Histology of the Oviduct of the Leopard Frog, *Rana pipiens* [1,2]

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ABSTRACT The first one to three mm of each of the paired female oviducts in *Rana pipiens* consists of a folded mucosa lacking tubular glands and enclosed by a serosa. Most of the remainder of the oviduct is lined by a folded mucosa richly endowed with simple tubular, jelly-secreting glands. The final one to two cm of the uterine portion of the oviduct, however, has a smooth mucosal lining which lacks tubular glands. Jelly-secreting glands penetrate to the base of the mucosa and open to the lumen between ridges capped with ciliated or mucous secreting epithelial cells. As cells of the jelly-secreting glands grow and differentiate during the summer, they accumulate a granular secretory product which exhibits basohpilia in hematoxylin-stained specimens. This is the essential change in the histological structure of the gland throughout the growing season.

The adult male oviduct is a solid cord of cells for about one-third of its length. The inferior two-thirds, however, is like the female oviduct at an intermediate stage of seasonal growth with gland cells containing secretory granules which exhibit basophilia.

The oviducts of mature females of *Rana pipiens* are a pair of convoluted tubules extending from the *ostia tubae*, their anterior openings dorsal to the lungs, posteriorly to their terminations in the lateral wall of the cloaca. The ducts are supported by dorsal mesenteries. One distinguishes an anterior, straight portion of the oviduct, the *pars recta*, a middle convoluted portion, the *pars convoluta*, and a dilated, thin-walled posterior portion, the *pars uterus* (Christensen, '30). The mature oviduct undergoes an annual cyclic change, with full growth by the end of the summer and regression after spawning in the spring. Christensen ('30) has correctly attributed the annual period of growth to an accumulation of secretory product in oviducal gland cells. He also has recorded the persistence of rudimentary oviducts in mature males of *Rana pipiens*. According to Moore ('44), there are geographical exceptions to the occurrence of male oviducts in *Rana pipiens*.

This report describes light microscopic features of the histology of both male and female oviducts.

MATERIALS AND METHODS

Adults of *Rana pipiens* were collected in Cheboygan County, Michigan, in the vicinity of the University of Michigan Biological Station during May, June, July and August or were purchased from E. G. Steinhilber, Oshkosh, Wisconsin. The latter animals were physiologically ready to be stimulated to ovulate. Oviducts from both sexes as well as ovaries from females were fixed in Bouin's or Carnoy's fixatives or in 10% formalin. They were dehydrated in alcohol, cleared in xylene, embedded in paraffin, and serially sectioned at 10 μ and stained with hematoxylin and eosin. Photomicrographs were taken with a Spencer photomicrographic camera.

RESULTS

The mature oviduct (fig. 1) near the anterior end of the superior straight portion has a folded mucosa, lined with a single layer of columnar epithelial cells, and is enclosed by a serosa (fig. 2). The epithelia lining is comprised of ciliated cells and mucous secreting cells. Coursing within the connective tissue of the mucosa

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is a capillary network. By examining a series of sections one finds that the lining epithelium is elevated into longitudinal or longitudinally oblique folds, often branching. Although there are scattered mucous secreting cells, most of the lining cells are ciliated columnar. Cilia in this region are sometimes as long as the cells are high.

Approximately 2 mm beyond the ostium, short, tubular glands appear in the *pars recta* of the oviduct (fig. 3), and longitudinal ridges become very numerous. The glands open at the bases of folds of the surface epithelium and extended radially almost the full thickness of the mucosa. The longitudinally oriented folds between the mouths of the glands are capped by ciliated and mucous epithelium.

Height and branching of the folds diminish as one progresses posteriorly into the convoluted portion. Concomitantly, the tubular glands (fig. 4) double their depth of penetration and increase their basal diameter. Most of the convoluted portion has a uniform appearance, but glands become larger and their bases enlarge at the lower end near the *pars uterus*. Throughout the *pars convoluta* the cilated and mucous secreting cells bordering the lumen of the oviduct form a pattern of ridges running longitudinally in a spiral fashion and are continuous anteriorly with the ridges in the upper non-glandular portion. Many glands are arranged so that their orifices open in obliquely oriented spiral rows in the furrows between the major epithelial ridges. Other glands open into lesser furrows branching from the major oblique spiralling furrows. The capillary network surrounding the bases of the glands ramifies through the connective tissue of the ridges formed by mucous secreting or ciliated lining cells.

Individual tubular glands are composed of columnar cells arranged radially around the lumen of a cylindrical tube (fig. 5). Basally the gland broadens out. At the anterior level where glands first appear, the lumina of the glands are relatively wide, but in most of the glandular portions of the oviduct, the lumina are virtually obliterated.

Secretory cells of a particular gland are approximately equal in size. As secretory products accumulate with seasonal growth, the gland cells become taller, and their nuclei are pushed more and more basally. Eventually most of the cytoplasmic region of the cell becomes loaded with hematoxylin-staining secretory granules (fig. 6). This accumulation of secretory product is the essential variation in the histological structure of the female oviduct as the seasonal growth cycle proceeds through the summer. Figure 4 shows a typical section for an oviduct of a frog collected in May whereas figure 6 illustrates the appearance of the glands loaded with secretory product collected in late July or August.

Throughout most of the glandular portion of the oviduct, all cells of a given gland contain abundant accumulations of secretory product. Approaching the posterior end of the *pars convoluta*, however, one notes a diminution in size of gland cells and a progressive decrease in the amount of secretory product, which becomes limited to the apical end of gland cells (fig. 7). Nuclei in these smaller cells remain spherical in the clear basal cytoplasm rather than flattening against the basal border of the cells.

There is a rather abrupt transition from a region in which all glands are full of granular secretion to a region which contains a mixture of stages of development of glands. The first evidence of transition is that the majority of glands contain cells with only very sparse numbers of granules, although a minority have cells still fully loaded with secretory granules. There follows another abrupt transition, now to a region in which only the basal cells of the glands contain appreciable accumulations of secretory product. The more apical cells of a gland in this region contain little or no secretory product. They are cuboidal with central nuclei. Successively more posterior glands possess less and less secretion product, and glands become progressively sparser. Ciliated and mucous secreting cells begin to extend down into the necks of glands as secretory cells regress. Both surface folds and the glands become progressively shallower so that with the *pars uterus* only a flat epithelial lining remains (fig. 8). The tunica propria of the mucosal lining of the undistended

pars uterus is relatively thick in comparison to that in the rest of the oviduct.

The oviduct of the adult male is smaller than that of the adult female with accumulated secretory product at the end of the summer season. The male structure possesses relatively undeveloped superior and inferior portions. The superior third is a tapering solid cord of cells, but its convoluted portion possesses tubular glands and resembles that of the female. Glands of the *pars convoluta* contain cells with secretory granules, although granules are absent from many cells (fig. 9). Cells containing granules tend to be limited to the basal portions of glands. When granules are present, they usually lie in the apical end of the cell toward the lumen.

DISCUSSION

Christensen ('30) observed that the earliest evidence of the oviduct of *Rana pipiens* is a thickening of the peritoneum lateral to the mesonephros in both male and female tadpoles 7.5 to 9.0 cm long. Growth and differentiation then proceed anteriorly and posteriorly. At metamorphosis the oviduct has a folded epithelial lining. Differences in the male and female oviducts become apparent subsequent to this time. The epithelial lining and the development of tubular glands is similar for both sexes, although the connective tissue tunic is much thicker in the female. Another obvious feature is that the female oviduct becomes convoluted *(pars convoluta)* throughout most of its length, while that of the male becomes convoluted only in the posterior part, from the level of the mesonephros backward. The present report supplements Christensen's by describing histological details of the ciliated, mucous secreting and jelly-secreting epithelial cells lining the oviducts in both females and males.

It is apparent from this study that the three principal regions of the oviduct described by Christensen ('30), e.g., the *pars recti, pars convoluta* and *pars uterus,* are not characterized internally by distinctively different types of epithelial lining. Jelly-secreting glands occur in all except the first few millimeters of the *pars recti,* throughout the *pars convoluta* and in a transitional zone of the anterior portion of the *pars uterus.* Cuboidal cells line the anterior few millimeters of the *pars recti* and the posterior dilatable portion of the *pars uterus.* There are indeed three regions of the oviduct on the basis of histology of the epithelial lining, i.e., a glandular portion and anterior and posterior nonglandular portions; but these three histologically characterized regions do not correspond to the three regions observed grossly.

Regions of oviducts in several urodeles can be described according to anatomical variation which correlate with the changes in histochemical properties. Humphries and Hughes ('59) have reported regional variation in the staining of epithelial cells lining the oviduct of *Triturus viridescens.* They described six regions, all of which are periodic acid-Schiff positive. Three of the six regions show metachromasia with toluidine blue and stain positively with Alcian blue. These staining reactions are not eliminated by pretreating sections with hyaluronidase nor ribonuclease. Kambara ('56, '57) has described three regions in the oviducts of the newt, *Triturus pyrrhogaster.* He states that the first two regions are PAS-positive and show variable metachromasia with toluidine blue. These two regions secrete the major portion of the jelly. The oviduct of *Triturus alpestris,* according to Vilter ('66) has seven sectors. Regions 3, 4 and 5 contain very dense granules uniformly distributed throughout the cytoplasm with the nucleus located basally. These granules stain with Alcian blue and are apparently glycoprotein in character. Finer granules which stain with PAS are present in the cells found in region 5. The seventh and most caudal sector contains granules which are protein. Another urodele, *Salamandra atra,* has an oviduct of five segments (Vilter, '67). This structure contains a variety of cells, including a granular mucocyte which, in the mature state, contains spherical granules which stain with Alcian blue. Humphries et al. ('68) found sialic acid in regions B and D of the oviduct of *Notophtalmus viridescens.* It was not present in regions A, C, and E.

In contrast to these salamanders, the frog, *Rana pipiens,* shows no regional localiza-

tion based on histochemical properties. The mucous-secreting cells lining the oviduct and jelly-secreting cells of the glands in the middle region of the oviduct of *Rana pipiens* both give PAS reactions (Lee, '67). There is no regional localization of specialized mucous cells or jelly-secreting cells with respect to staining by Alcian blue or metachromasia with toluidine blue, with mercury bromphenol blue and ninhydrin-Schiff, and with Barrnett and Seligman's method for sulfhydryl and disulfide groups (Lee, '67). A methylene blue extinction method, a dialyzed iron reaction, acetylation and deacetylation before the periodic acid-Schiff reaction and a reaction for the presence of sialic acid in the *Rana pipiens* oviduct (Lee, '67) was similar for the jelly glands examined at various levels. These procedures suggest no localization into separate zones to correlate with production of the three principal jelly layers surrounding each egg.

The effect of the jelly layers and contact of eggs with portions of the oviduct or contact with jelly upon maturation and fertilization of the egg has been studied by several investigators.

Katagiri ('65) reported that eggs removed from the first 5 cm of the oviduct in one experiment had only one jelly layer (J_1) or sometimes two (J_1 and J_2) around them. Eggs from 5–9 cm always had layers J_1 and J_2. Layer J_3 was sometimes present if eggs were removed at 9–15 cm from the ostial end of the oviduct and always present at 15–23 cm. All 4 jelly layers were present around eggs from more posterior levels of the oviduct (23–76 cm). I have removed eggs from approximately the middle of the oviduct of *Rana pipiens* and found that the three principal jelly layers were already present.

Katagiri ('65) found that eggs invested with J_1 alone did not cleave after insemination. Eggs with J_1 and J_2 became fertilized and cleaved normally. Coelomic eggs introduced into the lower end of the oviduct about 10 cm from the uterus were coated only with J_4, according to Katagiri; yet these underwent 85.2% cleavage after insemination. He concludes that either J_2 or J_4 may facilitate successful sperm penetration.

Shaver ('66) has tested jelly secretions from upper and lower levels of the oviduct for their effects on the fertilizing capacity of spermatozoa. He finds that jelly from the lower end of the oviduct inhibits the fertilizing capacity of sperm more than does jelly from the upper part of the oviduct. Despite the accumulating evidence for physiological differences in the jelly secreted at various levels of the oviduct, the present study reveals no morphological basis for such differences.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

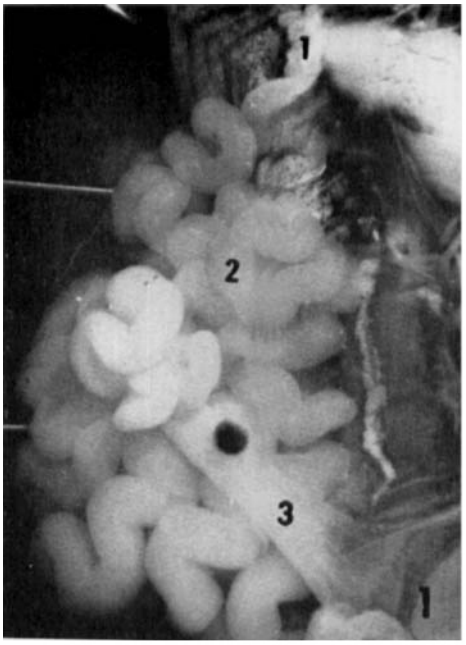
1 Gross appearance of a fully developed right oviduct of an adult female, which consists of three regions: (1) a straight upper portion (*pars recta*) opening anteriorly at the ostium (ostium indicated by a hair); (2) a middle, highly convoluted portion (*pars convoluta*); and (3) a lower, dilatable portion (*pars uterus*) emptying into cloaca (egg inserted to aid identification). × 2.

2 Upper non-jelly-secreting region of the oviduct. The tube at this level is composed of a highly folded mucosa, lamina propria and a serosa. Mucous-secreting cells and the heavily ciliated cells seen at lower regions are not evident. × 110.

3 Simple tubular glands from the upper portion of the middle, secretory portion of the oviduct. Glands at this level of the oviduct are relatively shallow. This oviduct is from a female less than one month after spawning. There is no obvious secretory product. × 115.

4 Middle, secretory portion of the oviduct showing the simple tubular morphology of the jelly-secreting glands. Some gland cells show secretory product (arrow). Ciliated and mucous-secreting epithelial cells line the lumen. × 200.

5 Longitudinal section of glands from secretory region of oviduct at an early stage of annual growth cycle. Gland cells show nuclei which are displaced basally. The gland lumen has been obliterated by the enlarged cells. Mucous and ciliated epithelial cells form the epithelial tufts protruding into the oviducal lumen. × 260.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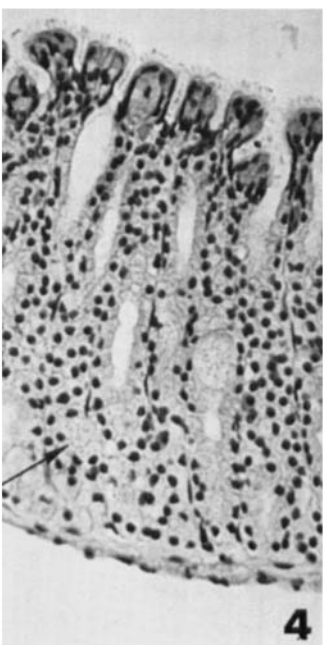
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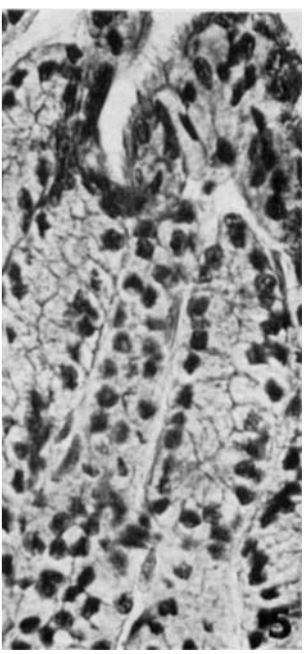
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PLATE 2

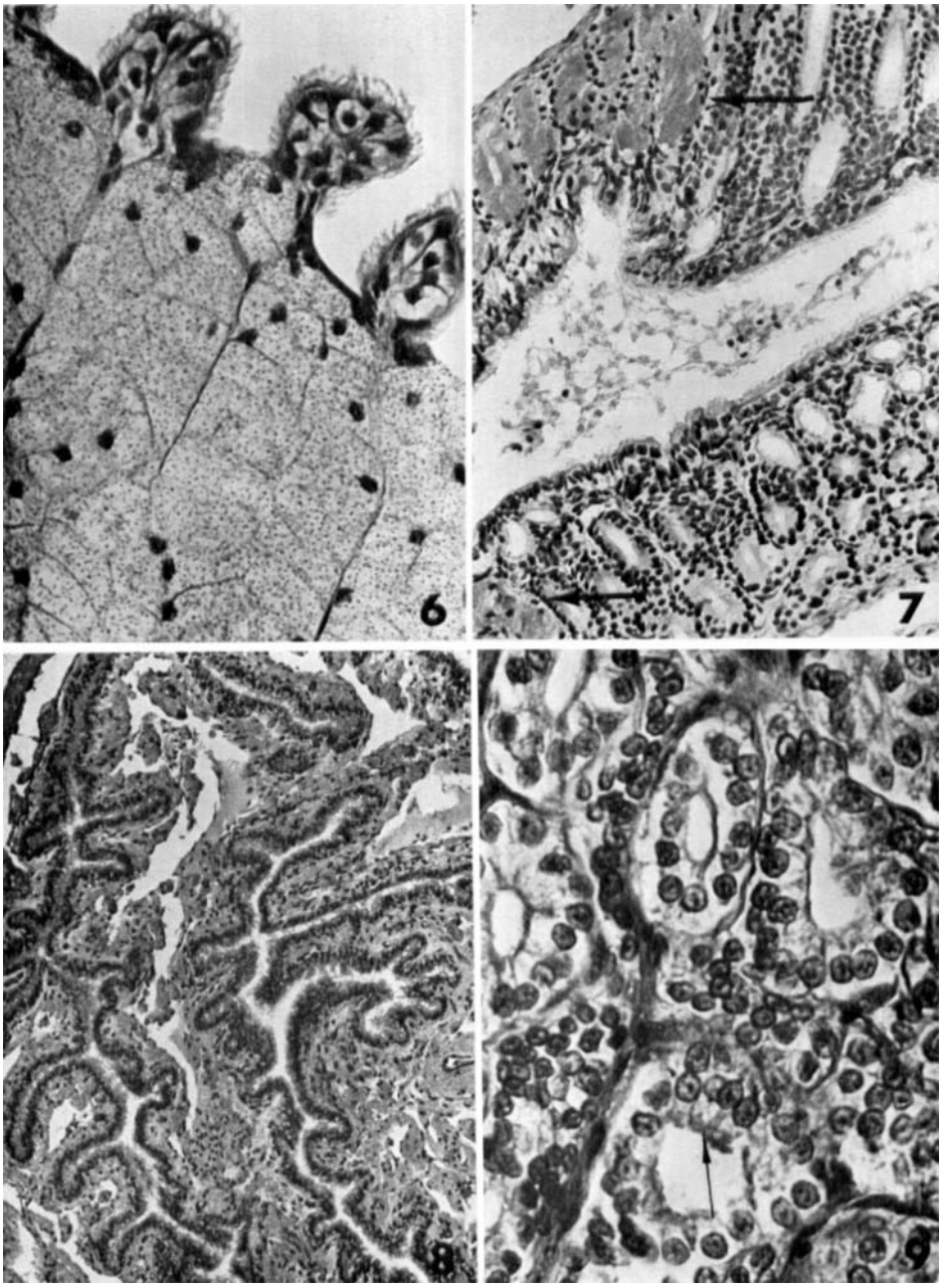
EXPLANATION OF FIGURES

6 Longitudinal section of glands from oviduct of female collected at the end of the seasonal growth showing cells with accumulation of secretory product, which includes fine basophilic intracellular granules staining with hematoxylin. Cells are larger and more fully developed than those in figure 4. Nuclei are further displaced basally. × 425.

7 Longitudinal section showing a region of transition (arrows) at lower end of *pars convoluta* of oviduct from gravid female. Glands in the jelly-secreting region toward the left contain abundant secretory product whereas those in the more caudal (right) region contain less stored product and have wider lumina. × 155.

8 Longitudinal section from the lower end of the *pars uterus* of the oviduct of a gravid female. The tunica propria is thick. Tubular glands are absent. × 100.

9 Section through middle of convoluted portion of a differentiated male oviduct. Centrally located nuclei and open lumina of glands reflect slight accumulation of secretory product (arrow). × 480.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