase in the testis was present in the cytoplasm and in the nuclei of cells of the intertubular tissue. A much weaker reaction of the enzyme was observed in the nuclei of germ cells which formed the basal layer in tubules. Very feeble reactions were given by the cytoplasm and nuclei of Sertoli cells, secondary

spermatocytes and spermatids. The acid phosphatase reaction was feeble in the basal cell nuclei. These findings would indicate that the fowl testis is similar to most mammalian testes with regard to the distribution of the alkaline and acid phosphatases (Rollinson, 1954; Wolf, Kabat & Newman, 1943; Wislocki, 1949).

From the seminiferous tubules to the rete testis the transitional stage in the change of the epithelium was from the typical stratified germinal type to the single layer, cuboidal cell type of the rete. At the transitional stage modified Sertoli cells predominated and their cytoplasm was marked by more positive reactions to FSA and Sudan black staining. Large secretory droplets from these cells, which stained positively with Sudan black after acetone treatment and embedding in paraffin wax, were often seen in the lumen. This is considered to indicate the presence of a compound lipid similar in nature to that produced in the seminiferous tubules. The lipid in the rete epithelium appeared to be mostly part of the cell structure and not secretions.

Epididymal region. Since there is no long, highly tortuous ductus epididymis in the cock, the term 'epididymal region' is used in the present work to describe the small group of tubules lying on the hilum of the testis. The structure is comprised of many short vasa efferentia joining the ductus epididymis (Stoll & Maraud, 1955). The whole is embedded in connective tissue and firmly anchored to the dorsal body-wall. On the left side most of the adrenal gland is embedded in the connective tissue of the proximal epididymal region.

The several types of vasa efferentia had a lining epithelium exhibiting in places intense holocrine secretion. Intra-epithelial glands occurred, and long tufts of stereocilia were carried on the free surface of some of the cells (Pl. 2, fig. 5). Abundant lipid-protein material was present (Pl. 2, fig. 6), and some of it, after fixation in formol-calcium and subsequent treatment with acetone before embedding in paraffin wax, was still revealed with Sudan black. FSA-positive material was present in the lipid droplets which indicates the presence of either aldehydes produced from a complex lipid or a mucopolysaccharide fraction (Pl. 2, fig. 7). There was no demonstrable phospholipid revealed by the acid-haematin test. A few smooth muscle cells appeared beneath the basement membranes of the vasa efferentia.

There were parts of tubules in close proximity to the basement membrane of the epithelium of some of the vasa efferentia, and large lipid droplets also occurred in their cytoplasm (Pl. 2, fig. 6). It is possible that these represent the satellite tubules of the vasa efferentia. During the active season the epithelial cells of the vasa efferentia were to be seen in various representative states of building up and breaking down. This points to marked functional activity, during the course of which it seems likely that much lipid-protein-polysaccharide, together with other cytoplasmic inclusions, is secreted.

Occasionally groups of cells appeared in the connective tissue of the epididymal region which were positive to the Schultz test and also birefringent, thus indicating the presence of cholesterol esters. These may represent either 'le paradidyme' or the 'vasa aberrantia of rete' (Stoll & Maraud, 1955). In the course of dissection isolated small yellow patches were noticed in the connective tissue of the epididymal region. Some of them were undoubtedly nodules of adrenal tissue, but some most likely represent rudimentary tubules from the mesonephros which produce the buff-

coloured droplets that appear in the semen, especially of young cockerels just beginning to be sexually active.

The ductus epididymis generally had a smooth, circular outline and had a lining epithelium of pseudo-stratified columnar cells (Pl. 2, fig. 8). There was no indication of secretion from the cells. There did not seem to be sufficient specific evidence of glycogen secretion in the ductus epididymis or vasa efferentia tubules comparable with what is thought to occur in the dog and rabbit (Nicander, 1954), and in man (Montagna, 1952). FSA-stained sections after saliva treatment did not produce any picture different from that of the untreated sections. In the ductus epididymis the lipid and FSA-positive material was greatly reduced in the epithelium, and appeared to be confined to the cell framework only.

A weak acid phosphatase reaction was observed in the secretions of cells in some of the vasa efferentia; in the ductus epididymis the reaction was negative. This is contrary to the condition found in man (Montagna, 1952), rabbit, guinea-pig and mouse (Wolf *et al.* 1943). Alkaline phosphatase appeared to be faintly scattered throughout the cytoplasm of all cells in the epididymal region.

Vas deferens. From the evidence already given it is likely that spermatozoa are bathed in a lipo-mucoprotein medium when entering the vas from the ductus epididymis. At the junction of the vas deferens with the ductus epididymis the epithelium was slightly folded, and large vacuoles appeared to be formed at the free surface of the cells and to be extruded into the lumen. The upper region, prior to the distended part, had a relatively wide lumen (Pl. 2, fig. 9) and an epithelium showing a little lipid material scattered throughout the framework of the cells only. The short distal region was characterized by an increased thickness of the fibrous tissue of the submucosa and the outer smooth muscle (Pl. 3, fig. 10). There was a scanty distribution of lipid in cells of the vas but it did not seem to form an extensive secretion to the seminal fluid. Only very weak or absent reactions for acid and alkaline phosphatases were demonstrated in the epithelium of most of the vas, but occasionally isolated areas in the distal region were found secreting some acid phosphatase. After performing the phosphatase tests a variable amount of yellow-brown coloration was seen in the tissue due to non-specific adsorption of sulphide precipitate on muscle, collagen, etc. The areas where the enzyme was taken to be definitely present were coloured dark brown or black and such was the case in the above-mentioned areas of the distal vas after the test for acid phosphatase. The reaction in most of the epithelium of the vas was more towards yellow-brown coloration of nuclei and cytoplasm, thus indicating little or no acid or alkaline phosphatase to be present. A little FSA-positive material was present in cell cytoplasm, and some was seen amongst spermatozoa when the latter were retained in sections of the distal vas deferens. Blebs of cell extremities were occasionally to be seen extruding into the lumen of the vas deferens at this level (Pl. 3, fig. 11), and thus it is likely that any intracellular enzyme would be shed into the lumen along with cytoplasmic constituents and their breakdown products.

At the junction of the vas deferens with the ejaculatory duct there was a noticeably small storage space for spermatozoa but the vas as a whole formed the main reservoir. There was no mucopolysaccharide present in the epithelium of this region and only a little faintly scattered lipid was visible within the cells. At the distal end

of the vas deferens there was no glandular epithelium comparable with that of the ampulla of mammals.

Ejaculatory ducts. Each vas deferens joined the corresponding erectile ejaculatory duct which protruded into the urodaeum of the cloacal chamber (Pl. 1, fig. 1). The ducts were similar in structure to the penis of mammals in respect of subepithelial sinuses and tortuous arterioles and venules in the deep fibrous connective tissue of the submucosa (Pl. 3, fig. 12). The small blood vessels, and subepithelial sinuses, become engorged with blood during erection. The swelling of each ejaculatory duct appeared to be partly brought about by a contraction of the muscle of the vas deferens, forcing the spermatozoa into it. During collection of semen this can be clearly seen on occasions when lumbar stimulation causes filling of the duct and only gentle squeezing with the fingers is necessary to eject the semen into a container. The inner lining epithelium of the ejaculatory duct was of a pseudostratified columnar type; only a little scattered lipid and no mucopolysaccharide material was identifiable within the epithelial cells. The epithelium was much folded at the proximal end but was straightened out distally. The epithelium of the external surface of the duct contained mucin-secreting cells (Pl. 3, fig. 13). There was no acid or alkaline phosphatase reaction in the cytoplasm of the latter.

Lymph folds, vascular bodies and phallus. These tissue structures are considered together since they form what the author considers the most controversial elements in comparing the morphological and functional relationships of the reproductive tracts of birds and mammals. Together with the erectile, ejaculatory ducts they form the copulatory organ of the cock.

The vascular bodies have a surface epithelium of pseudo-stratified columnar cells. Glands are formed which sometimes extend into the submucosa, and many goblet cells are present (Pl. 4, fig. 14). The cells of the deep glands secrete an abundance of FSA-positive material that stains metachromatically (γ red) with Toluidine blue, indicating the presence of sulphated mucopolysaccharide (Pl. 4, figs. 15, 16). A little acidic lipid material is present also which stains with Nile blue. Neither phosphatase reaction is given by the cells. Many aggregations of lymphocytes occur in the submucosa, especially near to the ureteral openings (Pl. 4, fig. 14). Sinuses lined with endothelium and filled with blood are present underneath the basement membrane of the surface epithelium.

The epithelial cells of the ureters, which open into the urodaeum at the base of the ejaculatory ducts, also contain abundant sulphated mucopolysaccharide which forms part of the mucinous secretion. Aggregations of lymphocytes are present in the submucosa. The metachromatic reaction in the epithelium is equal in intensity to that of the vascular body epithelium. Only the nuclei of the lymphocytes give feeble phosphatase reactions.

The surface epithelium of the lymph folds is of the pseudo-stratified columnar type, and some of the cells secrete FSA-positive material which gives only a very slight metachromatic reaction (Pl. 4, fig. 17). This indicates that the mucin is less acidic in nature. There are no submucosal glands. Blood sinuses are present in the submucosa but are not so extensive as those in the vascular bodies. Very few smooth muscle cells are present beneath the basement membrane of the epithelium; it is suggested, therefore, that during normal copulation there is no forcible ejection of

secretion from the epithelial cells. This secretion could be passively extruded, as a result of the engorgement of the organ, and together with spermatozoa and fluids from the ejaculatory ducts, is most likely to be a component of the seminal fluid.

The phallus (Pl. 1, fig. 1) is bounded exteriorly by stratified squamous epithelium of the mucous membrane variety. Immediately beneath this, abundant muscle tissue, representing the posterior retractor penis muscle, is firmly anchored. Towards the interior of the cloaca the epithelium becomes similar to that of the lymph folds.

DISCUSSION

Although the present study has been confined chiefly to morphological features of the male fowl reproductive tract, it has revealed several facts which have a direct bearing on the problem of the physiological function of the various reproductive organs. The first point to be considered is the close relationship of the abdominal air sacs to the anterior poles of the testes. In wild birds, according to Cowles & Nordstrom (1946), the abdominal air sacs serve in a similar capacity as the scrotum does in the mammal. From anatomical investigations on the domestic fowl it is difficult to understand how the air sacs in their position could have much of a cooling action on the testes. Without rapid respiration induced by flying, the incoming air must be appreciably warmed by its passage through the lungs. Further physiological work is needed to investigate this aspect of avian reproduction.

Next to be considered is the presence of so-called seminal vesicles in the fowl. The common conception that the swollen distal portion of the vas deferens serves as a storage organ for spermatozoa in the fowl appears unlikely since the increased size is mainly accounted for by increased musculature and connective tissue in the subepithelial layers. The whole vas serves as a transitory storage place in the absence of an extensive ductus epididymis. This suggestion is in partial agreement with the findings of Nishiyama (1951) who found the greatest concentration of spermatozoa in the proximal end of the vas in the cock and not at its distal end. Morphologically there does not seem to be any evidence for drawing an analogy between the seminal vesicles of mammals and the swollen, distal parts of the vasa deferentia of the fowl, since the latter lack the glandular structure of seminal vesicles. Also, in the mammal, the latter are generally outgrowths and separate organs derived from the vas deferens; no such development occurs in the fowl. There is no secretion of alkaline phosphatases which in mammals generally are produced by the seminal vesicles (Mann, 1954). Bailey (1953), studying Fringillid birds, stated that the only reason he had for calling the distal vasa deferentia by the name seminal vesicles was because it was conventional to do so. In the expanding field of semen physiology this can lead to confusion when comparing the function and chemical composition of seminal fluids from various species of animals, and so, on present evidence and until more data are available as to the chemical nature of secretions produced in the fowl reproductive tract, it is considered preferable not to liken the distal vasa deferentia in the fowl to the seminal vesicles of mammals. The junction of the vas deferens with the ejaculatory duct might be considered as a contracted region similar to the ampulla of mammals. However, there are no glands extending into the submucosa, and the general epithelium shows no signs of secretory activity.

In the cock, it has been impossible to demonstrate any gland that compares morphologically with the prostate. In the mammal the latter is derived from urogenital sinus epithelium during embryogenesis, and in the mature animal the organ is composed of numerous tubulo-alveolar glands embedded in connective tissue through which smooth muscle septa run. The glands secrete material into the urethra through independent ducts. Smooth muscle contractions during copulation govern the process.

Nishiyama (1954) stated that the lymph folds and vascular bodies were analogous to accessory reproductive glands of mammals on the basis of their positive growth response to androgenic hormone. It is well known, however, that in mammals the penis and associated glandular structures, as well as the seminal vesicles and prostate gland, will respond to such hormone. It is, therefore, necessary to be more specific in definition. Unlike the seminal vesicles or prostate gland, the cloacal glands do not produce secretions of acid or alkaline phosphatases. The acid phosphatase appears to be a universal feature of the prostate gland (Wolf *el al.* 1943; Rollinson, 1954). By their position and also by the presence of much sulphated-mucopolysaccharide in the secretions of their glands, the vascular bodies seem more likely to be analogous with the mammalian bulbo-urethral glands. It is significant that Lutwak-Mann & Rowson (1953) found that in the secretion of such glands in the bull there is a fair quantity of combined hexosamine and glucuronic acid indicative of the presence of mucopolysaccharide. The pH of the fluid obtained from the bull was highly alkaline, pH 7·8, which corresponds with the figure pH 7·9 given by Nishiyama (1952*b*, 1954) for that produced by the lymph folds and vascular bodies of the fowl. Lutwak-Mann & Rowson suggested that as the secretions of the bulbo-urethral glands are ejaculated first they serve to clear and neutralize the contaminants of the urethra prior to the passage of the fraction of the ejaculate containing spermatozoa. In the fowl it is feasible that the secretions of the epithelial glands of the vascular bodies subserve a similar purpose of protecting the cloacal muscosa from damage by obnoxious constituents of the faeces and urine. In the submucosa of the lymph folds, but particularly in the vascular bodies, there are also numerous foci of lymphocytes.

The vascular bodies in the Brown Leghorn appear to form an almost continuous ring of tissue around the base of the ejaculatory ducts in the urodaeum. On morphological evidence it is suggested that their surface epithelial glands are related to the cloacal structures formed in embryogenesis and associated with the distal development of the urinary ducts. Their erectile properties, and those of the lymph folds, indicate a close analogy of these two structures with the penile, urethral portions of the mammal.

Although histochemical methods alone are not always adequate to demonstrate specific chemical compounds in tissue it is considered that they have, at least, helped in the present work to throw light on the general type of secretory material elaborated by the various parts of the male reproductive tract. Sufficient information has been obtained to serve as a general guide to the consideration of a future chemical analysis of semen constituents in relation to fowl sperm activity *in vitro*. The cytoplasm of Sertoli cells, of spermatids during the formation of spermatozoa, and cells of the vasa efferentia all appear to contribute material of a complex

lipo-protein-polysaccharide nature to the seminal fluid of the fowl; without, as yet, confirmatory results of chemical analysis, it would appear that a compound containing lipid and not very soluble in organic solvents is secreted. Inositol-containing lipids and cerebrosides are worthy of consideration. It is not generally stressed that testis products contribute to seminal plasma, however, Regaud (1902) was of the opinion that lipid of Sertoli cells actively contributed to the seminal secretion of the rat, and Lynch & Scott (1951) reported that, in the rat, lipid was stationary in Sertoli cells at times when spermatogenesis was inhibited, but in active seminiferous tubules lipid droplets were shed into the lumina. It is not considered that the observations of Coombs & Marshall (1956), indicating no lipid in active fowl testis tubules, are at variance with the findings of lipid in the present work. From the photomicrographs of their paper, some lipid is indicated, but it is insignificant in amount and of the wrong type for their hypothesis.

The epithelia of the proximal and distal vas deferens exhibit intense holocrine secretion. There is only a slight positive reaction with Sudan black and FSA, and so the organic secretion is largely composed of some other non-lipid compound possibly of a protein or polypeptide nature.

Compared to the mammalian reproductive tract there is no major gland secreting phosphatases into the seminal plasma of the fowl. The epithelia of parts of the vasa efferentia and parts of the vas deferens shed blebs of cytoplasmic material from the apices of cells which could contribute some intracellular phosphatase to seminal plasma. This does not necessarily mean that such material plays any direct, significant part in the metabolism of spermatozoa. In a cytochemical examination, one must be extremely cautious in ascribing any enzyme present in the seminiferous tubule specifically to either spermatozoa, or the cytoplasms of Sertoli or basal spermatogenic cells, since it is difficult to identify the cell types with the staining procedure used.

It is feasible that the complex lipo-protein in fowl semen, since it is not diluted with much accessory secretion, affords protection to the surface of spermatozoa and accounts partially for their relative resistance to temperature shock compared with most mammalian spermatozoa (Smith & Polge, 1950; Skaller, 1951; Lake, 1954). This is in accord with the view that some of the beneficial effect of adding egg yolk to mammalian semen diluents is due to the presence of complex lipids or lipoproteins which can afford protection to the spermatozoa (Mayer & Lasley, 1944; Blackshaw, 1954). The role of seminal fluid constituents in the maturation phenomenon of the spermatozoa in the epididymis and vas deferens, as well as a biochemical analysis of fowl semen and cloacal gland fluids, are at present under investigation.

SUMMARY

The structure of the reproductive tract of the cock has been examined to determine the nature of fluids which can gain access to collecting vessels during the massage method of semen collection. Although future chemical analyses are necessary to examine the precise nature of substances secreted into the seminal fluid, the organic matter appears to be mainly composed of complex lipid and protein-polysaccharide materials. It is derived from the seminiferous tubules and vasa efferentia. The

apices of cells in parts of the vas deferens break down and appear in this way to contribute material to the seminal fluid. It has been confirmed that in the cock there are no glands which are analogous to the seminal vesicles or prostate glands of mammals. Cytologically, the vascular bodies in the cloaca bear the closest resemblance to bulbo-urethral glands in respect of secreting abundant mucopolysaccharide. The lymph folds secrete mucin of a similar nature but, probably, less acidic in reaction.

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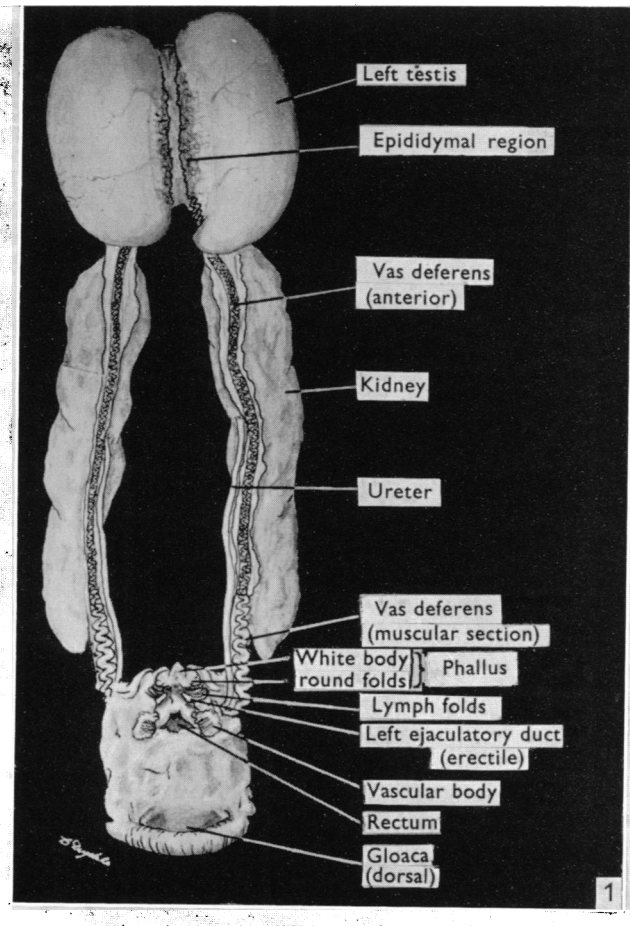

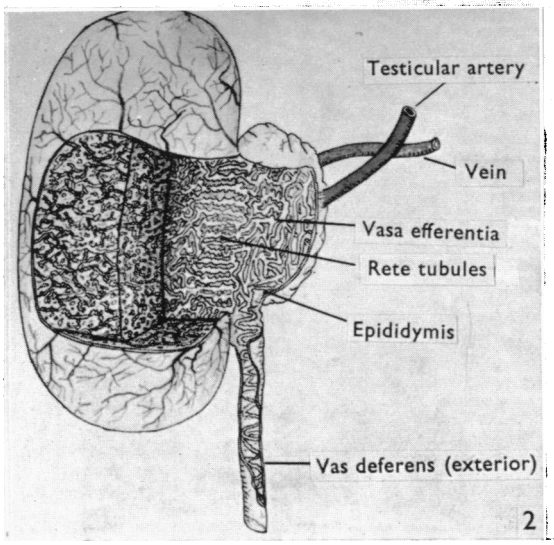

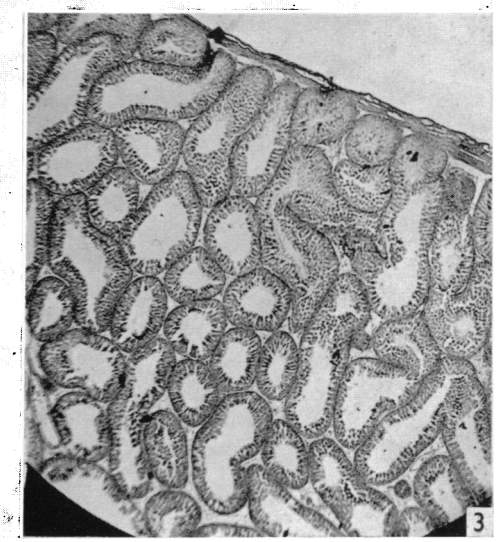

LAKE—THE MALE REPRODUCTIVE TRACT OF THE FOWL

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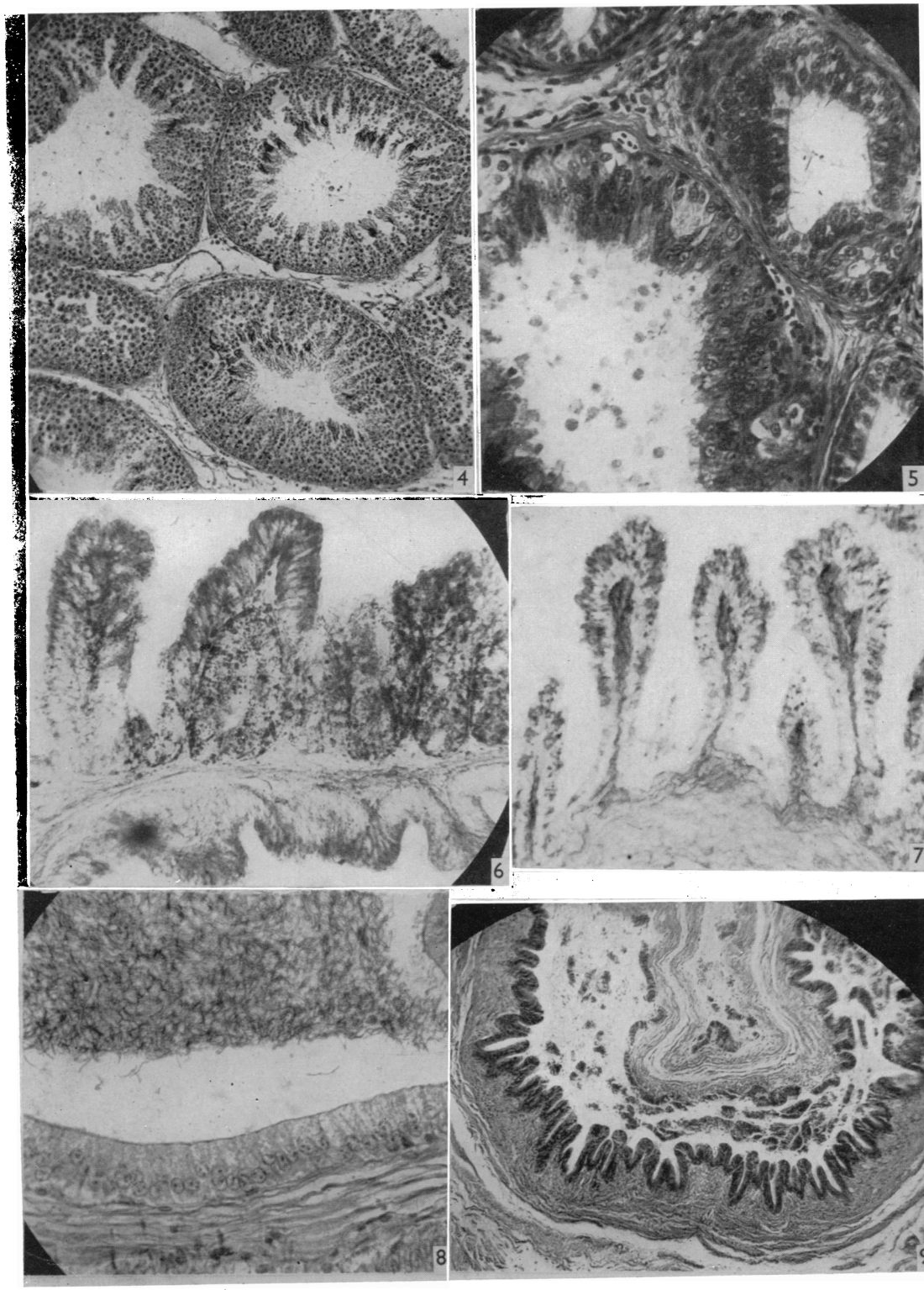

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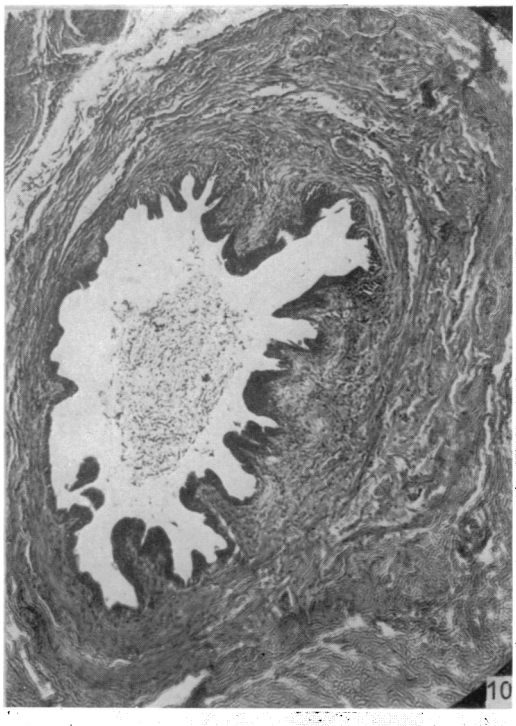
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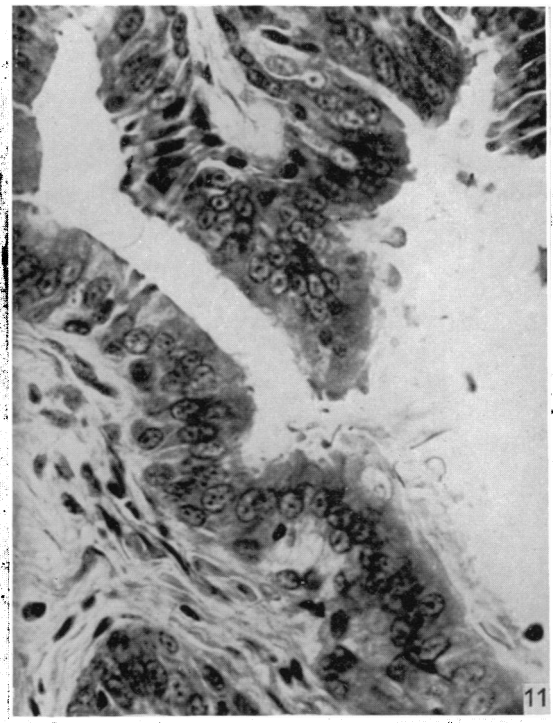
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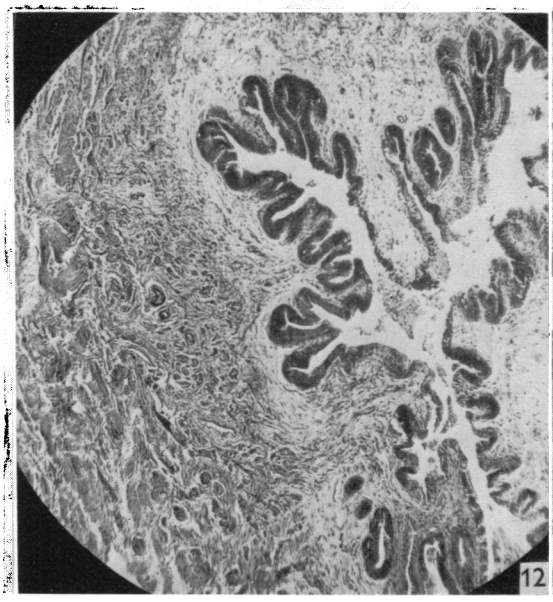
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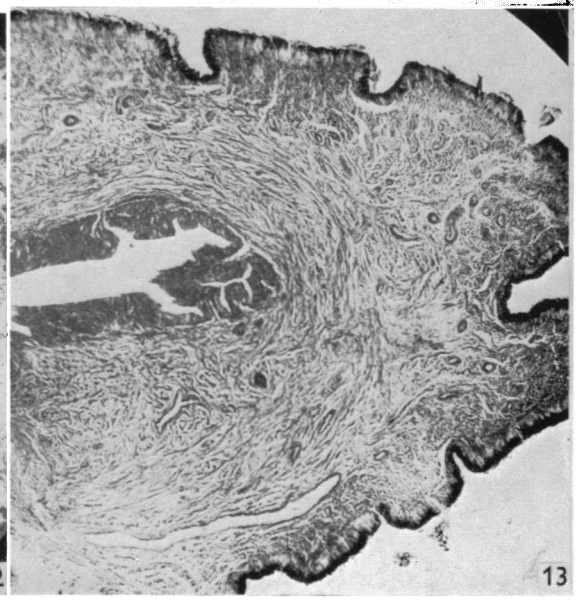
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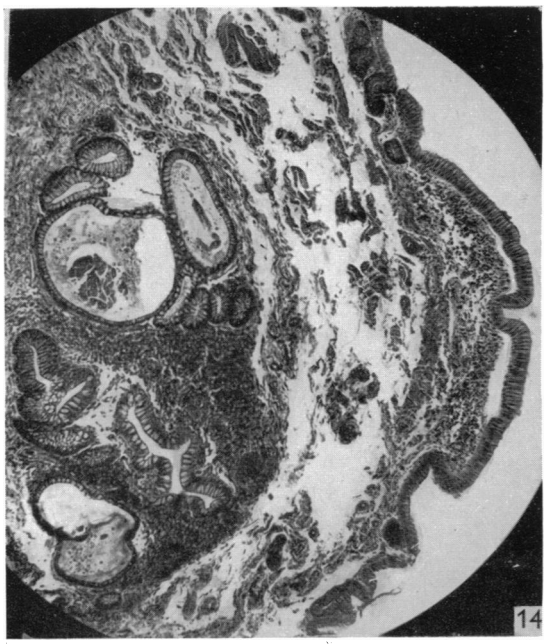

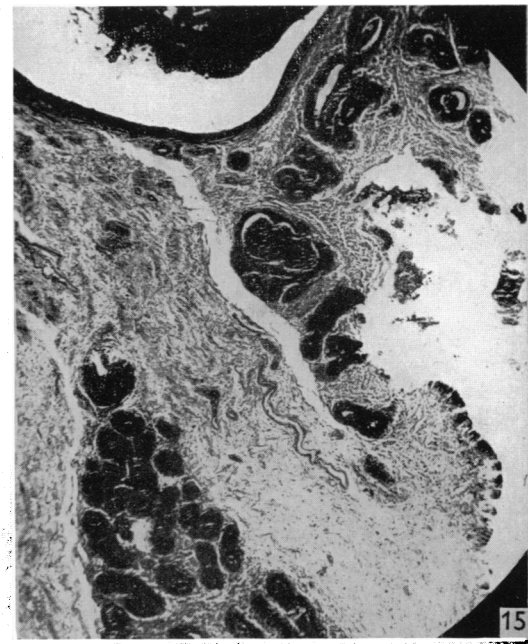

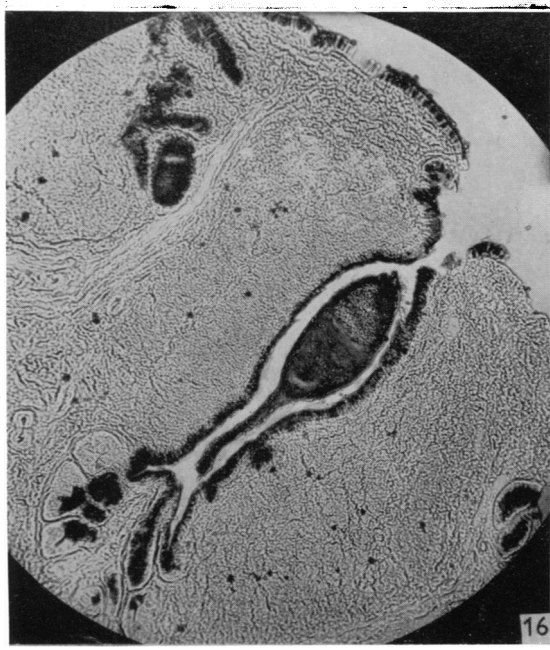

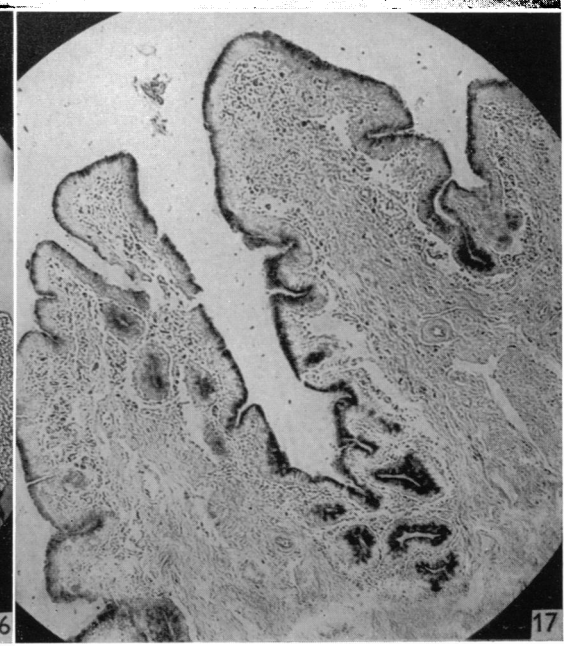

LAKE—THE MALE REPRODUCTIVE TRACT OF THE FOWL

EXPLANATION OF PLATES

All sections stained with haematoxylin and eosin unless otherwise stated.

Plate 1

Fig. 1. Drawing of the reproductive tract and associated organs. The cloacal chamber is represented as having been slit in a cranial direction along both sides as the cock was lying on its back. The ventral portion was then turned on its vertical axis, so that the anterior end is facing back into the bird.

Fig. 2. Rough sketch of the testis and epididymal region.

Fig. 3. T.S. Cock testis showing thin t. albuginea and relatively large diameter tubules. No connective tissue septa are present which group tubules into lobules. × 50.

Plate 2

Fig. 4. T.S. Cock testis showing numerous spermatozoa in each tubule. Spermatocytes are small. × 120.

Fig. 5. T.S. One type of vasa efferentia epithelium showing the extrusion of large droplets of cell contents. Intra-epithelial glands are visible. × 600.

Fig. 6. Sudan black B stain. Vasa efferentia epithelium showing lipid in the cells. A section of a satellite tubule is seen in the subepithelial tissue of one villus. × 500.

Fig. 7. FSA stain. Another type of vasa efferentia epithelium showing positive granular material in cells. × 500.

Fig. 8. Columnar epithelium of ductus epididymis. × 600.

Fig. 9. T.S. Middle region of vas deferens. Note tortuous nature of duct and relatively wide lumen. × 40.

Plate 3

Fig. 10. T.S. Distal, swollen region of vas deferens. Note increased thickness of fibrous tissue in the submucosa, and of the outer muscle layers. × 40.

Fig. 11. Portion of epithelium of distal vas deferens to show extrusion of cellular apices. × 1200.

Fig. 12. T.S. Ejaculatory duct in contracted state to show numerous small blood vessels in submucosa. × 80.

Fig. 13. FSA stain. Epithelium on external surface of ejaculatory duct shows positive material in cells. × 80.

Plate 4

Fig. 14. T.S. Vascular body in region of the base of ejaculatory duct near to ureter opening. Note goblet cells in epithelium, aggregations of lymphocytes surrounding the deep-lying glands and the vast blood sinuses in the sub-epithelial tissue. × 80.

Fig. 15. FSA stain. Positive material in the glands of the vascular body. × 80.

Fig. 16. Toluidine blue metachromasia. Section of vascular body to show acid mucopolysaccharide in gland cells. × 120.

Fig. 17. Toluidine blue metachromasia. Part of lymph fold to show limited amount of acid mucopolysaccharide in epithelial cells. × 120.