THE EMBRYONIC DEVELOPMENT OF THE PROCTODEAL GLAND OF *COTURNIX COTURNIX JAPONICA*

by

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ABSTRACT

The proctodeal gland of the Common Coturnix (Coturnix coturnix japonica) in located within the dorsal wall of the proctodeum; it has been described as an aggregate gland composed of numerous glandular units which secrete, in the sexually active male, a foamy exudate of unknown function.

Two studies which summarize certain aspects of the embryogeny of the proctodeal gland have been published. These papers are contradictory in description of the development. Further, the papers contain little indepth information to foster acceptance of either of the postulated theories on embryonic development.

This study was undertaken to present the detailed embryology of the proctodeal gland from day of first appearance to day of hatching. A total of 57 embryos were collected at 12 hour intervals from day 7 to day 16 (3 embryos/12 hour time period), serially sectioned and stained with either a hematoxylin and eosin stain or a connective tissue stain. Eight embryos from day 11 to day 16 were prepared for study on the Scanning Electron Microscope.

The first indication of proctodeal gland development is in the 9.5 day embryo in which solid epithelial buds (gland primordium) proliferate from the dorsal proctodeal epithelium into the lamina propria. At 11 days incubation the solid epithelial buds have developed into solid convoluted epithelial cords. At 12 days the epithelial cords begin to branch as the result of expansions along their length; concurrently the solid branched epithelial cords (glandular units) show the first evidence of cellular degeneration in the core of the cord. As a result of the cellular degeneration, vacuoles are formed in the glandular units by 12.5 days. By 15 days,

coalescence of the vacuoles has formed a continuous lumen throughout the glandular units. At 16 days the glandular units can be recognized to be individual simple branched alveolar glands.

At about 13 days of incubation epithelial caps begin to develop on the proctodeal surface. These caps consist of a single layer of squamous epithelium which separates the vacuolizing glandular units from the proctodeal cavity. These epithelial caps begin to disappear at 16 days and thus open the lumen of the glandular units to the proctodeal cavity.

The lamina propria associated with the dorsal proctodeal wall is composed of only reticular fibers prior to 10.5 days. Subsequent to this time collagen fibers begin to develop in the lamina propria and extend throughout the lamina propria by 11.5 days. The presence of elastic fibers in the lamina propria was not observed until 11.5 days. The connective tissue fibers of the lamina propria including those associated fibers surrounding the glandular units are interwoven with the epimysium and perimysium of M. sphincter cloacae located dorsal and lateral to the gland.

At 16 days incubation the future secretory cells are cuboidal and present no indication of secretory function.

The proctodeal gland is derived from the ectoderm as an inward growth of epithelium of the dorsal proctodeal wall. The lamina propria which underlies and surrounds first the epithelial buds, then the epithelial cords and finally the glandular units is a derivative of the mesoderm.

INTRODUCTION

Embedded within the dorsal wall of the proctodeum is a gland which is present in members of both sexes of the Common Coturnix (Coturnix coturnix japonica, Temminck and Schlegel). This glandular tissue however, is greatly enlarged in the sexually active male (Coil and Wetherbee, '59); Klemm et al., '73). A variety of names have been given to this gland: e.g., foam gland (Ikeda and Taji, '54), cloacal gland (Coil and Wetherbee, '59), paracloacal gland (Perez and Sandoval, '66) and most recently proctodeal gland (Klemm et al., '73). The gland will be identified throughout this paper as the proctodeal gland (Glandula proctodealis).

The proctodeal gland has been reported to be functionally active only in members of the genus Coturnix to which the Common Coturnix belongs (Klemm et al., '73).

The Common Coturnix (Coturnix c. japonica) was imported from Japan in 1955 by the Missouri Conservation Commission to determine if it could be established as a supplement to other game bird species in the country (Padgett, '58; Padgett and Ivey, '59; Howes and Ivey, '61). Though the wildlife releases ended in failure (Keeler, '60), the Common Coturnix increased in value as a poultry research animal (Padgett and Ivey, '59; Wilson et al., '61; Howes and Ivey, '61; Landsdown et al., '70). This value as a research animal as stated by Wetherbee ('61) is such that its potential for avian research is equal to that of the fruit fly and the white rat in other disciplines.

Despite the growing significance of this bird as an experimental pilot animal for poultry research, especially in the area of reproduction, very little basic information is available concerning either this bird in general

or the proctodeal gland. The specific function of the secretion of the proctodeal gland is still unknown, but one suggested possibility is that it has an, as yet unknown, effect upon the reproductive process. If the gland has a function in the reproductive process, it may be questionable whether the Common Coturnix is an ideal pilot animal for poultry reproductive studies. In order to answer this question much more basic information on the origin and function of this gland is needed than is presently available. One area requiring detailed study is the embryogenesis of the gland.

Studies on the embryonic development of the proctodeal gland have been published by Nagra et al. ('59) and Perez and Sandoval ('66). These authors differ in opinion on the developmental process of the proctodeal gland and their papers contain little indepth information to permit acceptance of the data in either of these publications on embryonic development. This study was undertaken to present a detailed study of sequential development of the proctodeal gland from time of first appearance to its condition at hatching.

This study of the embryonic development is one of a series of morpho-functional studies being carried out in the Laboratory of Veterbrate Morphology, College of Veterinary Medicine, Kansas State University, to extend the present knowledge of the proctodeal gland of the Common Coturnix.

LITERATURE REVIEW

A review of the literature produced few papers on the morphological structure of the proctodeal gland. Most of the morphological data contained in the papers reviewed was ancillary to studies on other aspects of the gland or its secretion.

The proctodeal gland extends from the opening of the cloacal bursa almost to the caudal end of the dorsal lip of the cloaca; it continues only slightly down the lateral walls of the cloaca (Klemm et al., '73).

In the sexually active male the entire cloacal region is swollen (Klemm et al., '73) as the result of hypertrophy of the glandular mass and surrounding musculature (Nagra et al., '59). A comparable development of either gland or musculature (Klemm et al., '73) is lacking in the mature female. The proctodeum of the male, in its stage of hypertrophy, shows a single layer of glandular units located beneath the stratified squamous epithelium (Nagra et al., '59). Coil and Wetherbee ('59) reported that enlargement of the glandular tissue was associated with enlarged testes. Sachs ('67) demonstrated a direct correlation between testicular regression and recrudescence and regression and hypertrophy of the proctodeal gland. Apparently, the state of development of the proctodeal gland is correlated with the amount of testosterone produced by the testes. As pointed out by Coil and Wetherbee ('59), this hypertrophy of the cloaca is not to be confused with the cloacal protuberance described for some passerine birds; this cloacal protuberance, as described by Wolfson ('52; '54), results from enlargement of the convoluted sperm ducts. In the Common Coturnix (Coturnix c. japonica) the noticeable swelling is related to the development of the proctodeal gland (Coil and Wetherbee, '59).

The dorsal and lateral portions of the gland are surrounded by and have an intimate relationship with M. sphincter cloacae (Klemm et al., '73). Ventrally the gland is covered by a stratified squamous epithelium continuous with the dorsal lining of the proctodeum (Coil and Wetherbee, '59; Klemm et al., '73).

Tamura and Fujii ('67) were the first to describe the proctodeal gland as "agregates (sic) of small glands...". Klemm et al. ('73) stated that the gland lacks the lobular pattern and the common excretory duct required of a gland to be classified as a compound gland. They further defined it as an "aggregate gland" composed of numerous glandular units which are comparable in structure to that of simple branched alveolar glands. However, according to Klemm et al. ('73) the proctodeal gland has not been studied in sufficient detail to permit assignment of the glandular unit to any extant anatomical classification.

A connective tissue capsule, formed from the lamina propria covers the entire aggregate gland (Klemm et al., '73). The individual glandular units are long and narrow (Coil and Wetherbee, '59) and are separated by thin intraglandular septae which are extensions of the connective tissue capsule around the gland (Klemm et al., '73).

Composition of the capsule and intraglandular septae according to Klemm et al. ('73) includes, in order of abundance, collagen fibers, reticular fibers and elastic fibers. Collagen and reticular fibers are always present in the primary folds, while reticular fibers are always present in the secondary folds. Collagen fibers are not abundant in the secondary folds (Klemm et al., '73). Elastic fibers are abundant in the septae (Tamura and Fujii, '67) and may be present in the primary and secondary folds (Klemm et al., '73). Klemm et al. ('73) divide the connective tissue capsule into two regions:

1) subglandular--between the gland and overlying musculature and 2) supra-glandular--that portion which underlies the proctodeal epithelium.

The subglandular connective tissue is continuous both with the sagittal raphé from which fibers of the M. sphincter cloacae originate and with the perimysium and endomysium of that muscle. Thus the gland and the overlying musculature are physically and functionally inseparable (Klemm et al., '73).

Each glandular unit has a short terminal papilla in which is located the "excretory canal" (Klemm et al., '73). Secretion product of the glandular unit passes through this canal and exits through an excretory pore into the proctodeal cavity. A continuation of the stratified squamous epithelium of the dorsal surface of the proctodeum lines both the excretory pore and canal (Klemm et al., '73).

The secretory epithelium begins at the termination in the canal of the lining of the proctodeal epithelium as a low columnar cell and increases in height to a typical high columnar secretory cell (Klemm et al., '73). The tall slender primary and secondary folds of the glandular units which are lined with this high columnar epithelium (Nagra et al., '59) project into the lumen of the glandular unit (Klemm et al., '73). The cytoplasm of the secretory cell contains a "network with small granules" (Coil and Wetherbee, '59) and a round nucleus located near the base of the cell (Tamura and Fujii, '67).

The secretion product has been reported (Schleidt and Shalter, '72) to be more abundant in the Common Coturnix than in the European Coturnix (Coturnix coturnix coturnix); in the latter, the secretion is only a fraction of that obtainable from the Common Coturnix.

Function of the secretory product of the proctodeal gland is yet unknown (Klemm et al., '73). Ikeda and Taji ('54) suggested that the secretion of the gland might be an ejaculate fluid and it is documented that

secretion from the proctodeal gland is transferred into the female cloaca at the time of copulation (Sachs, '67). Klemm et al. ('73) however, reported that the glandular secretion is not an ejaculate because there is no exclusive relationship between copulation and secretory discharge. Ikeda and Taji ('54), for example, stated that the foamy "secretions" are also found mixed with the feces from the birds. In addition Knight ('67, as reported by Klemm et al., '73) has shown that the ejaculate fluid is a product of the corpora paracloacalis vascularis. Perez and Sandoval ('66) suggusted that the secretion "seems to be" connected with occlusion of the oviduct after coitus to prevent loss of sperm when the female takes flight. Coil and Wetherbee ('59) postulated that the penis, located on the ventral wall of the cloaca, is inserted into the cloaca during copulation, and it is possible that the secretion of the gland may serve as a lubricant. Wetherbee ('61) subsequently stated that the secretion "probably acts as an analog to secretions of the mammalian Cowper's gland, aiding in sperm transfer". Klemm et al. ('73) stated that glandular exudate may exert an influence, as yet unknown, on the reproductive process under normal breeding conditions.

The chemical composition of exudate of the proctodeal gland until recently has been the subject of little investigation. Perez and Sandoval ('66) reported that it is rich in nitrogen and lipoids. The secretion product is strongly PAS positive (Coil and Wetherbee, '59; Fujii and Tamura, '67; Tamura and Fujii, '67; Klemm et al., '73) and stains violet with toluidine blue (specific test for mucin) (Coil and Wetherbee, '59). The pH of the exudate ranges from 6.3 to 6.6 (Ikeda and Taji, '54). Fujii and Tamura ('67) identified that the secretion of the proctodeal gland as a sulfated acid mucopolysaccharide in the sexually active male and a nonsulfated acid mucopolysaccharide in the female and immature male.

The possibility of the secretion being either a sulfated or nonsulfated acid mucopolysaccharide was questioned by Klemm et al. ('73) because Zugibe ('70) demonstrated that acid mucopolysaccharide will exhibit a PAS positive reaction only after a prolonged oxidation period. Based on the PAS reaction and an absence of lipids, Klemm et al. ('73) suggested that the secretion was either a neutral polysaccharide, a mucoprotein or glycoprotein. Renzoni ('71), on the basis of chromatographic studies, reported the foamy exudate to be a glycoprotein.

The information pertaining to the embryonic development of the proctodeal gland is scant and the information which is available is contradictory. For example, Perez and Sandoval ('66) reported that the first buds of a glandular nature appear after 7 days of incubation as a derivative from the mesonephros. These buds continued to develop to form two individual genital glands located under the roof of the cloaca and which eventually fuse to form one single gland. Nagra et al. ('59) however, stated that the glands began to differentiate in the proctodeum, between the 11th and 12th day of incubation as solid epithelial buds which subsequently develop into epithelial spirals. A lumen begins to develop in these spirals during day 13 and by day 15 "a lumen is present throughout the entire gland". At hatching the units are sparsely located in the mucosa as slightly convoluted tubular glands (Tamura and Fujii, '67).

MATERIALS AND METHODS

Incubation of Eggs

Eggs from the breeding stock retained by the Laboratory of Vertebrate Morphology, College of Veterinary Medicine, Kansas State University were collected each morning for a period of ten days. To prevent variability in the embryogenesis due to delayed incubation (Manner and Granik, '67) the eggs were immediately placed in a rocker incubator after collection. The incubation environment was maintained at a temperature of 99° to 100°F with a relative humidity of 45 to 55 percent (wet bulb reading of 79° to 85°F).

The eggs were turned automatically every four hours to prevent the blastodisc and young embryos from adhering to the egg shell membranes (Padgett, '58). Logging of developmental time began at the time the eggs were placed in the incubator.

Collection of Embryos

Embryos were collected at each of the following times:

Collection Times	No. of Embryos-Light Microscopy	No. of Embryos-Scanning Electron Microscopy
7 days	3	-
8 days	3	-
9 days	3	-
9.5 days	3	-
10 days	3	-
10.5 days	3	-
11 days	3	1
11.5 days	3	-
12 days	3	-
12.5 days	3	-
13 days	3	1
13.5 days	3	-

Collection Times	No. of Embryos-Light Microscopy	No. of Embryos-Scanning Electron Microscopy
14 days	3	2
14.5 days	3	-
15 days	3	2
15.5 days	3	-
16 days	3	2

Hatching of the embryos began at 16 days and was complete by 16.5 days. Eggs unhatched at this time failed to hatch.

The embryos were removed from the egg by carefully dissecting away the shell in order to prevent damage to the embryos. After removal the embryos were decapitated, the yolk sac and embryonic membranes peeled away, the hind limbs removed and that portion of the embryos caudal to the wings was retained.

Fixation and Processing of Embryos

Light Microscopy. Embryos were fixed in an unbuffered 10% formalin solution for 24 hours at room temperature, then washed in running tap water for 24 hours.

Embryos of 8 days and older were then decalcified in RDO[1] commercial decalcifier as indicated below:

Age of Embryo	Time Immersed* in RDO
8 days	3 hours
9 and 9.5 days	4 hours
10 and 10.5 days	5 hours
11 and 11.5 days	7 hours
12 and 12.5 days	10 hours
13 and 13.5 days	11 hours
14 to 16 days	12 hours

*Note: Prolonged immersion in RDO (12 hours or more) will weaken the basophilic reaction of the tissue.

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[1]DuPage Kinetic Laboratories, Inc., P. O. Box 416 Downers Grove, Illinois 60515.

After decalcification the embryos were placed in a 70 percent ethanol solution for storage. Standard schedules for processing tissue for paraffin sectioning gave extremely poor results; infiltration was poor as was dehydration. Therefore it was necessary to develop specific processing schedules for these tissues. These schedules were as follows:

A. Embryos younger than 10 days

1) 80% Ethanol	55 minutes
2) 95% Ethanol	35 minutes
3) 95% Ethanol	40 minutes
4) Abs. Ethanol	40 minutes
5) Abs. Ethanol	40 minutes
6) Abs. Ethanol and Toluene (1:1)	40 minutes
7) Toluene	40 minutes
8) Toluene	40 minutes
9) Toluene and Paraffin (1:1)	40 minutes
10) Paraffin (57°F, low vacuum)	1 hour
11) Paraffin (57°F, high vacuum)	1 hour
12) Embed	

B. Embryos older than 10.5 days

1) 80% Ethanol	1 hour
2) 95% Ethanol	1 hour 15 minutes
3) 95% Ethanol	1 hour
4) Abs. Ethanol	1 hour
5) Abs. Ethanol and Methyl Salicylate*	1 hour 30 minutes
6) Abs. Ethanol and Methyl Salicylate*	2 hours 15 minutes
7) Abs. Ethanol, Toluene and Methyl Salicylate*	2 hours
8) Toluene and Methyl Salicylate*	2 hours 30 minutes
9) Toluene	30 minutes

10) Toluene and Paraffin (1:1)	2 hours 30 minutes
11) Paraffin (57°F, low vacuum)	4 hours
12) Paraffin (57°F, high vacuum)	overnight
13) Embed	

*Methyl Salicylate, 4 drops/10 ml of solution.

The three embryos from each period prepared for light microscopy were serially sectioned at 10 micron thickness; two of these were sectioned transversely, one was sectioned sagittally. The tissue ribbons were cut into lengths that would be accommodated on 3" x 1" or 3" x 2" slides depending on stains to be used. A thin layer of albuminized water (2 drops of egg albumin/10 ml of water) was spread over the surface of the slide and the ribbons placed on the albuminized water. The slides were then heated on a hot plate at 62°F until the bubbles that had formed disappeared completely. Following removal from the hot plate the slides were air dried for 24 hours, with a final drying prior to staining for 30 minutes in a Lipshaw forced air slide dryer.

For light microscopy study of serial sections, one embryo sectioned transversely and one sagittally from each collection time were stained in either Harris or Mayers hematoxylin and counterstained in eosin (Humason, '67). Since standard connective tissue stains such as Mallory's Triple and Masson's Trichrome did not allow detection of collagen fibers, elastic fibers and reticular fibers in the same section, Humason and Lushbaugh's ('60) stain for elastic fibers, reticular fibers and collagen fibers was attempted. Results from the technique were unsatisfactory and it was necessary to modify the procedure. Therefore, the remaining embryo from each time period was stained for connective tissue fibers by the following procedure:

1) Toluene	2 minutes
2) Toluene	3 minutes

3) Abs. Ethanol	1 minute
4) Abs. Ethanol	1 minute
5) 95% Ethanol	1 minute
6) Pyridine and 95% Ethanol (1:1)	15 minutes
7) 95% Ethanol	2-3 seconds
8) Running H_2O	5 minutes
9) Periodic Acid (0.5% aqueous)	15 minutes
10) Distilled H_2O (Fresh)	3 minutes
11) Silver Nitrate (2% aqueous; Fresh)	20 minutes
12) Distilled H_2O (Fresh)	rinse
13) Distilled H_2O (Fresh	rinse
14) Ammonical Silver Solution* (Fresh)	10 minutes
15) Distilled H_2O	dip
16) Formalin (30%) agitate gently	3 minutes
17) Distilled H_2O (4 changes)	rinse
18) Gold Chloride (0.25% aqueous) tone from brown to gray	
19) Distilled H_2O	rinse
20) Sodium Thiosulfate (5% aqueous)	3 minutes
21) Running tap H_2O	5 minutes
22) Distilled H_2O	1 minute
23) Orcein**	30 minutes
24) Distilled H_2O (Fresh)	rinse
25) 95% Ethanol	1 minute
26) Abs. Ethanol	until red stops fading
27) 95% Ethanol	1 minute
28) 70% Ethanol	1 minute
29) Distilled H_2O	1 minute

30) Phosphomolybdic Acid (5% aqueous)	15 minutes
31) Distilled H_2O	rinse
32) Light Green SF Yellowish***	35 seconds
33) Distilled H_2O	rinse
34) Distilled H_2O	rinse
35) 95% Ethanol	1 minute
36) 95% Ethanol	1 minute
37) Abs. Ethanol	1 minute
38) Abs. Ethanol	1 minute
39) Toluene	2 minutes
40) Toluene	2 minutes

*Ammonical Silver Solution

1. 5% Silver Nitrate (Aqueous)-60ml.
2. Add 10% Sodium Hydroxide-60 drops.
3. Add 28% Ammonia Hydroxide drop by drop until precipitate which was formed has just a few grains remaining.
4. Add distilled water to bring volume to 180 ml.
5. Stain at room temperature in poorly lighted room.

**Orcein Solution

1. Add 1 ml of conc. Hydrochloric Acid and 100 ml of 70% Ethanol.
2. Add Orcein-0.4 gm.
3. Dissolve Orcein completely.
4. Check and adjust pH; it should be in the range of 1.2 to 2.0.

***Light Green SF Yellowish Solution

1. Add 0.3 ml of Glacial Acetic Acid and 100 ml distilled H_2O.
2. Add Light Green SF Yellowish-0.5 gm.
3. Dissolve completely.

In all silver solutions use chemically clean glassware. Do not let metal come into contact with silver solutions.

Staining reaction of connective tissue fiber types:

Fiber Type	Color
Reticular	Black
Elastic	Reddish-brown
Collagen	Green

All sections were mounted in Histoclad[2]. After cover slipping, the slides were then placed on a warming tray for five minutes to insure even spreading of the mounting media.

All tissues were studied, and selected sections photographed, on a Ziess RA microscope.

Scanning Electron Microscopy. Embryos were fixed in a solution of (2%) glutaraldehyde at 4°C for a minimum of three hours.

The ventral surface of the entire embryo was removed after fixation to expose the dorsal surface of the proctodeum. The dorsal surface of the proctodeum was then washed with avian saline to remove the mucoid coating on the dorsal proctodeal epithelium. The tissue was then further trimmed to facilitate dehydration and critical point drying. Dehydration was carried out in a graded series of ethanol as follows:

1) 50% Ethanol	3 hours
2) 70% Ethanol	3 hours
3) 90% Ethanol	3 hours
4) 95% Ethanol	3 hours
5) 95% Ethanol	3 hours
6) 100% Ethanol	3 hours

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[2]Clay Adams, Inc., Parsippany, New Jersey 07054.

7) 100% Ethanol	3 hours
8) 100% Ethanol	3 hours
9) Amyl Acetate	30 minutes
10) Amyl Acetate	15 minutes
11) Critical point dry	

The tissue was then mounted on a stub, carbon and gold plated and examined in an ETEC Autoscan scanning electron microscope.

Clay Model

It was difficult to classify the structure of the glandular unit by observing serial sections by light microscopy. Therefore, a serial reconstruction was prepared of a glandular unit using the 16 day stage. The reconstruction was made from 14 serial sections, at 10 microns per section, which were projected onto paper using a B & L Microprojector. These paper sections were then cut and clay reconstructions of each were prepared and combined to form a model of the glandular unit.

Terminology

Wherever possible, the terminology used in this study is that of the Nomina Anatomica Veterinaria ('73).

RESULTS

At the first stage of glandular development, the mucosa of the dorsal proctodeal wall consists of only two components; the epithelial lining and the lamina propria. No submucosa is present. The lamina propria extends dorsally to the developing M. sphincter cloacae, thus binding the lamina propria to the connective tissue fibers around the developing muscle. Prior to 9.5 days of incubation there is no indication of proctodeal gland development.

For the purposes of this study, the embryonic development of the proctodeal gland is divided into five identifiable stages: 1) formation of epithelial buds, 2) formation of solid convoluted epithelial cords, 3) branching of the convoluted epithelial cords, 4) cellular degeneration and vacuolization of the epithelial cords to produce a lumen, and 5) opening of the excretory pore.

Nine and One-half Day Embryo

Gland. The first evidence of proctodeal gland formation is the development of solid epithelial buds as slight protrusions into the lamina propria from craniolateral portions of the dorsal proctodeal epithelium at 9.5 days (figs. 1, 2). Located within the basal layers of the epithelial buds and dorsal proctodeal epithelium are numerous cells undergoing mitotic activity which can produce cells for continued expansion and for further formation of epithelial buds. The cells of the epithelial buds are cuboidal or prismatic in shape; they contain a centrally located nucleus and an acidophilic cytoplasm with scattered basophilic "granules" (fig. 1).

Lamina propria. Reticular fibers are located throughout the lamina propria of the 9.5 day embryo. However, they are most abundant cranially in

the median region of the dorsal proctodeal wall (figs. 3, 4). The fine reticular fibers form a netlike appearance throughout the lamina propria (fig. 3). In the caudal region of the dorsal proctodeal wall the reticular fibers are evenly distributed, with no distinct concentration in any one area. There is no separation between the reticular fibers of the lamina propria and those surrounding the developing M. sphincter cloacae (fig. 4). Beneath the solid epithelial buds and the dorsal proctodeal epithelium is a distinct layer of reticular fibers (reticular lamina) (fig. 3). Collagen and elastic fibers are not present in the 9.5 day embryo.

Ten Day Embryo

Gland. There has been no apparent change in the development of the epithelial buds (figs. 5, 6).

Lamina propria. There has been no apparent change in the connective tissue fiber development (fig. 7).

Ten and One-half Day Embryo

Gland. The solid epithelial buds have further penetrated the lamina propria and are numerous throughout the craniolateral portions of the dorsal proctodeal wall (figs. 8, 9, 10). New solid epithelial buds are developing medial to the original point of development (fig. 8). In addition development of solid epithelial buds is also progressing caudally, across the entire length of the dorsal proctodeal wall. There is no development of any epithelial buds in the caudal third of the dorsal proctodeal wall (fig. 9). At this time development of the solid epithelial buds is in a sequence of lateral to medial and cranial to caudal.

Lamina propria. There has been an increase in the number of reticular fibers throughout the lamina propria, with the midline of the cranial region

showing the largest increase in fibers. The reticular lamina is present beneath the solid epithelial buds and dorsal proctodeal epithelium (fig. 11). Substances which react to a typical collagen stain are present in the amorphous ground substance of the midline of the cranial region of the dorsal proctodeal wall; however, individual collagen fibers cannot be identified under the light microscope. There are no elastic fibers yet present in the lamina propria.

Eleven Day Embryo

Gland. The solid epithelial buds are elongating and are forming solid convoluted epithelial cords in the craniolateral portion of the dorsal proctodeal wall (figs. 12, 13). With the exception of the extreme caudal region the entire dorsal proctodeal wall now contains numerous solid epithelial buds (fig. 13).

Lamina propria. Collagen and reticular fibers are present throughout the dorsal wall of the proctodeum; the collagen fibers are concentrated in the midline, while reticular fibers are evenly distributed. The reticular lamina is present around the solid convoluted epithelial cords. Elastic fibers are present in association with M. sphincter cloacae, however, they are not present in the lamina propria. As the solid convoluted epithelial cords develop, the connective tissue fibers are being arranged in a pattern which conforms to the shape of the solid convoluted epithelial cords. There is no indication of connective tissue fibers being present within the solid convoluted epithelial cords (fig. 14).

Eleven and One-half Day Embryo

Gland. Both convoluted epithelial cords and epithelial buds are present throughout the entire dorsal proctodeal wall. The solid epithelial

cords are beginning to show evidence of epithelial expansions along their length; each such expanding cord is structurally isolated from all other cords and will now be termed glandular units. In both the expanding glandular units and the non-expanding convoluted epithelial cords there is a distinct basal layer consisting of cuboidal or prismatic shaped epithelium (fig. 15).

Lamina propria. As the glandular units continue to expand into the lamina propria, there is an organization of the connective tissue fibers around them. Elastic fibers are making their first appearance; specifically in the subglandular region--an area between glandular units and the M. sphicter cloacae. These elastic fibers as well as the collagen and reticular fibers are interwoven with the perimysium and epimysium of the M. sphincter cloacae. Reticular fibers are abundant throughout and form a reticular lamina around the glandular units. Collagen fibers are still not as abundant in the caudal region of the lamina as they are in the cranial region (fig. 16).

Twelve Day Embryo

Gland. A definite branched appearance of the glandular units is developing as a result of continued epithelial expansion at various levels along their length. Concurrently cellular degeneration is taking place throughout the length of the internal portions of some of the glandular units (fig. 17). The glandular units are not as numerous in the caudal region of the proctodeal wall as in the cranial region; however, there are numerous epithelial buds and convoluted epithelial cords now present in the caudal region.

Lamina propria. The three types of connective tissue fibers are present. In order of abundance the fibers are: reticular, collagen and elastic. There is an indication in the subglandular region of a connective tissue organization forming a layer which runs parallel to the dorsal proctodeal epithelium (fig. 18).

Twelve and One-half Day Embryo

Gland. An increase in cellular degeneration in the center of the glandular units in the cranial region of the dorsal proctodeal wall results in the formation of small vacuoles in the portion of the glandular units nearest the proctodeal cavity (fig. 19). In the caudal region of the dorsal proctodeal wall cellular degeneration is taking place in the glandular units, although no definite vacuoles have been formed (fig. 20). Cellular degeneration is confined to the central cells of the glandular units; the distinct basal layer of cells consisting of cuboidal and prismatic shape cells shows no sign of degeneration (figs. 19, 20).

Lamina propria. Reticular and collagen fibers are abundant throughout. Elastic fibers are present only in the subglandular region. In the midline area of the subglandular region there is a cranial to caudal directional alignment of the collagen fibers (fig. 21).

Thirteen Day Embryo

Gland. Numerous vacuoles are present within the glandular units nearest the proctodeal cavity. In the most advanced areas of cellular degeneration more than one vacuole is present, but each vacuole is isolated from other vacuoles by remnants of cells that have not completely degenerated. At the proctodeal surface, epithelial caps consisting of a single layer of squamous epithelium are beginning to develop; these caps separate the vacuolizing glandular units from the proctodeal cavity. The cells of the basal layer seen at 12.5 days are now beginning to take on a flattened appearance and may range from low cuboidal to squamous in appearance (fig. 22).

Lamina propria. There has been no noticeable change in the organization of connective tissue fiber constituents, since day 12.5 of incubation (fig. 23).

Thirteen and One-half Day Embryo

Gland. The glandular units continue to increase in size due primarily to epithelial expansion. The previously isolated vacuoles of the glandular units are now coalescing to form larger vacuoles. This coalescence is more pronounced in the region closest the proctodeal cavity. The basal epithelial cells of the glandular units lining the vacuoles continue to be squamous to cuboidal. Epithelial caps have become more numerous than in the 13 day embryo (figs. 24, 25).

Lamina propria. Due to increased development of the glandular units, the lamina propria is being compressed between these units. The compressed lamina propria may be termed "embryonic interglandular septae" because at this point each glandular unit appears as a separate and distinct gland. These septae are connected to the subglandular and supraglandular (area between the glandular units and dorsal proctodeal epithelium) connective tissue regions. With continued branching of the glandular units, and the "molding" of the connective tissue covering thus produced, portions of the interglandular septae are compressed between the epithelial branches and can best be described as septal extensions into the glandular units. Only reticular and collagen fibers are present in the interglandular septae and the septal extensions (fig. 26).

Fourteen Day Embryo

Gland. A definite lumen is being formed in some of the glandular units as the coalescence of the vacuoles continues (fig. 27). Vacuolization is taking place throughout each entire glandular unit as the result of degeneration of the central cells. As the development of these glandular units continue, they begin to resemble the general structure of a branched alveolar gland; no secretory epithelium is present. The lumen of each glandular unit

remains separated from the proctodeal cavity by the thin epithelial cap described at day 13 (figs. 27, 28, 29, 30).

Lamina propria. Elastic fibers are developing in the interglandular septae, in the septal extensions and are extending into the supraglandular region. Except in the subglandular region, the concentration of elastic fibers is not equal to that of the reticular and collagen fibers. The connective tissue fibers in the subglandular region are being compacted and the fibers are becoming aligned parallel to the proctodeal surface, as the result of growth and expansion of the glandular units (fig. 31).

Fourteen and One-half Day Embryo

Gland. There has been no noticeable change in the development of the glandular units (figs. 32, 33).

Lamina propria. There has been an increase in the number of elastic fibers present. The connective tissue fibers in the supraglandular region are now also becoming aligned parallel to the dorsal proctodeal epithelium. The lamina propria associated with the glandular units is arranged in two layers, the fibers of which run parallel to the dorsal proctodeal epithelium. One layer is the supraglandular layer--between the glandular units and the dorsal proctodeal epithelium, the other is the subglandular layer--between the glandular units and the overlying musculature. These two layers are connected by the interglandular septae separating the glandular units. The two parallel layers of connective tissue fibers become continuous with the lamina propria of the non-glandular lateral region of the proctodeum without coming into contact with each other (fig. 34).

Fifteen Day Embryo

Gland. All vacuoles have almost completely coalesced forming a lumen throughout each glandular unit. Though the lumina are present, degeneration

of the epithelial cells surrounding the lumina is still taking place in the glandular units. Where there is no evidence of any further cellular degeneration within the units, the lumina are lined with a single layer of cuboidal epithelial cells. There is now no apparent difference in the degree of development of the cranial glandular units compared to that of the caudal glandular units. The epithelial caps have become quite prominent and are bulging into the proctodeal cavity (figs. 35, 36).

Lamina propria. Numerous septal extensions are present as the result of the continued branching and development of the glandular units. A prominent reticular lamina is present around the epithelium of the glandular units. There is no evidence of the connective tissue fibers infiltrating between the epithelial cells of the glandular units; however, as the branches of the glandular units continue to expand the fibers of the reticular lamina are being compressed between the expanding branches (fig. 37).

Fifteen and One-half Day Embryo

Gland. There has been no apparent change in the gland development since day 15 (figs. 38, 39).

Lamina propria. The number of elastic fibers in the supraglandular region have increased, but still are not as numerous as the reticular and collagen fibers. The elastic fibers of the supraglandular region appear to be circling the glandular units in the region of the adult "excretory canal" (fig. 40).

Sixteen Day Embryo

Gland. Cellular degeneration and the resultant vacuolization is nearly complete leaving the glandular units lined with a single layer of epithelial cells. The epithelial cells are cuboidal in shape, have a centrally located

nucleus and a cytoplasm with basophilic "granules". There is no indication of secretory activity within these cells. The glandular units themselves can now be classified as simple branched alveolar glands as illustrated from the clay model reconstruction (figs. 44, 45). The epithelial cells forming the caps appear to separate, leaving the lumen of the glandular units open to the proctodeal cavity. For each glandular unit, a small papilla is present on the dorsal proctodeal epithelium by which the duct opens into the proctodeal cavity (figs. 41, 42).

Lamina propria. All three types of connective tissue fibers are present, with reticular fibers being the most abundant, followed by collagen fibers, then elastic fibers. Between each glandular unit there is an interglandular septum with numerous septal extensions. The interglandular septae and septal extensions are comprised of reticular, collagen and elastic fibers in order of abundance. The connective tissue fibers in the lamina propria of the supraglandular and subglandular regions are organized parallel to the dorsal proctodeal epithelium, producing a connective tissue sheet covering the dorsal and ventral areas of the glandular units. A prominent reticular lamina surrounds each glandular unit. The connective tissue fibers of the lamina propria are inseparable from the epimysium and perimysium of the M. sphincter cloacae (fig. 43).

DISCUSSION

The salient and sequential points of the results of this study are summarized here as a basis for comparison of the previous studies of embryogeny of the proctodeal gland.

The first indication of glandular formation was the presence, in the 9.5 day embryo, of solid epithelial buds (gland primordia) proliferating from the dorsal proctodeal epithelium. The cells within the epithelial buds were either cuboidal or prismatic in shape, with a centrally located nucleus. From day 9.5 to day 11 there was little development in the epithelial buds; at day 11 they began to develop into solid convoluted epithelial cords. Embryos of 12 days of incubation exhibited a branching of the solid convoluted epithelial cords as a result of expansions along their lengths. Concurrently, the cells in the central portion of the branched cords (glandular units) began to show the first evidence of degeneration. This degeneration was characterized by the loss of cytoplasmic and nuclear staining. By day 12.5 degeneration had progressed to the point where all evidence of cellular structure was lost and instead discrete vacuoles were formed. By day 15, coalescence of these vacuoles resulted in formation of a lumen in each of the glandular units.

On day 13, epithelial caps had begun to form in the epithelium of the proctodeal surface. These caps, consisted of a single layer of squamous epithelium, which separated the vacuolizing glandular units from the proctodeal cavity. By the 16th day of incubation, the cells comprising these caps had separated and opened the lumen of the glandular units to the proctodeal cavity.

At day 16 the epithelial cells lining the lumina were simple cuboidal in structure and nowhere was there any evidence of the typical high columnar

secretory cells of the functional adult as described by Klemm et al. ('73); neither was there any evidence of any secretory function by these cells. It is presumed that these cells, will ultimately form future secretory cells. These epithelial cells which lined the lumina had not, at anytime, shown signs of degeneration and vacuolization which characterized other cells of the glandular units.

The studies of Perez and Sandoval ('66) and those of Nagra et al. ('59) on the embryonic development of the proctodeal gland were contradictory to each other. Based on the results of this study, it is possible to evaluate these discrepancies and clarify the questions which they posed.

Perez and Sandoval ('66) reported that development of the first glandular buds (epithelial buds) were "already significant after seven days of incubation". My results indicate that the first glandular buds were found at 9.5 days. The reported difference in time of development is 60 hours or 16 percent of total incubation time. The average time to hatch for the Coturnix is 16 to 16.5 days (Wilson et al., '61). The hatching time of our specimens which were not taken for research samples was consistently 16 to 16.5 days, indicating that the aging of the specimens used in my study were quite close to the actual incubation ages. The discrepancy of 60 hours between the first reported development of the epithelial buds by Perez and Sandoval ('66) and those reported in this study is far greater than one would expect either from individual variation or from differences in "timing" the stages of development. No explanation can be put forth for this time discrepancy.

Perez and Sandoval ('66) also stated that the gland is a derivative of the mesonephros. My results would contradict this. By definition, the mesonephros is mesodermal in origin and never extends posterior to the body

cavity. Yet the proctodeum lies beyond the body cavity and is in fact, lined with ectoderm rather than with the mesoderm which forms the lining of the body cavity (coelom). Since the epithelial buds proliferate from this proctodeal epithelium, the proctodeal gland is, exclusive of its related connective tissue, ectodermal in origin.

Finally, Perez and Sandoval ('66) state that the glandular buds develop as two individual structures, which they identify, as paracloacal or paragenital glands, located under the roof of the cloaca. These glands were reported to ultimately fuse into a single "paracloacal gland". Development of the gland is always associated with the dorsal wall of the proctodeum, not the lateral. In the earliest stage of development the buds are located lateral to the midline. However, within 36 hours epithelial buds are located across the entire dorsal wall of the proctodeum. Thus, while initial development could be considered to begin in a paracloacal position the gland itself is never paracloacal and never consists of two separate structures which later fuse in the midline into a single gland. Perhaps, Perez and Sandoval ('66) may have confused development of the proctodeal gland with development of the Corpora paracloacalis vascularis which do develop as two separate structures. Probably the major cause for confusion lies in the fact that Perez and Sandoval ('66) studied only the 7 and 14 day embryos. As the results of this study indicate, there are complex major developmental sequences occurring between days 7 and 14 and the attempt to reconstruct the sequential development of the gland, on the basis of only these two stages, produced significant problems.

According to Nagra et al. ('59) the glands began to differentiate between the 11th and 12th day of incubation as solid epithelial buds in the dorsal region of the proctodeum. These buds subsequently develop into

"spirals", during day 13 and by day 15 "a lumen is present throughout the entire gland". This developmental process is similar to that reported in this study. However, in this study the epithelial buds differentiate after 9.5 days of incubation rather than between the 11th and 12th day as reported by Nagra et al. ('59).

As my data show, initial development of the glandular tissue is confined to the craniolateral portion of the proctodeal epithelium followed in 36 hours by extensions of buds in the median region of the cranial portion of the proctodeum. With completion of this cranial development, further differentiation then progresses across the entire cloacal wall in a sequential caudal progression. If the data reported by Nagra et al. ('59) was obtained solely from cross sections made in the caudal region of the embryo, they could report first development of epithelial buds at approximately 11 days.

The description by Nagra et al. ('59) of the epithelial buds eventually developing into "spiral" epithelial cords is perhaps questionable. The use of the term "spiral" implies formation of an helical arrangement of the cords; no evidence of any such arrangement could be seen. It appeared that these cords were thrown into convolutions rather than forming spirals. This convoluting of the cords most probably results from impedence of their inward growth by the development of the overlying M. sphincter cloacae, by the extensive lamina propria, or both.

The presence of a lumen in each glandular unit by the 15th day of incubation as reported by Nagra et al. ('59) were confirmed by this study.

Tamura and Fujii ('67) state that at hatching the glandular units are slightly convoluted tubular glands. According to this study, the glandular units do pass through a stage in development (11 to 11.5 days) where they resemble convoluted tubular glands, however, at this stage of development

they are still nothing more than solid convoluted epithelial cords. Subsequently, each unit then begins to expand, to form lateral branches (day 11.5) and ultimately to conform to a simple branched alveolar gland. In fact, by serially reconstructing a glandular unit as it was at day 16, immediately prior to hatching, it could be demonstrated that the unit is a simple branched alveolar gland (fig. 45). This result correlates with the postulation of Klemm et al. ('73), based on the complex adult configuration of the gland, that the units were most probably "modified simple branched alveolar glands".

The development of the glandular units in the dorsal proctodeal wall is similar to that of the esophogeal mucus glands as described by Romanoff ('60). He states that the mucus glands first develop from buds which project into the lamina propria; a lumen is ultimately formed in the solid epithelial cords by the process of vacuolization. Finally, a modification of the esophogeal epithelium (epithelial cap) separates the lumina of the glands from the esophogeal lumen. This epithelial cap "stretches" and finally ruptures as the result of an increase in pressure from the accumulation of extracellular fluid in the lumen (Romanoff, '60). Thus, the development of glandular units of the proctodeal gland basically resembles that described for the esophogeal mucus glands. In this respect, it is possible that the epithelial caps of the proctodeal glandular units also open as a result of accumulation of extracellular fluid as suggested by Romanoff ('60). Verification of the actual mechanism which results in separation of the cells of the epithelial caps requires studies beyond the scope and intent of this study.

The overall sequence of development of the connective tissue fibers associated with the lamina propria surrounding the glandular units is as

follows: reticular, collagen and elastic. This sequence is the same as that stated by Copenhaver et al. ('71) for the usual sequential development of connective tissue fibers in the human embryo. Reticular fibers were already present in large numbers in the lamina propria of the dorsal proctodeal wall by 9.5 days of incubation. A "collagen-like" staining reaction appeared at 10.5 days of development in the amorphous ground substance in the cranial region of the dorsal proctodeal wall. Individual collagen fibers, however, could not be identified. The possibility exists that the reaction taking place might be associated with tropocollagen macromolecules (Copenhaver et al. '71), which have not yet polymerized to form collagen fibers. Collagen fibers do become present throughout the lamina propria by 11.5 days of incubation, with development beginning cranially and ultimately progressing simultaneously laterally and caudally.

The elastic fibers first become present in the subglandular region of the lamina propria on day 11.5 of incubation. These elastic fibers appear to be a continuation of those which first appeared in association with M. sphincter cloacae. Copenhaver et al. ('71) state that elastic fibers develop as the result of terminal accretion of additional individual elastic tissue units upon an original elastic unit. This would explain how the fibers tend to grow ventrad from region of association with the M. sphincter cloacae. Elastic fibers in the supraglandular region of the lamina propria appear to be circling the region of the future "adult excretory canal" in the 15.5 day embryo. This observation correlates with that of Klemm et al. ('73), who reported that elastic fibers encircle the base of the excretory canal in the adult bird.

Terminology of the various portions of the connective tissue structures of the lamina propria associated with the glandular units is a problem.

Klemm et al. ('73) defined the adult connective tissue configuration as one which forms a connective tissue capsule surrounding the entire gland complex. This capsule then forms intraglandular septae between the individual units. In the adult, connective tissue fibers pass from the intraglandular septae into "primary and secondary folds", as defined by Klemm et al. ('73), of the secretory epithelium of the unit.

In the embryo there is no evidence of any connective tissue capsule surrounding the mass of glandular units, uniting them into a single gland of the "aggregate type" (Tamura and Fujii, '67; Klemm et al., '73). Therefore, since at hatching there is no limiting capsule associated with the embryonic proctodeal gland, verification of the classification of the gland as an "aggregate gland" cannot be made. Instead the dorsal proctodeal wall at this time in development is composed of what can be stated as numerous separate proctodeal glands which differ little from those found in the esophagus. The esophogeal glands are usually grouped together in most histological classifications as a "multiple gland". The appearance of the proctodeal gland at hatching does not provide a basis for confirming or rejecting classification of the adult gland. This is especially true since hormonal influence have been reported to further influence the anatomic configuration (Sachs, '67; Nagra et al., '59). Confirmation of classification as an "aggregate gland", or the existance of primary and secondary folds, or of the formation and terminology of the septal arrangement as reported by Tamura and Fujii ('67) or Klemm et al. ('73) remains difficult. Therefore, at the time of hatching the "gland" is in fact only an accumulation of separate glandular units (= multiple gland) with interglandular septae between them and septal extensions between the alveolar-like expansions (branches) of these units. The undeveloped gland (functionally) at hatching does not show the primary

and secondary folds described by Klemm et al. ('73). However, there are indications of what may develop into the primary and secondary folds. As the glandular units continue to expand one can speculate that the septal extensions will be further compressed between the branches of the glandular units and form primary septae. In addition, the reticular lamina which was located distal to the septal extensions and between the expanding branches was compressed and "isolated" so as to form the anlagè of what may become the "secondary fold" described by Klemm et al. ('73). As evidence of this hypothesis Klemm et al. ('73) state that the secondary folds consist primarily of reticular fibers.

This study has expanded the knowledge of the sequential development of the "proctodeal gland" and its association with the enveloping lamina propria. The structure of the "proctodeal gland" at hatching differs structurally from that described for both the adult male and female. Whether the maturation process alters the configuration seen at day 16 to that described for the adult will require additional study of the maturation process. It is believed that this study has resolved the differences in developmental patterns as previously reported by Nagra et al. ('59), Perez and Sandoval ('66) and Tamura and Fujii ('67); in so doing, however, questions have been raised which will require analysis of the post-hatching development.

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FIGURES

Key to figure abbreviations

a = allantois
b = solid epithelial bud
bf = Bursa of Fabricius
br = branch of glandular unit
c = collagen fiber
CA = caudal
ce = convoluted epithelial cords
co = coprodeum
CR = cranial
D = dorsal
dg = degenerating cell
e = elastic fiber
ee = epithelial expansion
es = epithelial caps
g = glandular unit
is = embryonic interglandular septa
lp = lamina propria
lu = lumen of glandular unit
m = M. sphincter cloacae
p = proctodeum
po = pore of glandular unit
r = reticular fiber
rl = reticular lamina
s = septal extension
sb = subglandular connective tissue region
sp = supraglandular connective tissue region
u = urodeum
V = ventral
va = vacuole
vt = vertebra

EXPLANATION OF FIGURES

Fig. 1. Transverse section of 9.5 day Common Coturnix embryo. First sign of proctodeal gland formation is the presence of epithelial buds on the craniolateral portion of the dorsal proctodeal wall. Cells of the epithelial buds are cuboidal or prismatic in shape. (H & E, X90; insert, H & E, X1140)

Fig. 2. Sagittal section of 9.5 day Common Coturnix embryo. First sign of glandular formation is the presence of epithelial buds on the craniolateral portion of the dorsal proctodeal wall. (H & E, X114; insert, H & E, X730)

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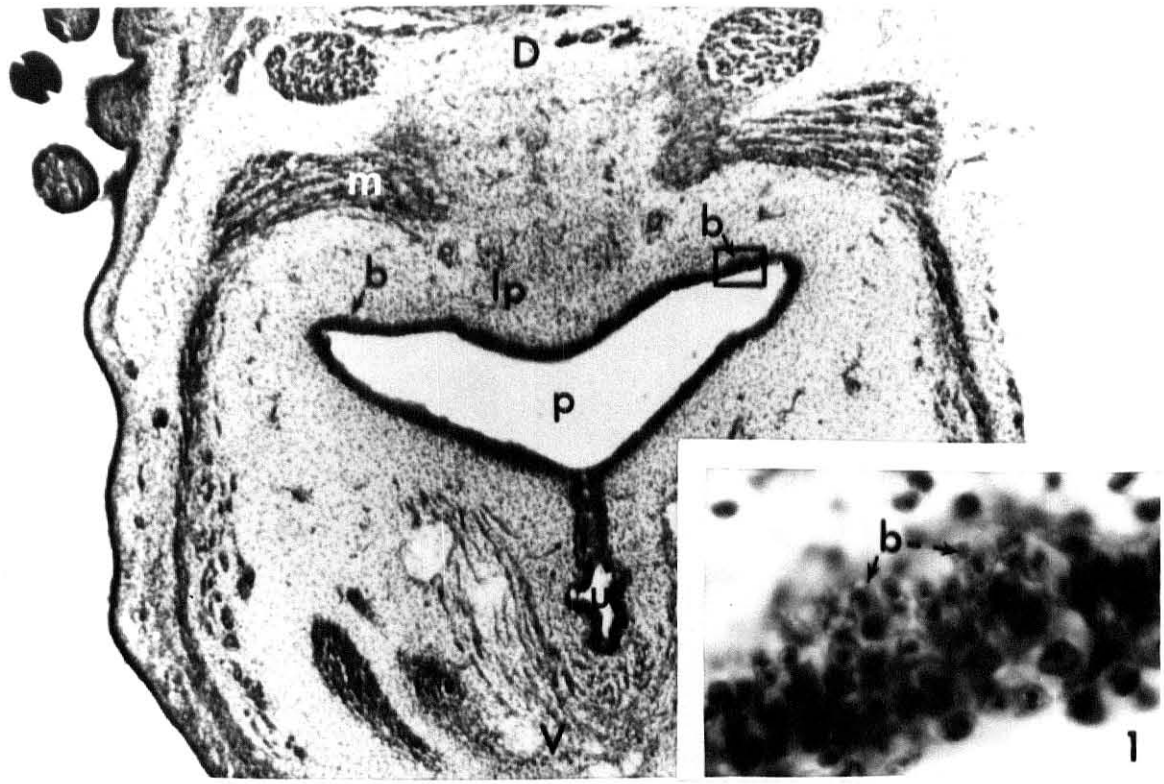
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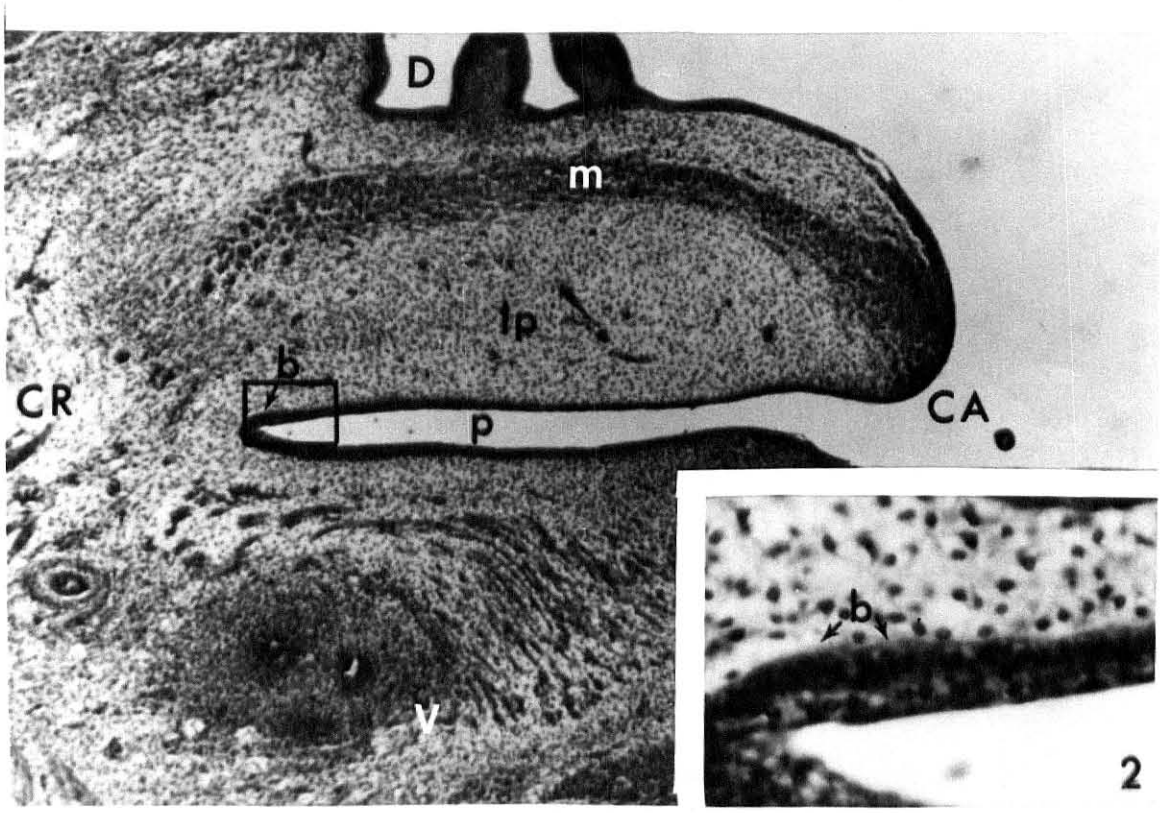
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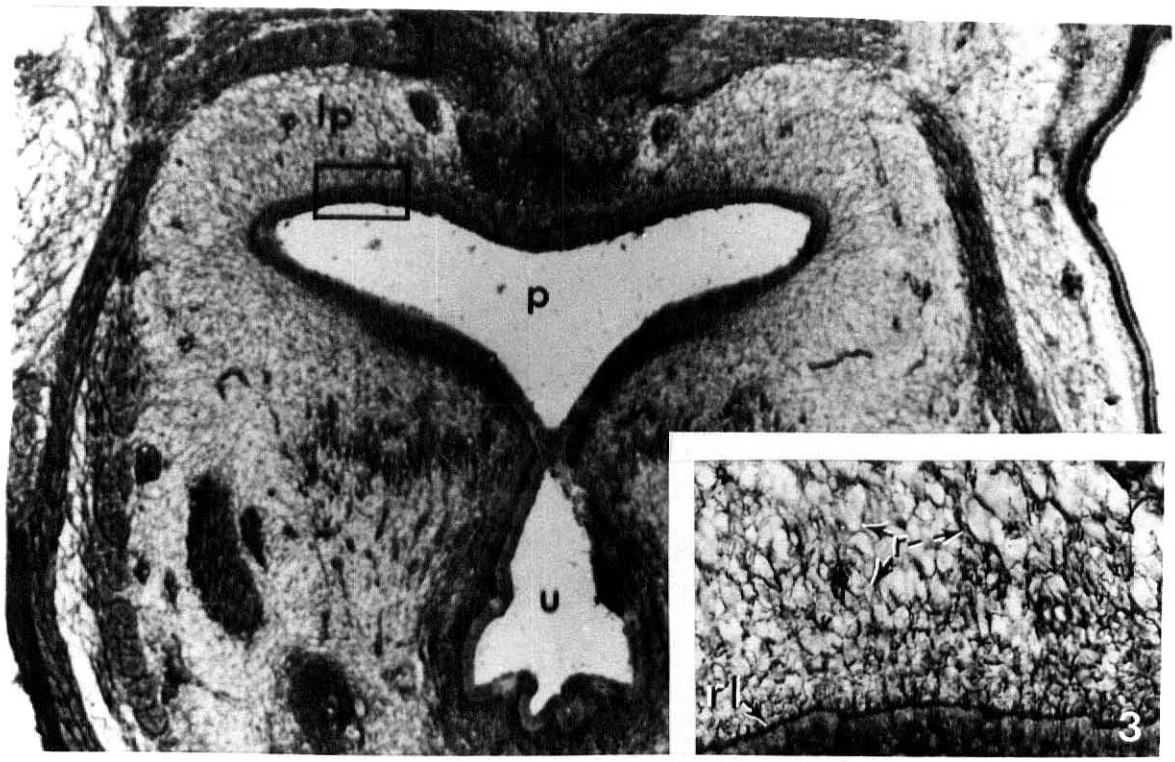
EXPLANATION OF FIGURES

Fig. 3. Transverse section of 9.5 day Common Coturnix embryo. Reticular fibers are located throughout the lamina propria. They are most abundant cranially in the median region of the lamina propria. Insert shows fine structure and netlike appearance of the reticular fibers. Beneath the solid epithelial buds and dorsal proctodeal epithelium is a distinct layer of reticular fibers (reticular lamina). (C.T. Stain, X114; insert, C.T. Stain, X456)

Fig. 4. Transverse section of 9.5 day Common Coturnix embryo. The lamina propria of the dorsal proctodeal wall consists only of reticular fibers. There is a concentration of the reticular fibers in the median region of the lamina propria. (C.T. Stain, X228)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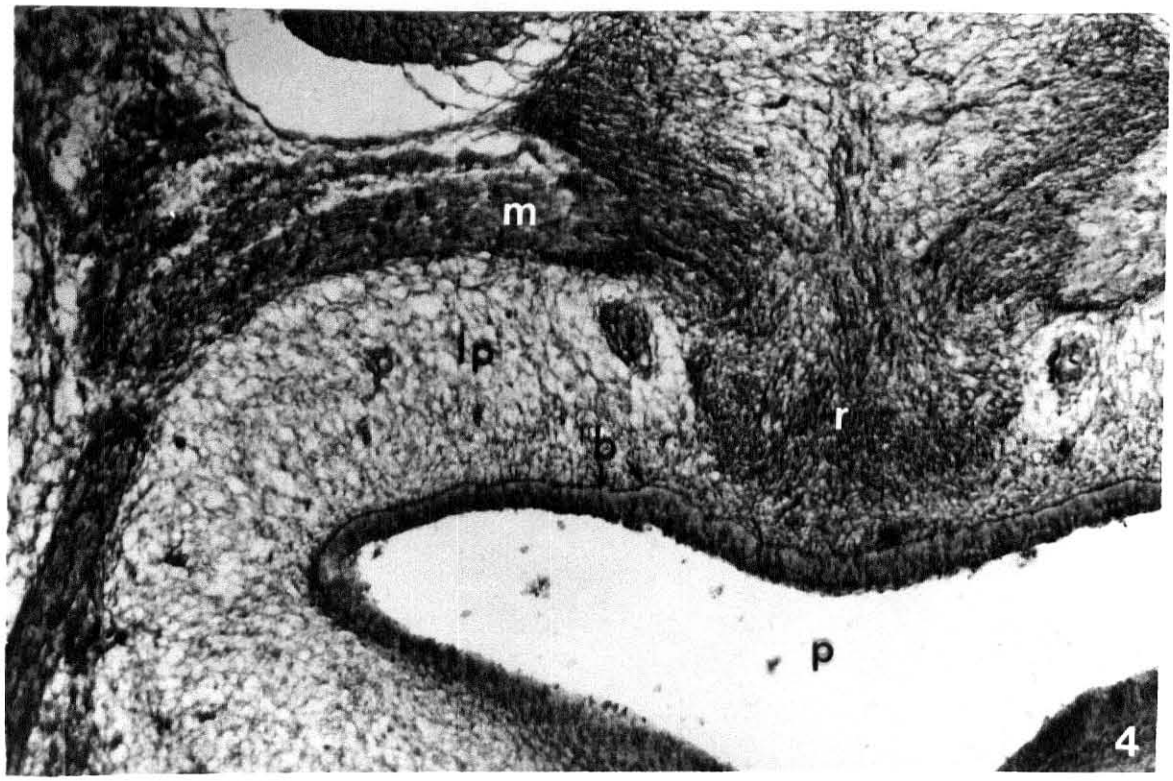
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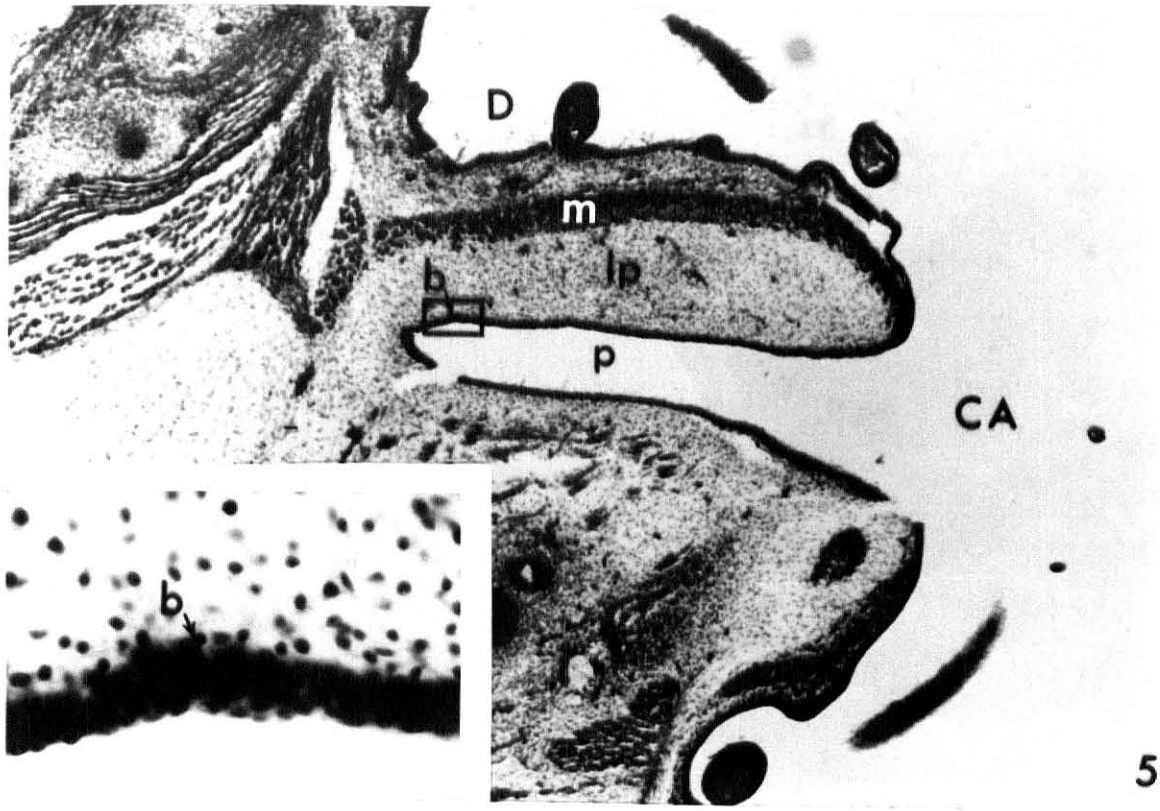
EXPLANATION OF FIGURES

Fig. 5. Sagittal section of 10 day Common Coturnix embryo. These are epithelial buds located in the craniolateral region of the dorsal wall of the proctodeum. No epithelial buds are located in the caudal region. (H & E, X71; insert, H & E, X730)

Fig. 6. Median section of 10 day Common Coturnix embryo. There are no epithelial buds present in the median region of the dorsal proctodeal wall. (H & E, X71; insert, H & E, X730)



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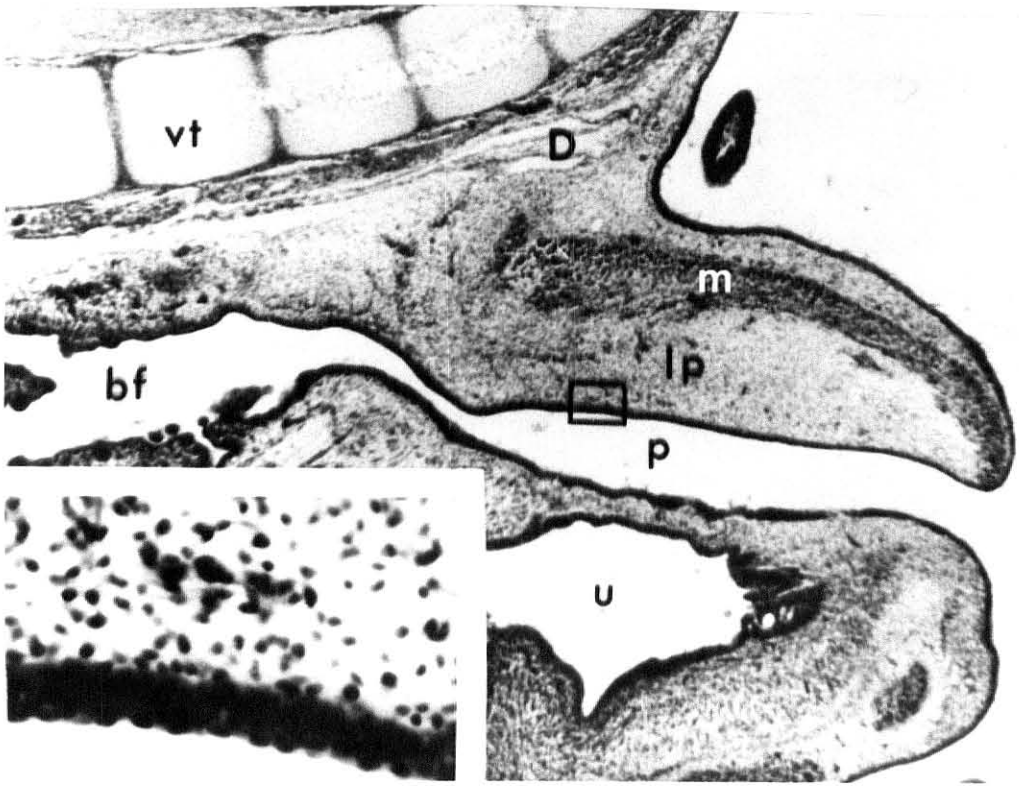

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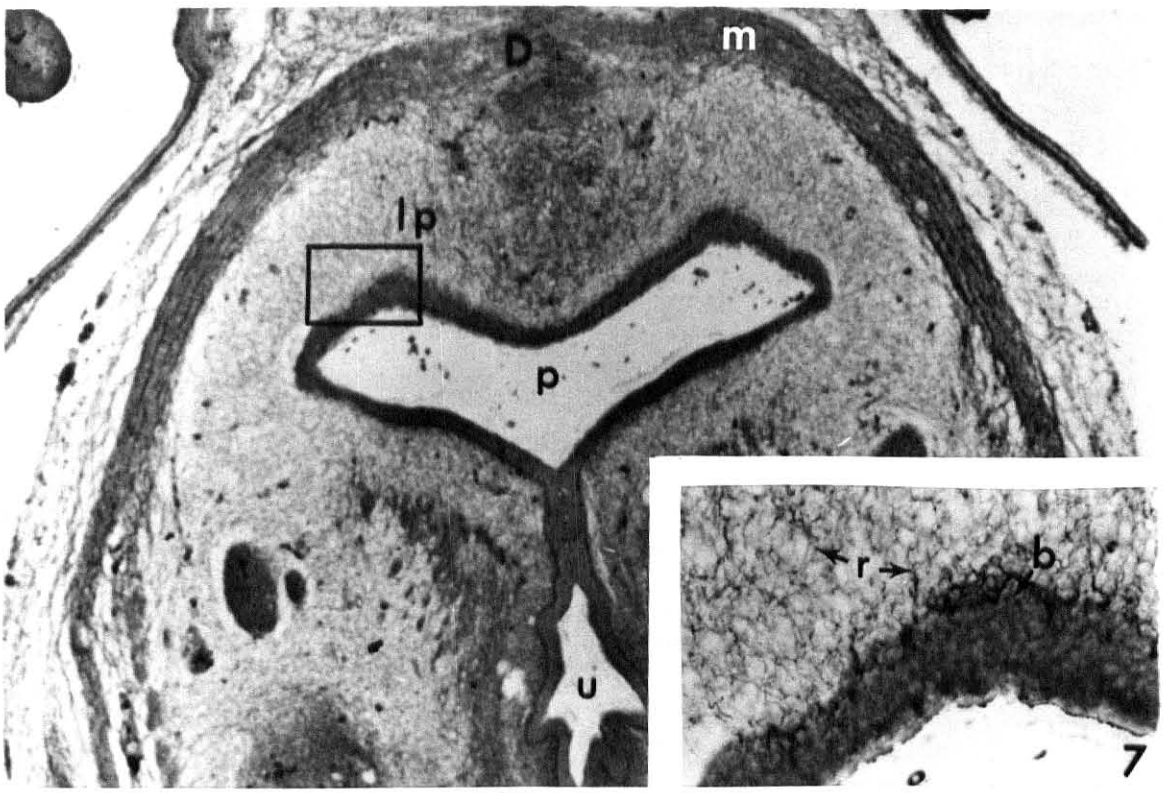
EXPLANATION OF FIGURES

Fig. 7. Transverse section of 10 day Common Coturnix embryo. Reticular fibers are located throughout the lamina propria. There is a reticular lamina present around the epithelial buds. Collagen and elastic fibers are not present. (C.T. Stain, X114; insert, C.T. Stain, X456)

Fig. 8. Transverse section of 10.5 day Common Coturnix embryo. There has been an increase in the number of epithelial buds in craniolateral region of the dorsal proctodeal wall. (H & E, X71; insert, H & E, X350)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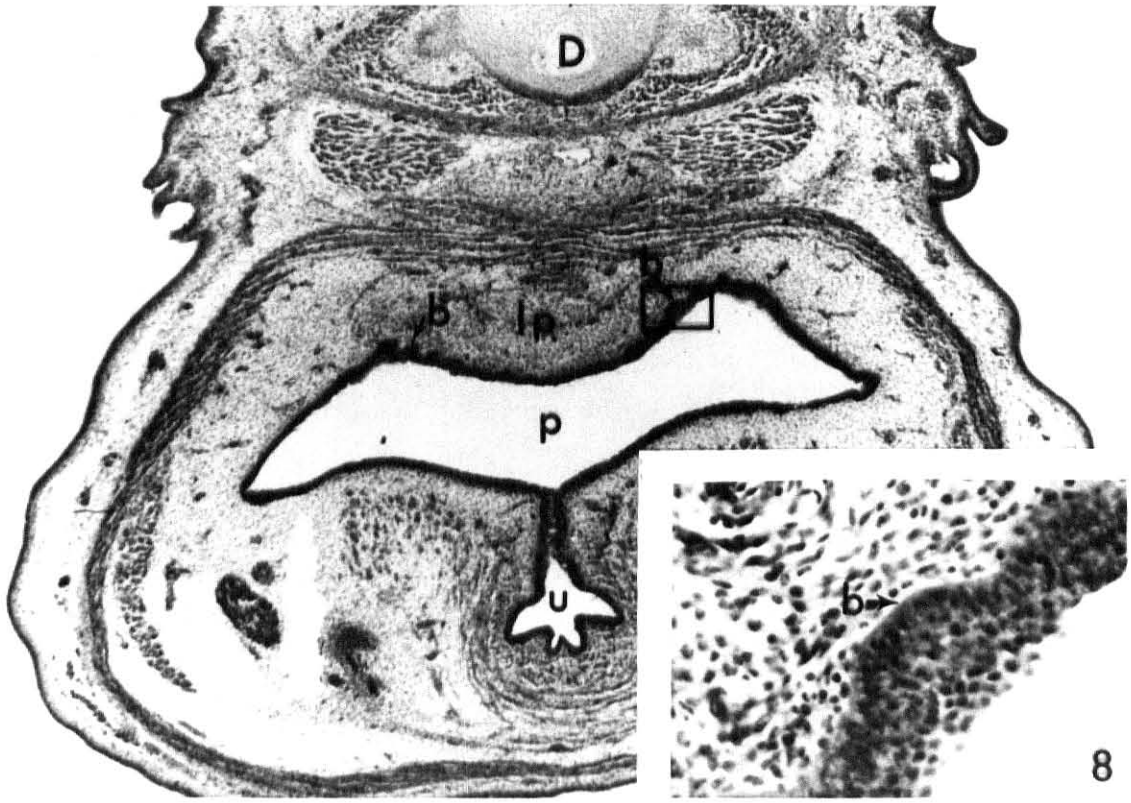
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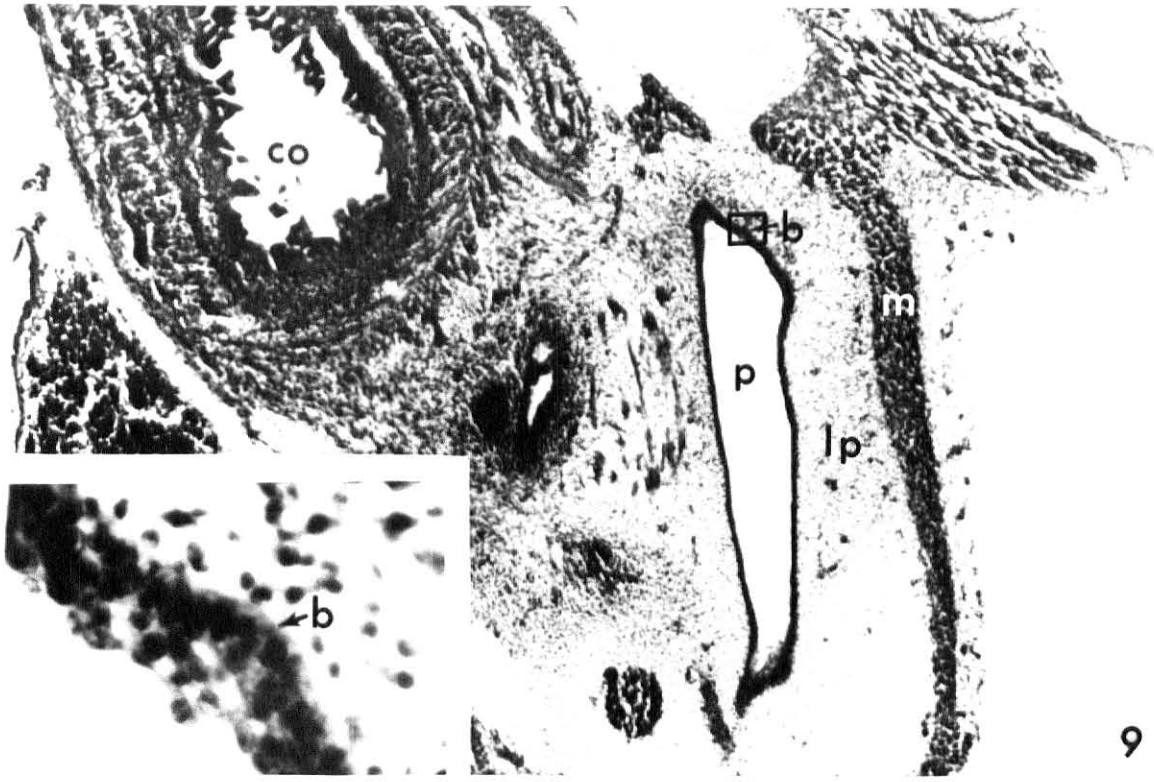
EXPLANATION OF FIGURES

Fig. 9. Sagittal section of 10.5 day Common Coturnix embryo. Epithelial bud located in craniolateral region of the dorsal proctodeal wall. (H & E, X91; insert, H & E, X912)

Fig. 10. Median section of 10.5 day Common Coturnix embryo. No epithelial buds are present in the median region of the dorsal proctodeal wall. (H & E, X71; insert, H & E, X456)



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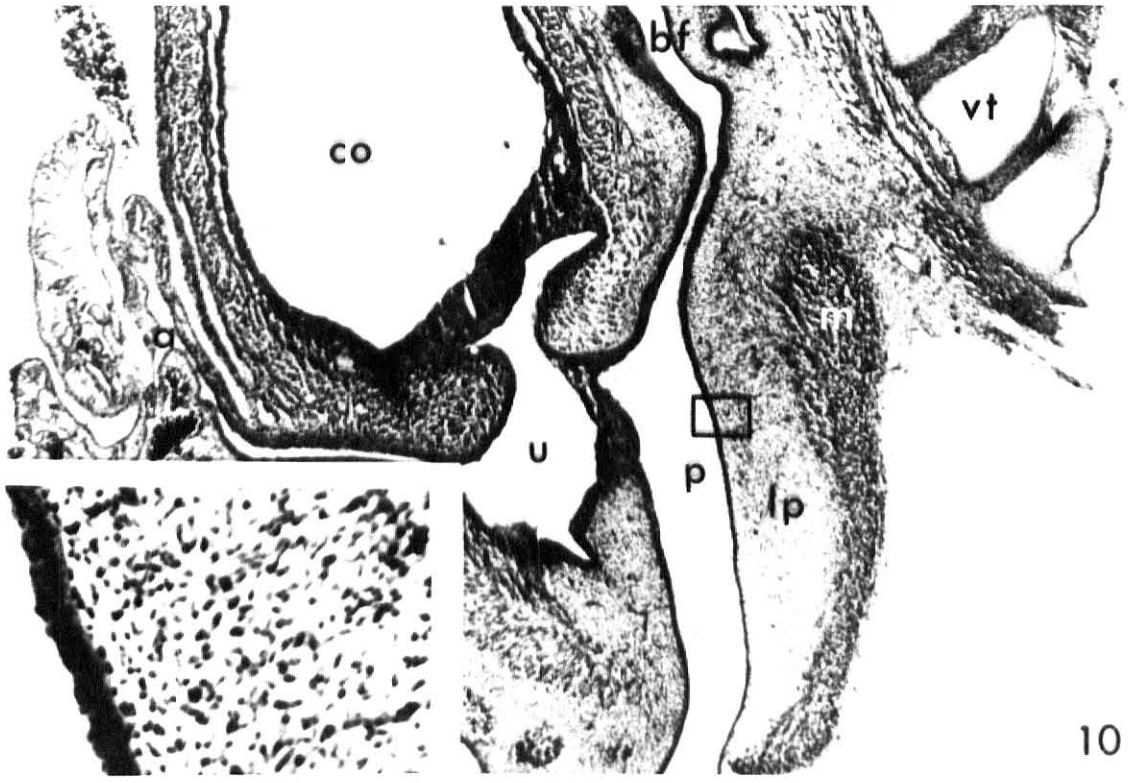

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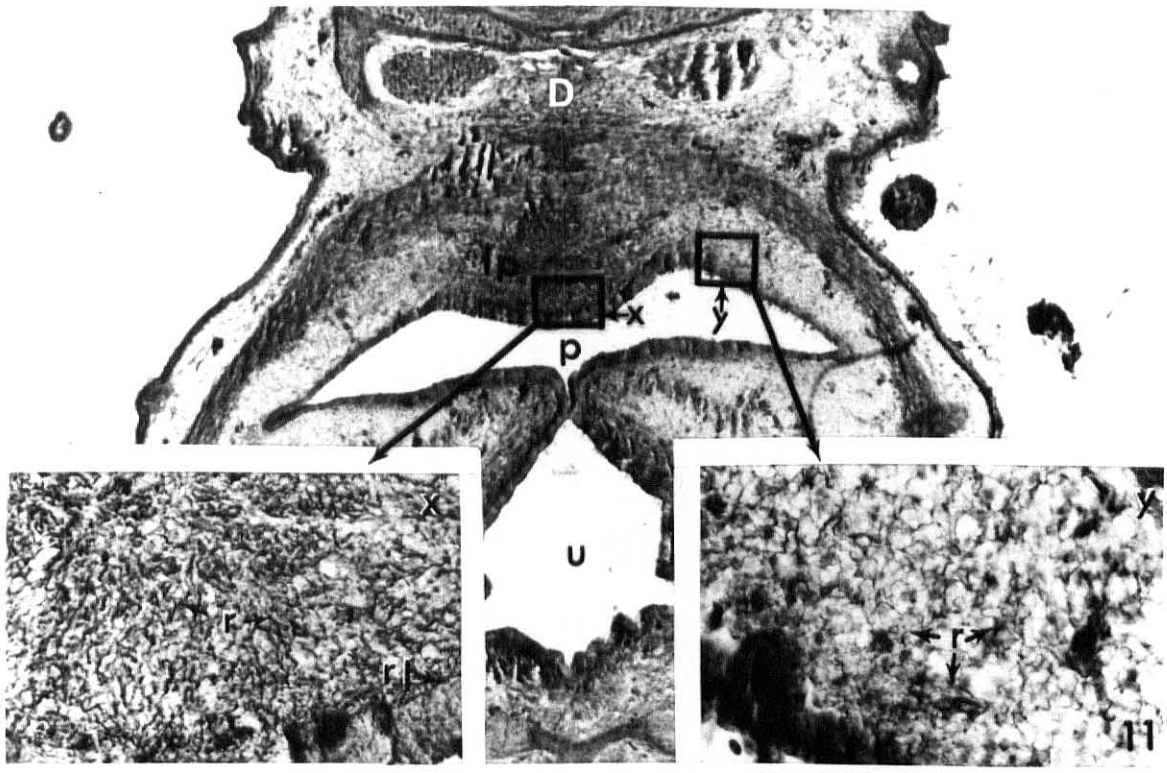
EXPLANATION OF FIGURES

Fig. 11. Transverse section of 10.5 day Common Coturnix embryo. Reticular fibers have become more numerous in the lamina propria. Substances which react to a typical collagen stain are present, but collagen fibers cannot be identified under the light microscope. (C.T. Stain, X71; insert x, C.T. Stain, X456; insert y, C.T. Stain, X456)

Fig. 12. Transverse section of 11 day Common Coturnix embryo. Solid epithelial buds have elongated and are forming solid convoluted epithelial cords. Epithelial buds are very prominent in the dorsal wall of the proctodeum. (H & E, X71; insert x, H & E, X456; insert y, H & E, X456)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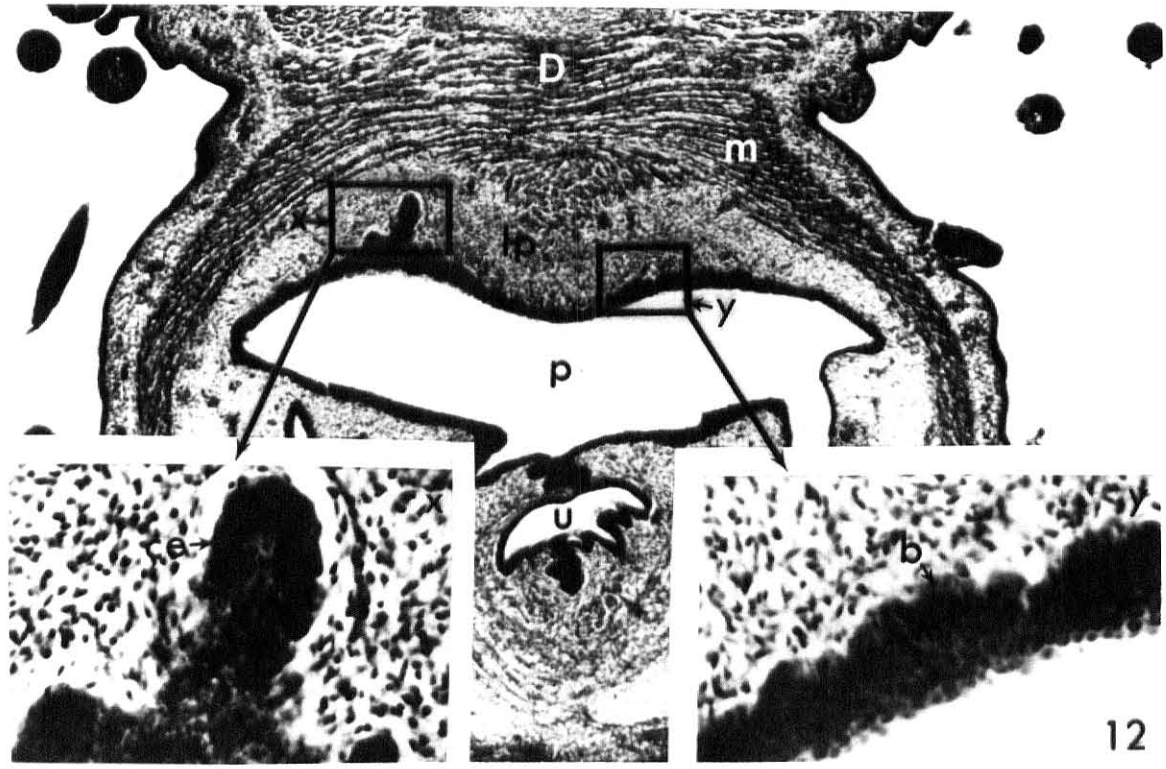
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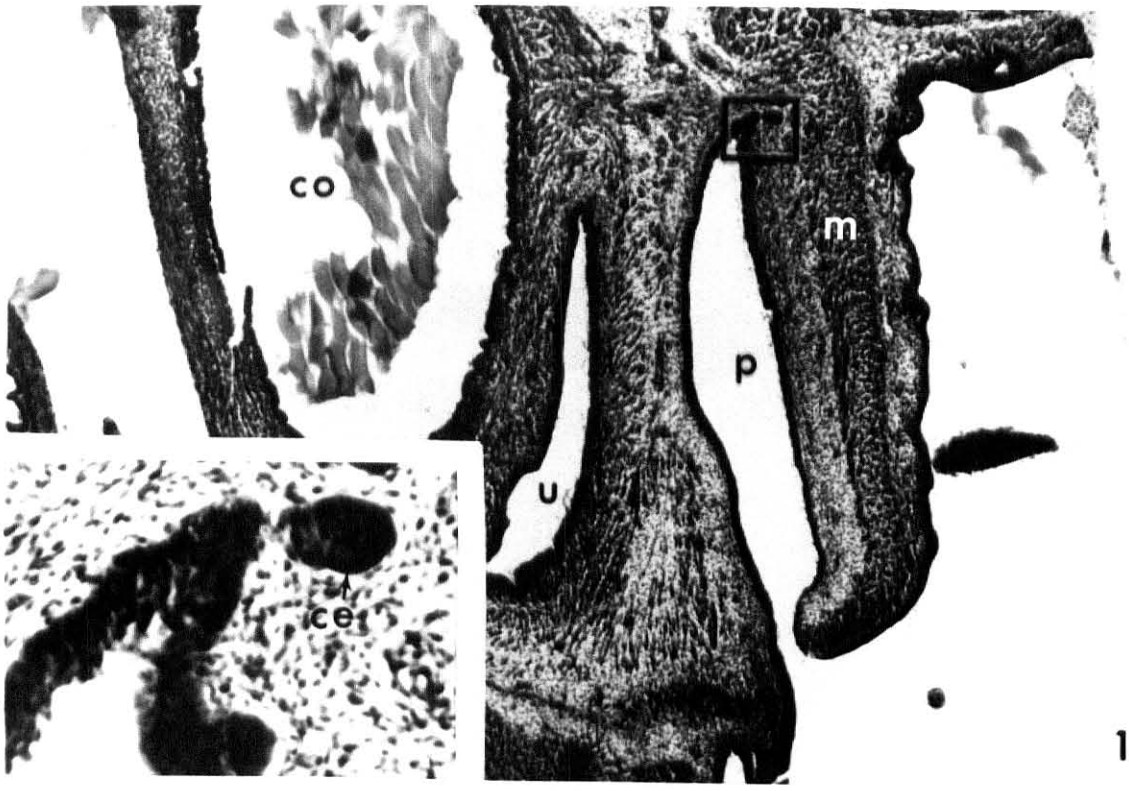
Fig. 13. Sagittal section of 11 day Common Coturnix embryo. Solid convoluted epithelial cords are developing in the craniolateral region of the dorsal proctodeal wall. (H & E, X71; insert, H & E, X456)

Fig. 14. Transverse section of 11 day Common Coturnix embryo. Reticular and collagen fibers are present in the lamina propria. Elastic fibers are present in the M. sphincter cloacae. There is an alignment of the connective tissue fibers around the solid convoluted epithelial cords. (C.T. Stain, X228; insert, C.T. Stain, X456)



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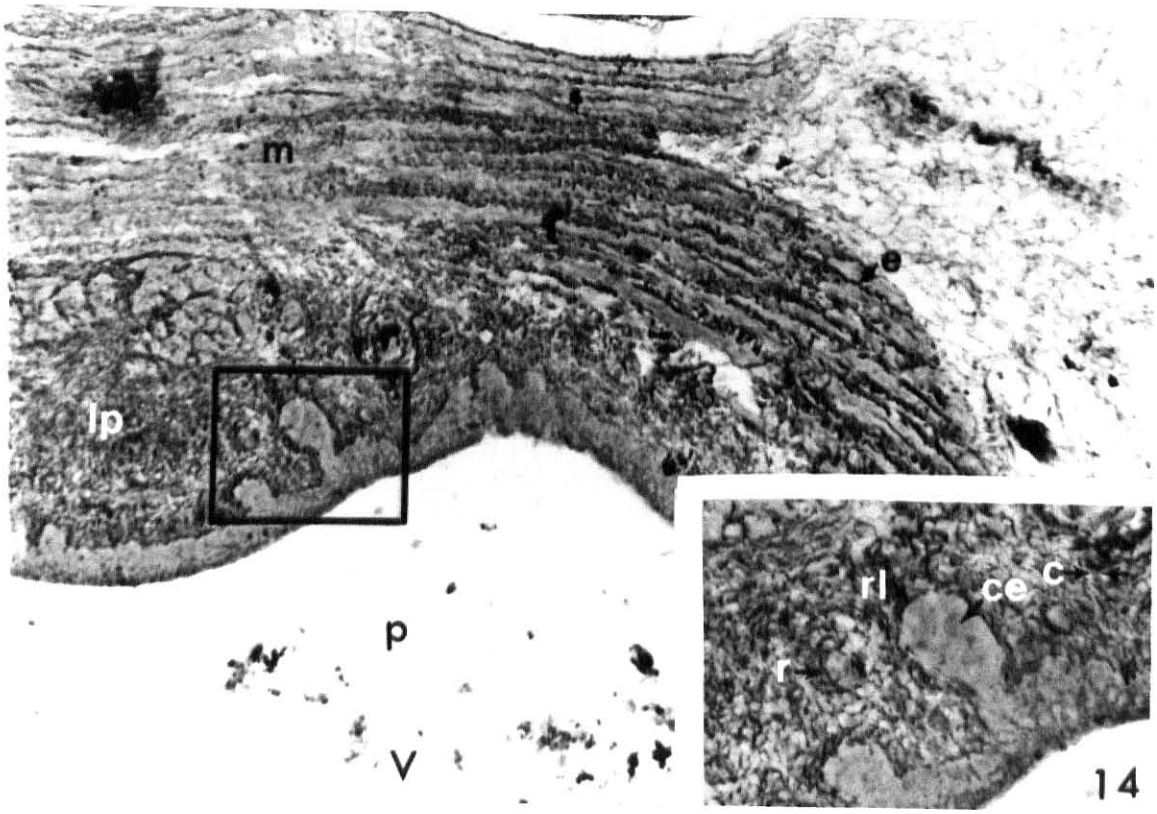

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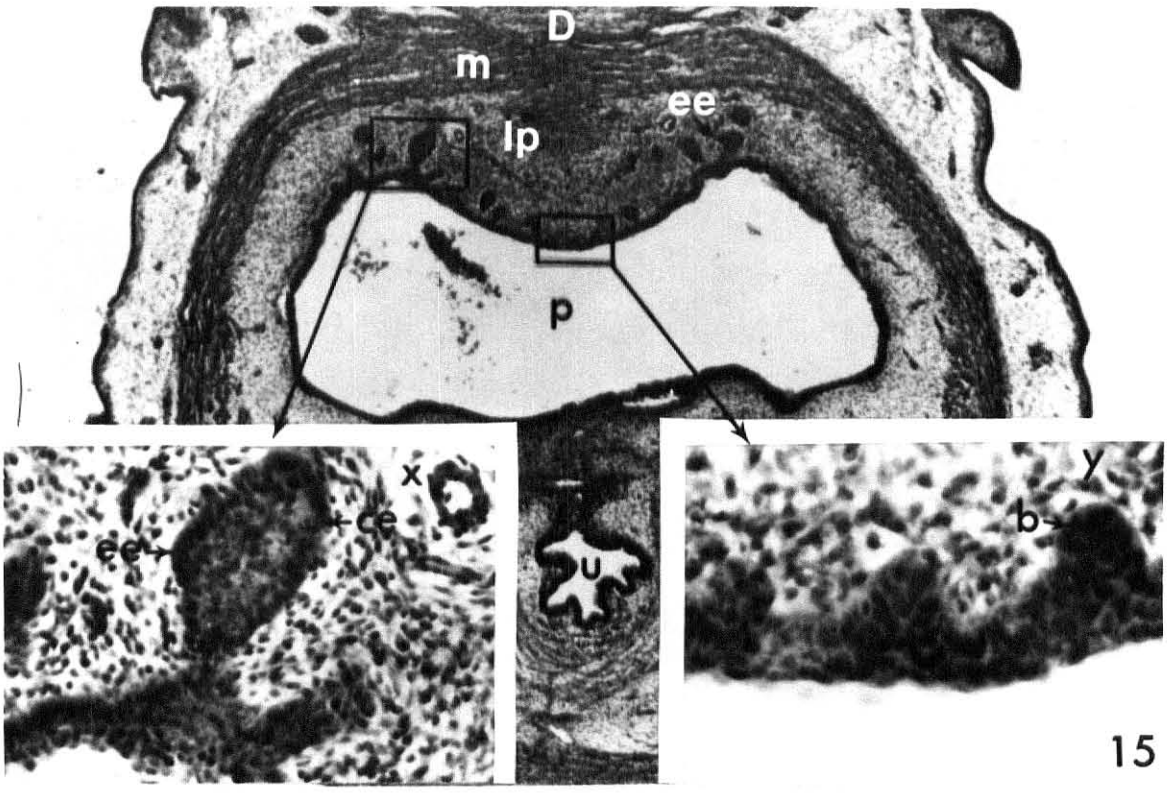
EXPLANATION OF FIGURES

Fig. 15. Transverse section of 11.5 day Common Coturnix embryo. Convoluted epithelial cords and epithelial buds are present throughout the entire dorsal proctodeal wall. The epithelial cords are beginning to develop a branched appearance as the result of expansions along their length. (H & E, X57; insert x, H & E, X730; insert y, H & E, X730)

Fig. 16. Transverse section of 11.5 day Common Coturnix embryo. Reticular and collagen fibers are present throughout the lamina propria. Elastic fibers are making their first appearance in the lamina propria. (C.T. Stain, X71; insert x, C.T. Stain, X456; insert y, C.T. Stain, X456)



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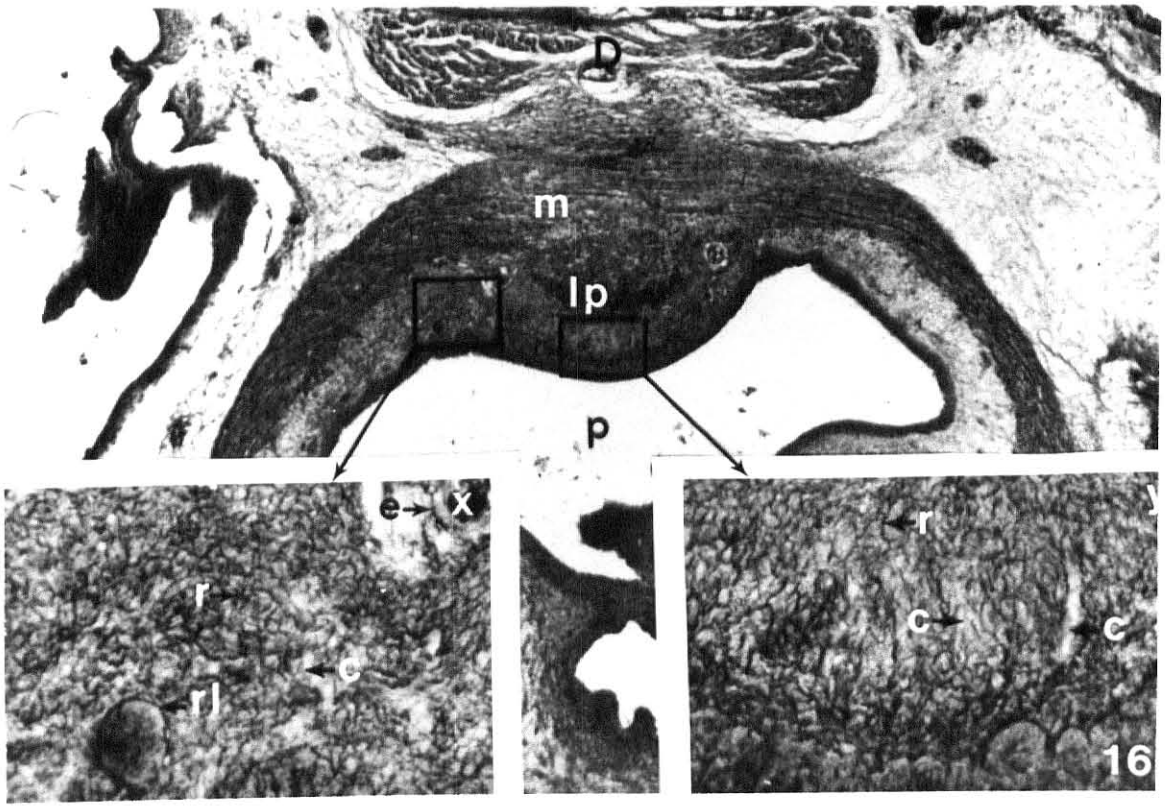

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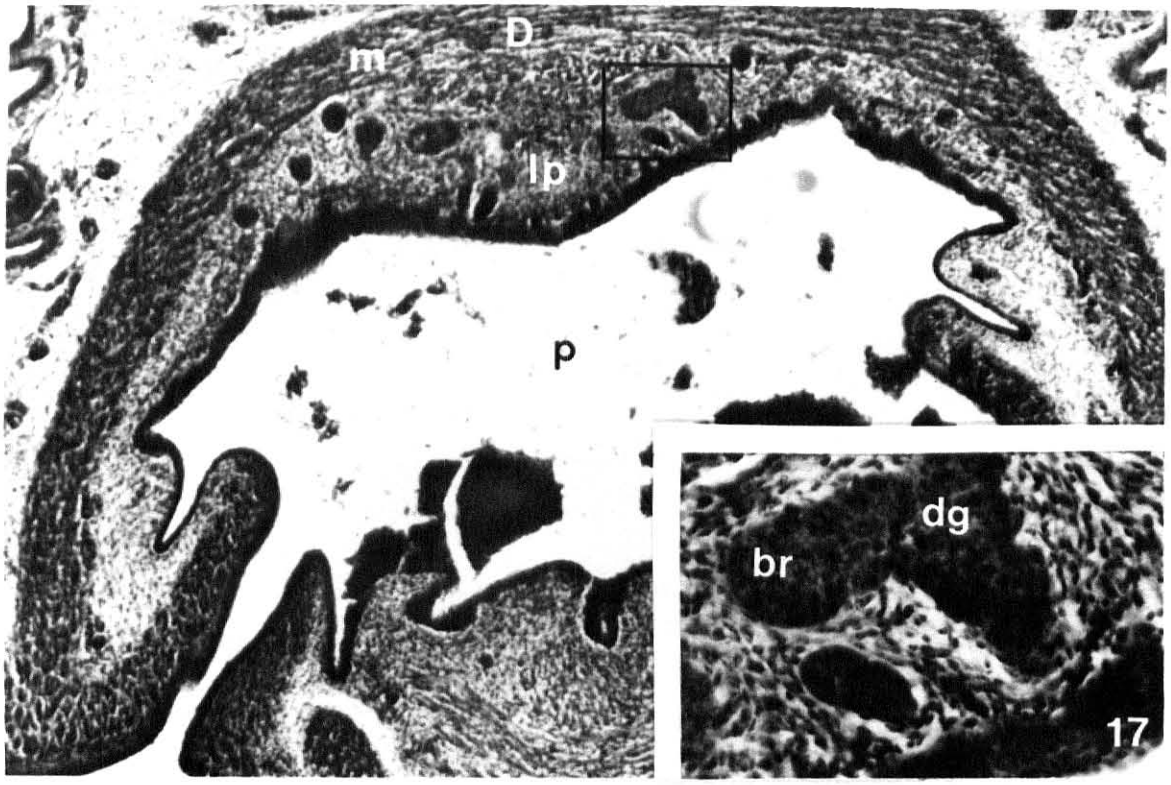
EXPLANATION OF FIGURES

Fig. 17. Transverse section of 12 day Common Coturnix embryo. A branching appearance of the glandular units is developing as a result of continued epithelial expansion. Cellular degeneration is taking place in the core of the glandular units. (H & E, X71; insert, H & E, X456)

Fig. 18. Transverse section of 12 day Common Coturnix embryo. Reticular and collagen fibers are located throughout the lamina propria. Elastic fibers are only present in subglandular region of the lamina propria. (C.T. Stain, X91; insert C.T. Stain, X456)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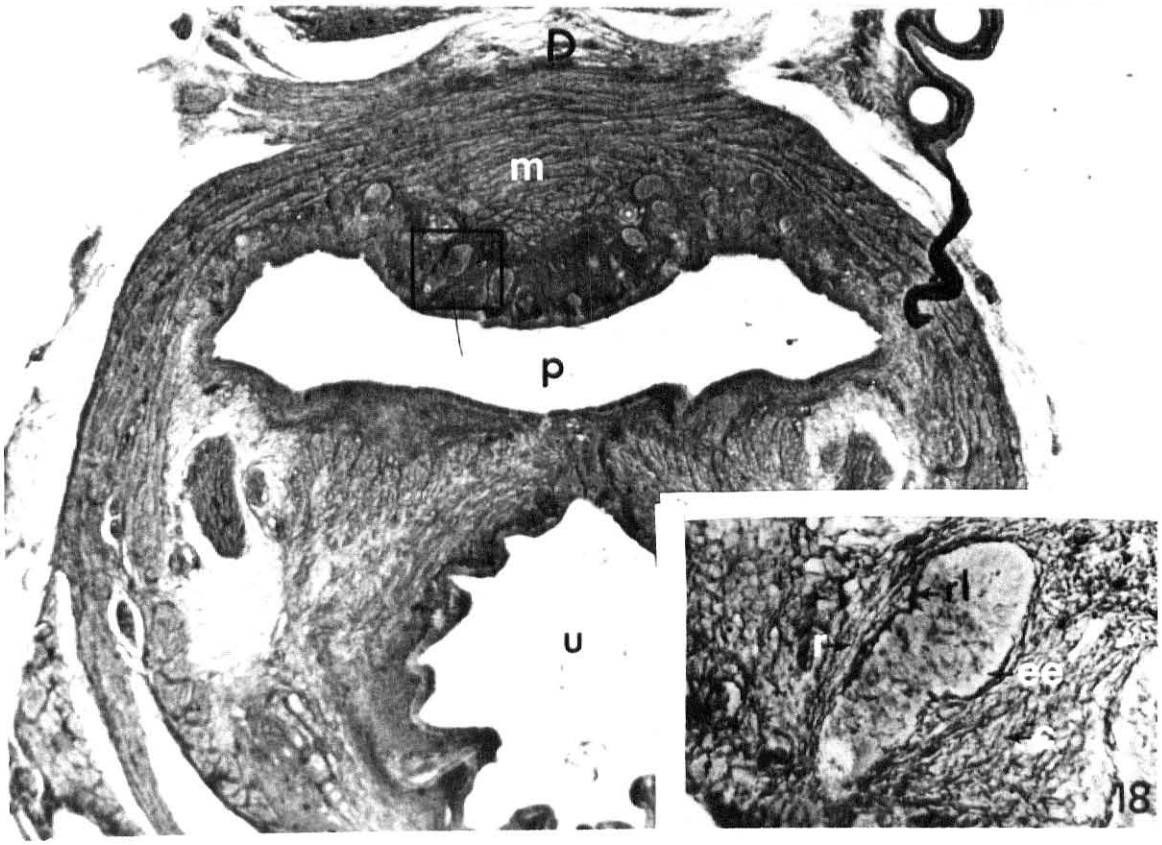
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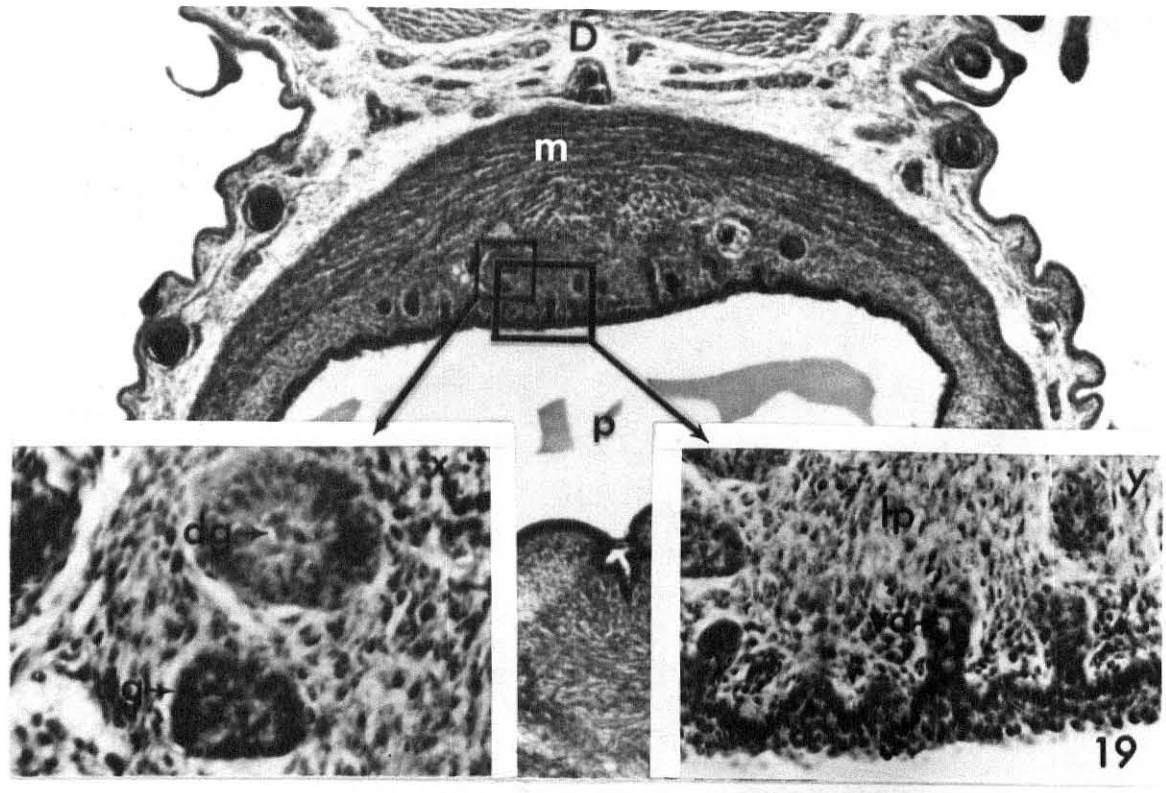
EXPLANATION OF FIGURES

Fig. 19. Transverse section of 12.5 day Common Coturnix embryo. There is an increase in cellular degeneration in the center of the glandular units nearest the proctodeal cavity. Basal layers of cells do not show signs of degeneration. (H & E, X71; insert x, H & E, X730; insert y, H & E, X456)

Fig. 20. Sagittal section of 12.5 day Common Coturnix embryo. Cellular degeneration is taking place in the unit throughout the entire dorsal proctodeal wall. (H & E, X71; insert, H & E, X456)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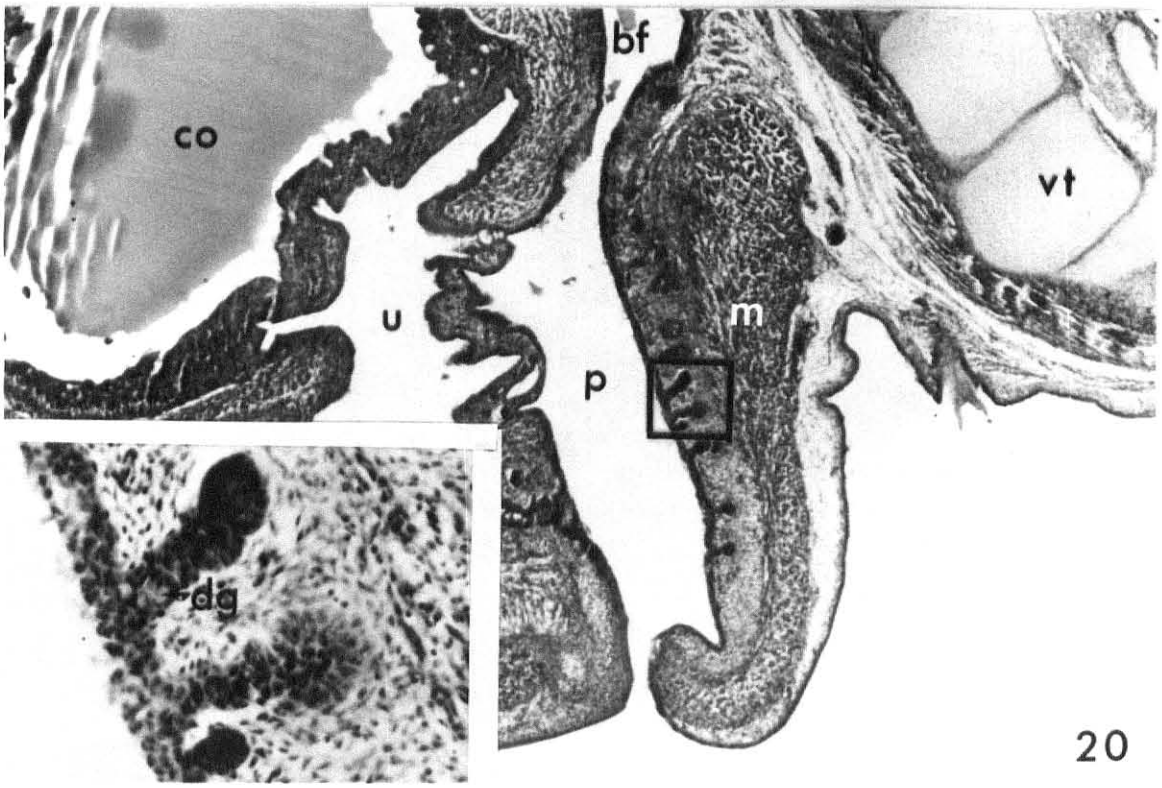
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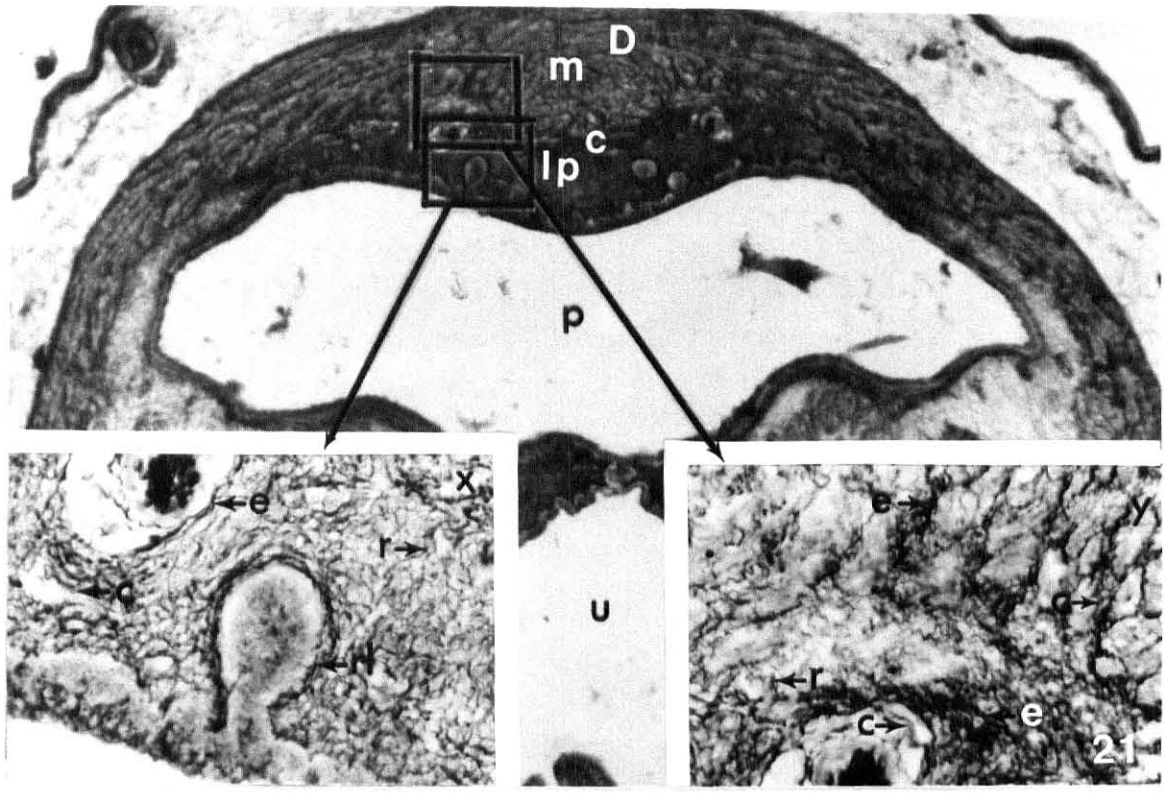
EXPLANATION OF FIGURES

Fig. 21. Transverse section of 12.5 day Common Coturnix embryo. Reticular and collagen fibers are located throughout the lamina propria. Elastic fibers are located only in the subglandular region of the lamina propria. There is a cranial to caudal directional alignment of the collagen fibers in the midline of the subglandular region. (C.T. STain, X71; insert x, C.T. Stain, X456; insert y, C.T. Stain, X456)

Fig. 22. Sagittal section of 13 day Common Coturnix embryo. Vacuoles are present in the glandular units; the majority of the vacuoles are located in the region of the glandular units nearest the proctodeal cavity. Epithelial caps are present. (H & E, X71; insert, H & E, X456)



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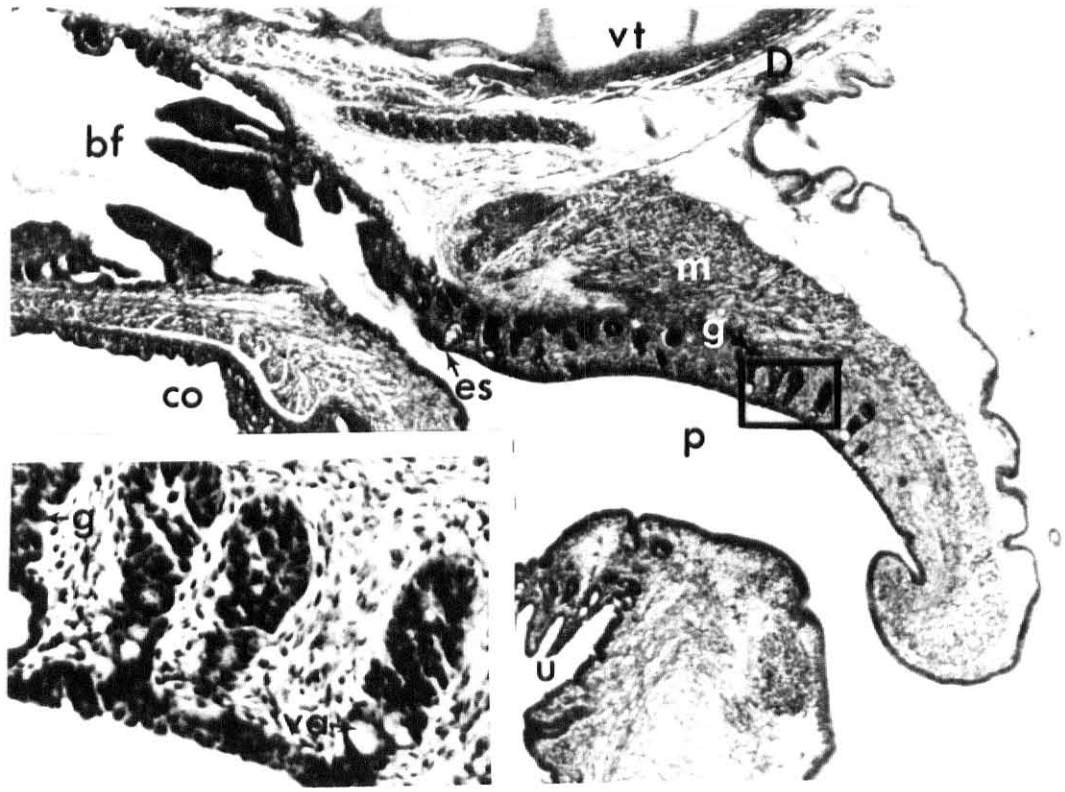

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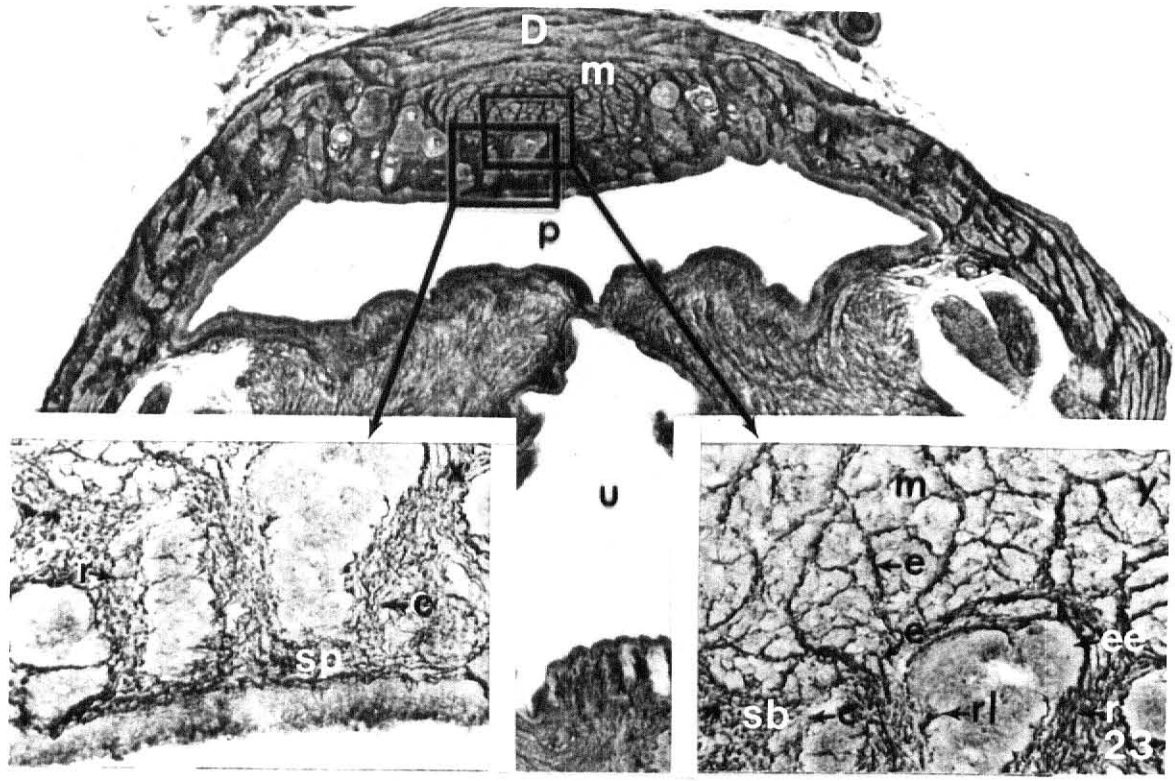
EXPLANATION OF FIGURES

Fig. 23. Transverse section of 13 day Common Coturnix embryo. All three types of connective tissue fibers are located in the subglandular region. Elastic fibers are located only in the subglandular region of the lamina propria. (C.T. Stain, X456)

Fig. 24. Transverse section of 13.5 day Common Coturnix embryo. There has been an increase in epithelial expansion along the lengths of the glandular units. The vacuoles are coalescing to form larger vacuoles. Epithelial caps are present. (H & E, X91; insert, X456)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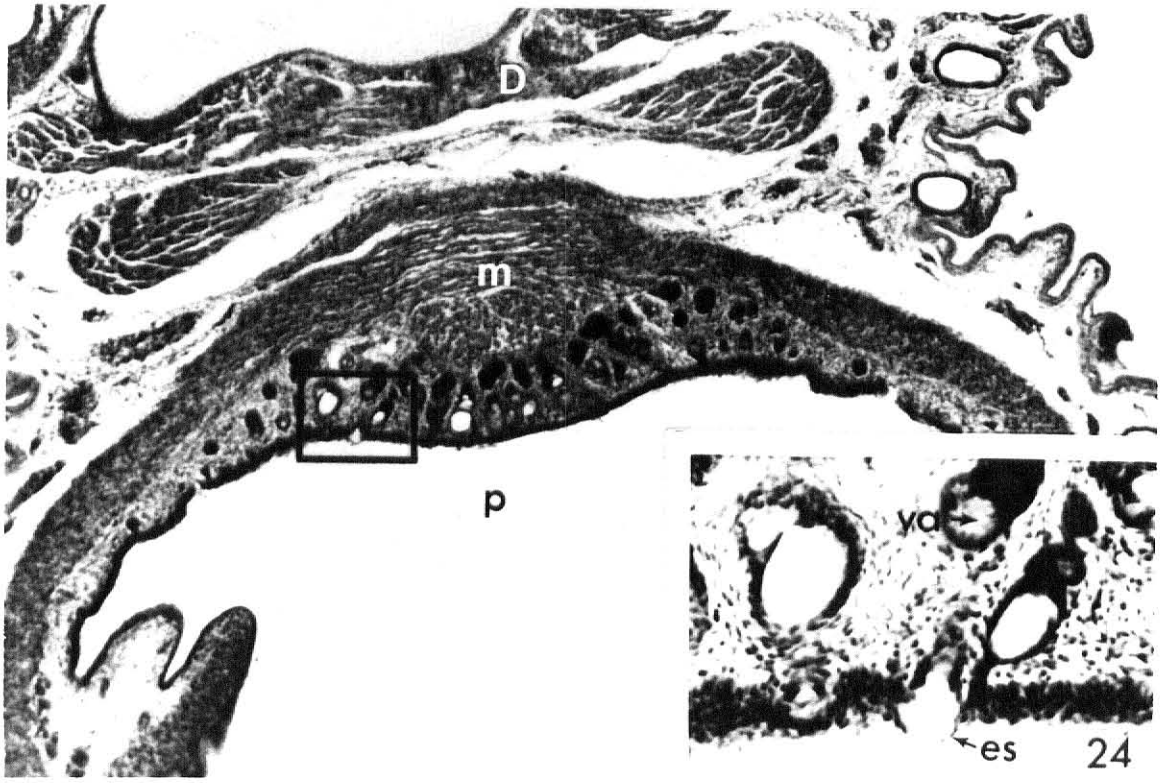
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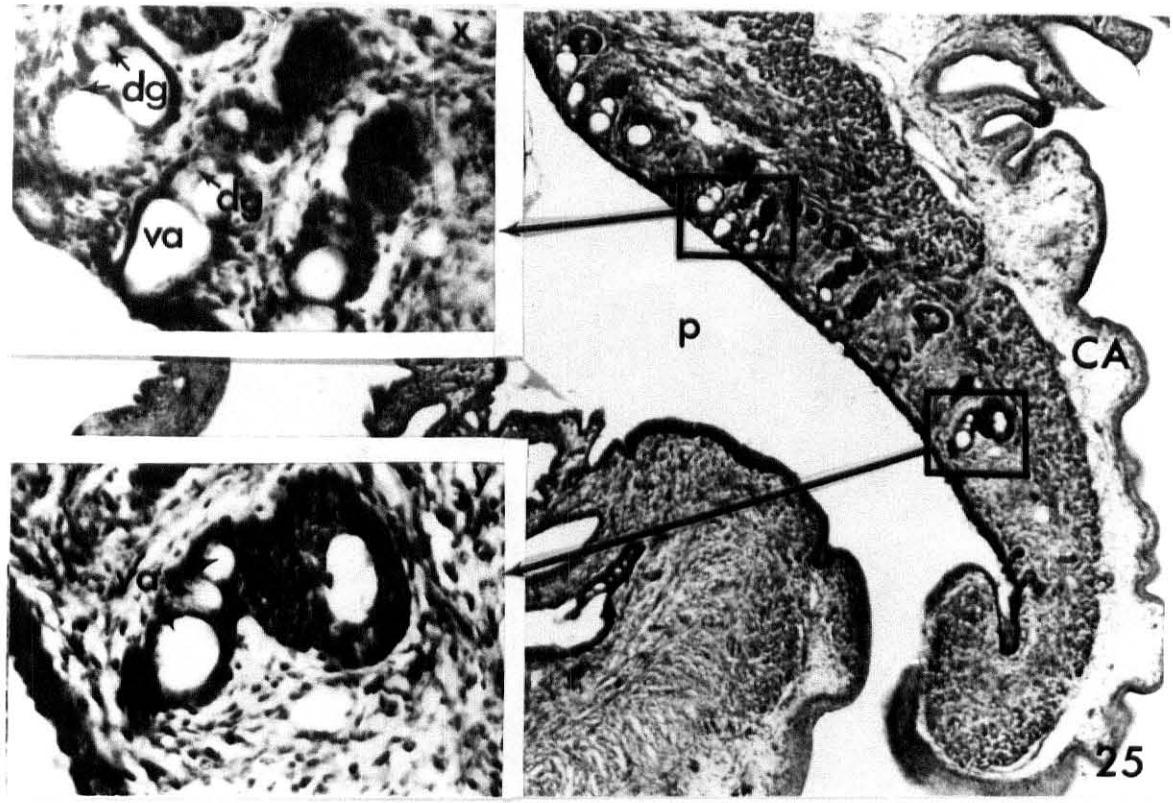
EXPLANATION OF FIGURES

Fig. 25. Median section of 13.5 day Common Coturnix embryo. Smaller vacuoles are coalescing to form larger vacuoles. Basal epithelium of the glandular units surrounding the vacuoles are squamous to cuboidal in structure. Epithelial caps are more numerous. (H & E, X71; insert x, H & E, X456; insert y, H & E, X456)

Fig. 26. Transverse section of 13.5 day Common Coturnix embryo. Embryonic interglandular septae are being formed. Septal extensions of the embryonic interglandular septae are present as a result of connective tissue compression between the epithelial branches. (C.T. Stain, X111; insert x, C.T. Stain, X456; insert y, C.T. Stain, X456)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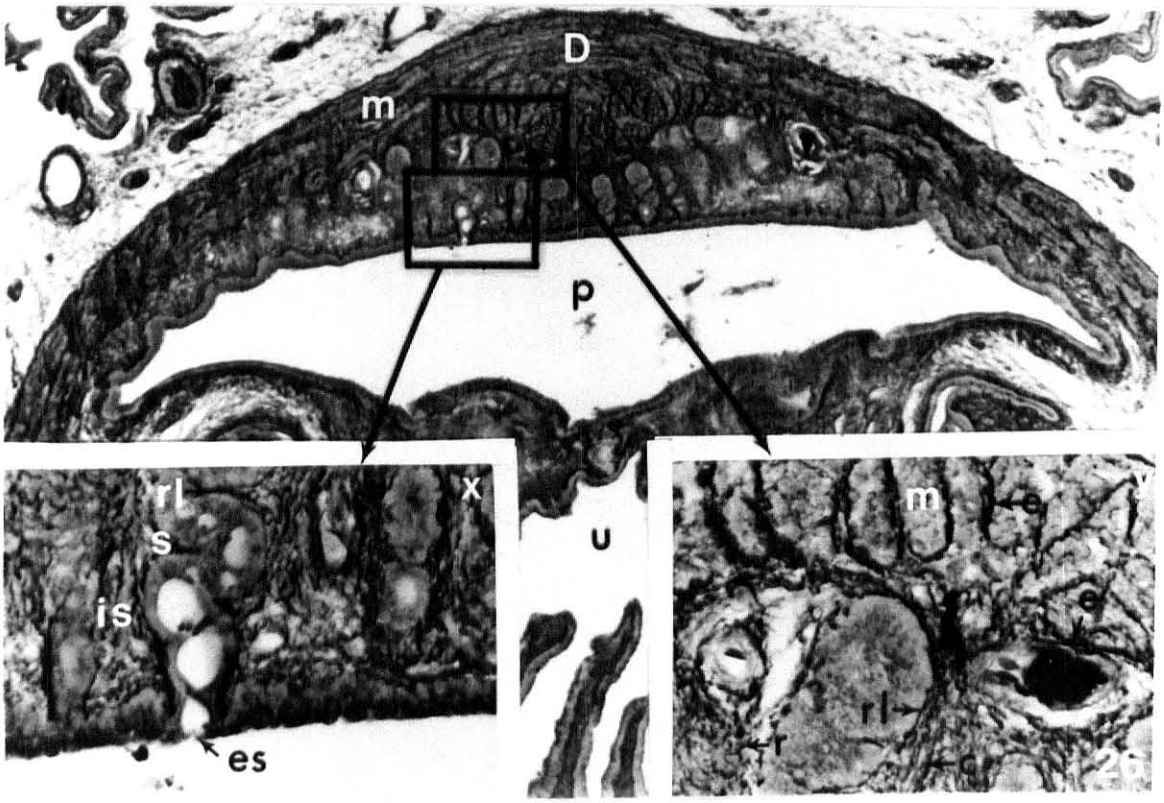
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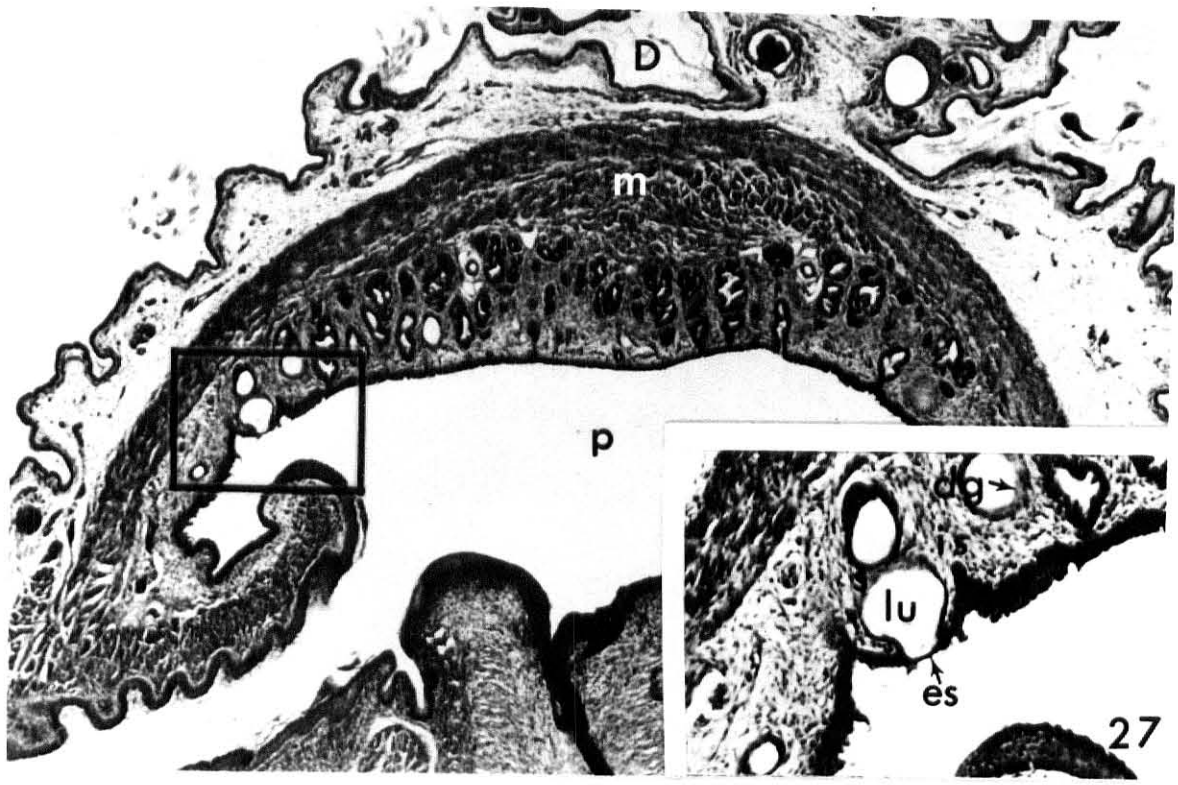
EXPLANATION OF FIGURES

Fig. 27. Transverse section of 14 day Common Coturnix embryo. A lumen is beginning to form in some of the glandular units as a result of coalescence of the vacuoles. The units are beginning to resemble simple branched alveolar glands. A thin epithelial cap is still present which separates the lumen of the glandular unit from the proctodeal cavity. (H & E, X71; insert, H & E, X228)

Fig. 28. Sagittal section of 14 day Common Coturnix embryo. Epithelial caps are prominent. A lumen is beginning to develop from coalescence of vacuoles. (H & E, X91; insert, H & E, X456)



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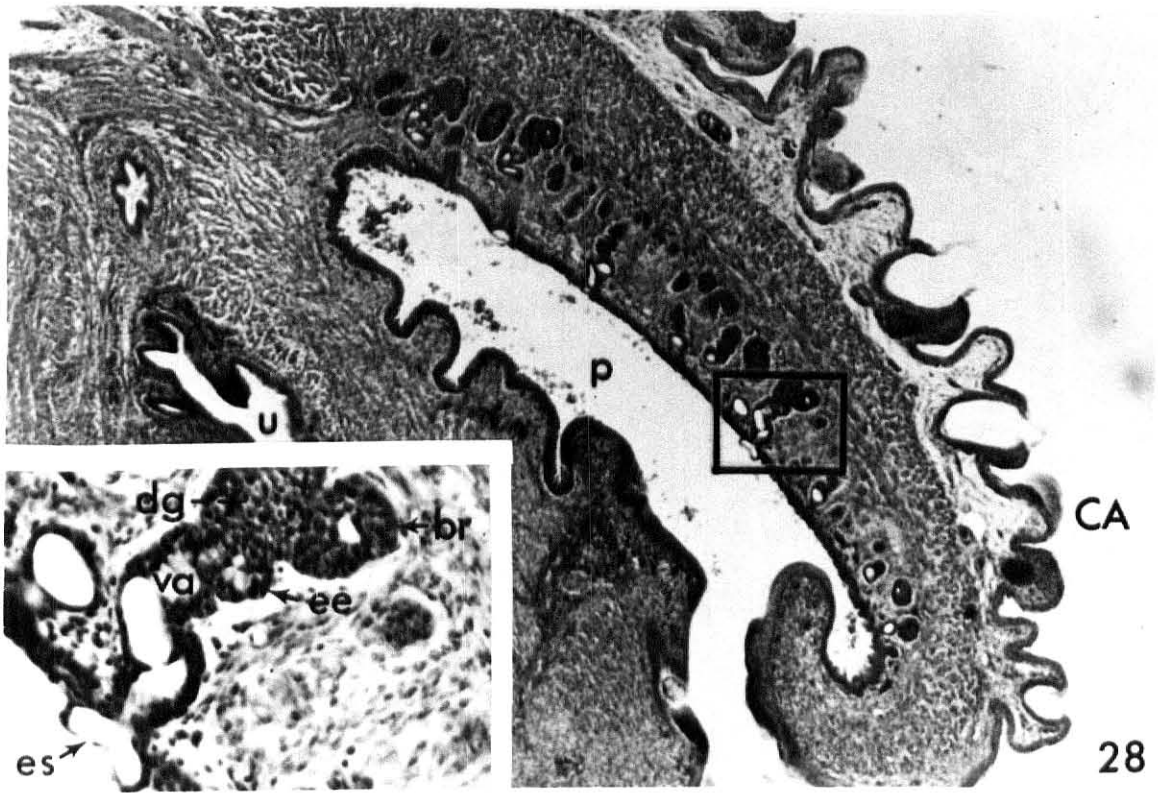

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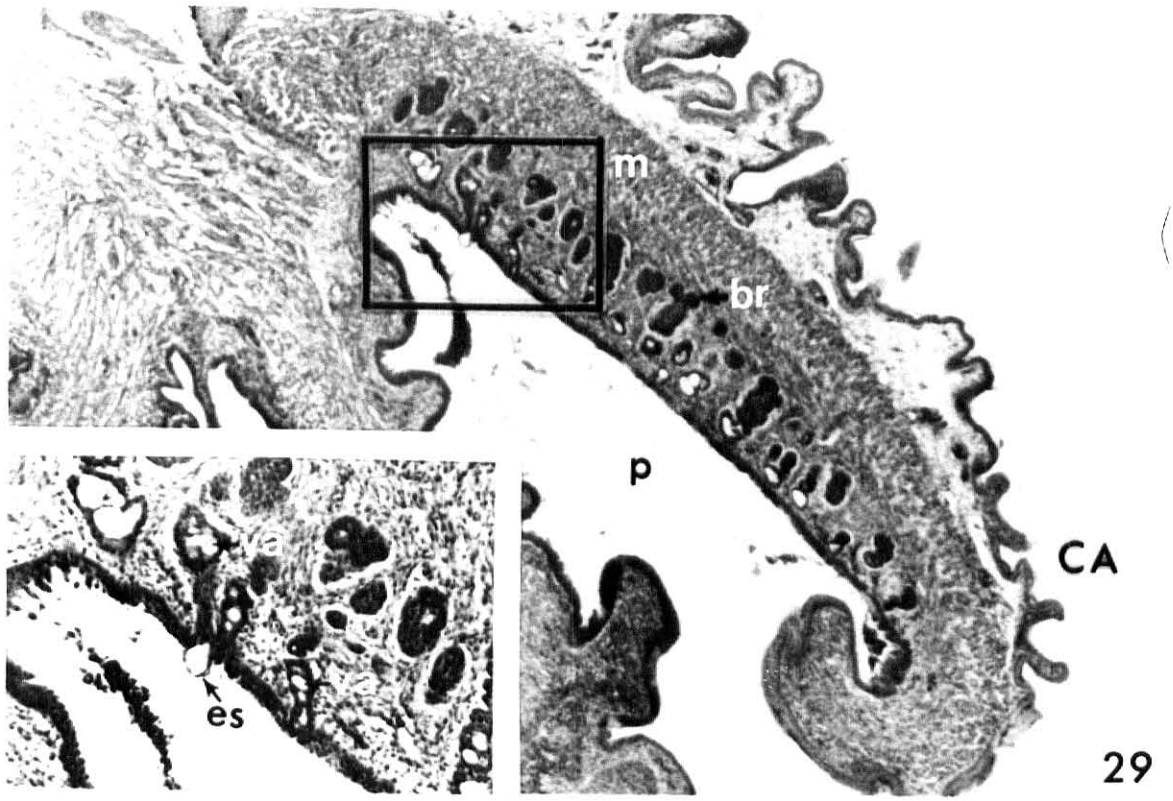
EXPLANATION OF FIGURES

Fig. 29. Sagittal section of 14 day Common Coturnix embryo. Epithelial caps are present as enlarged bulges projecting into the proctodeal cavity. A lumen is beginning to form as a result of coalescence of vacuoles within the glandular units. (H & E, X91; insert, H & E, X456)

Fig. 30. A Scanning Electron Micrograph of the dorsal proctodeal surface of 14 day Common Coturnix embryo. Epithelial caps are shown to be protruding from the dorsal proctodeal surface. (X873)



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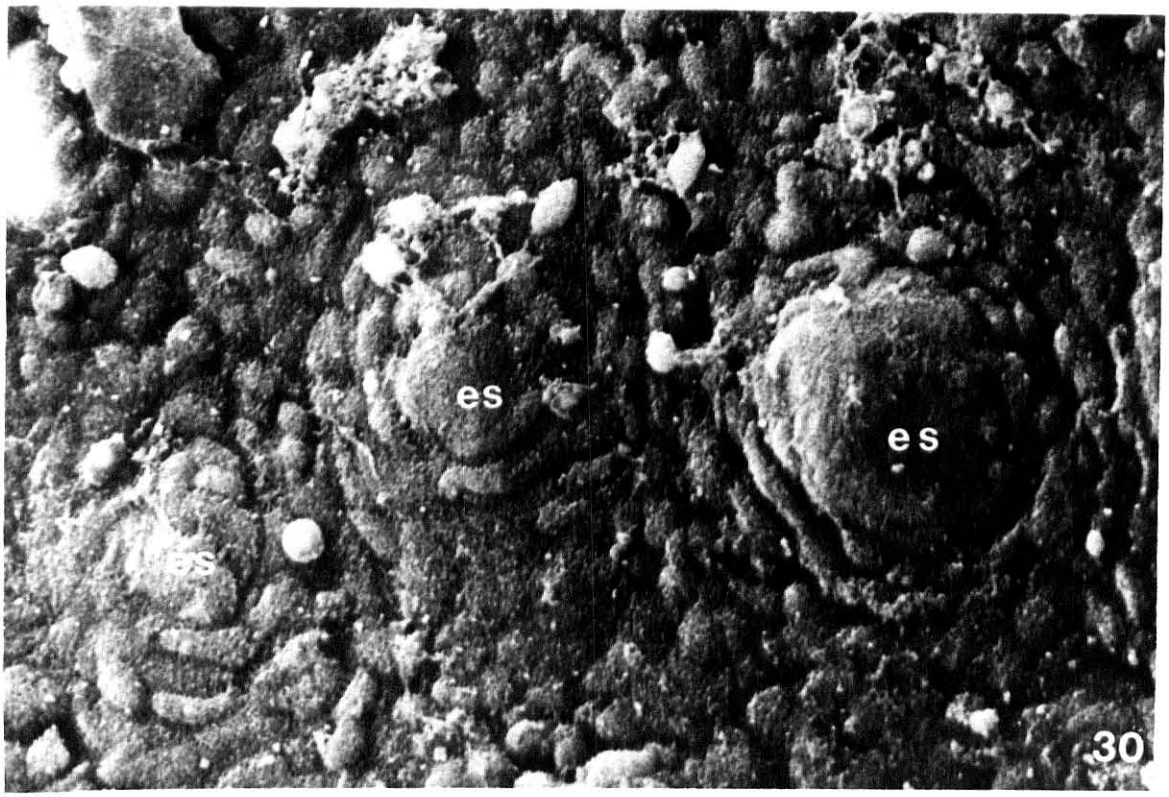

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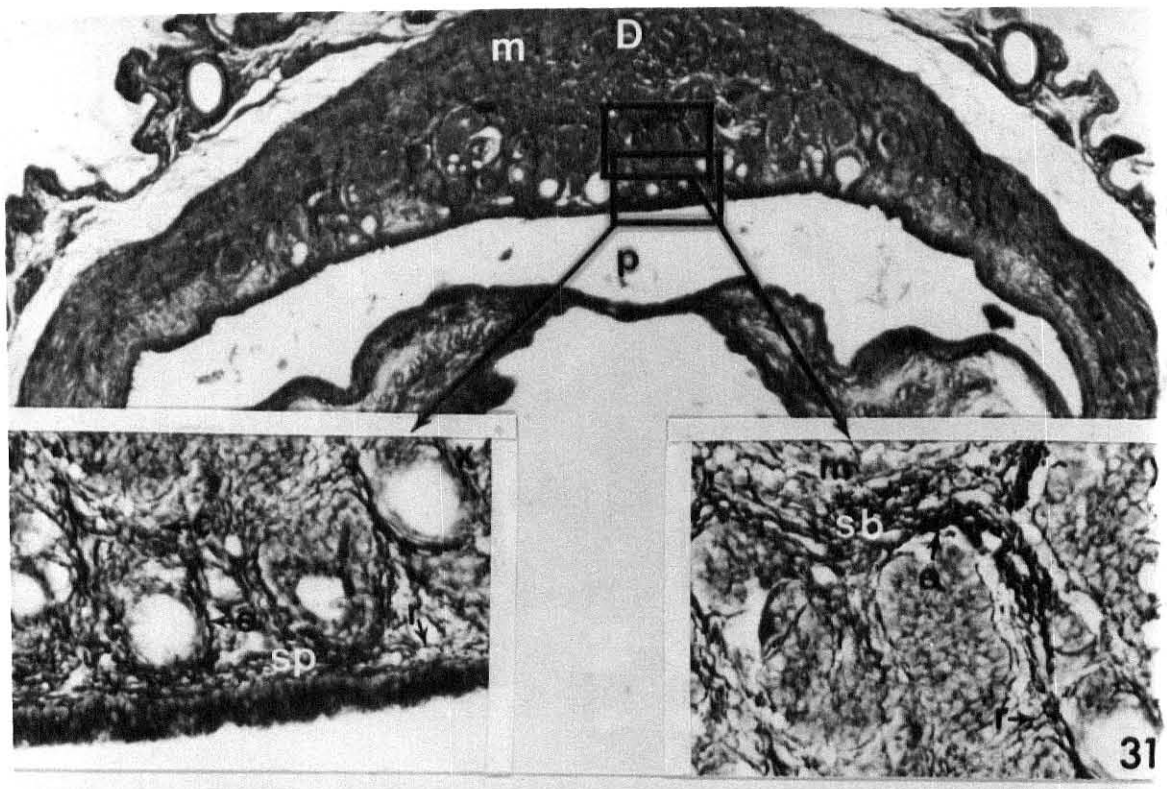
EXPLANATION OF FIGURES

Fig. 31. Transverse section of 14 day Common Coturnix embryo. Elastic fibers are developing in the interglandular septae, septal extensions and are extending into the supraglandular region. The connective tissue fibers are being compacted and the fibers are becoming aligned parallel to the proctodeal epithelium. (C.T. Stain, X71; insert x, C.T. Stain, X456; insert y, C.T. Stain, X456)

Fig. 32. Transverse section of 14.5 day Common Coturnix embryo. Epithelial caps are present and vacuoles are coalescing to form a lumen in the glandular units. (H & E, X91; insert x, H & E, X456; insert y, H & E, X456)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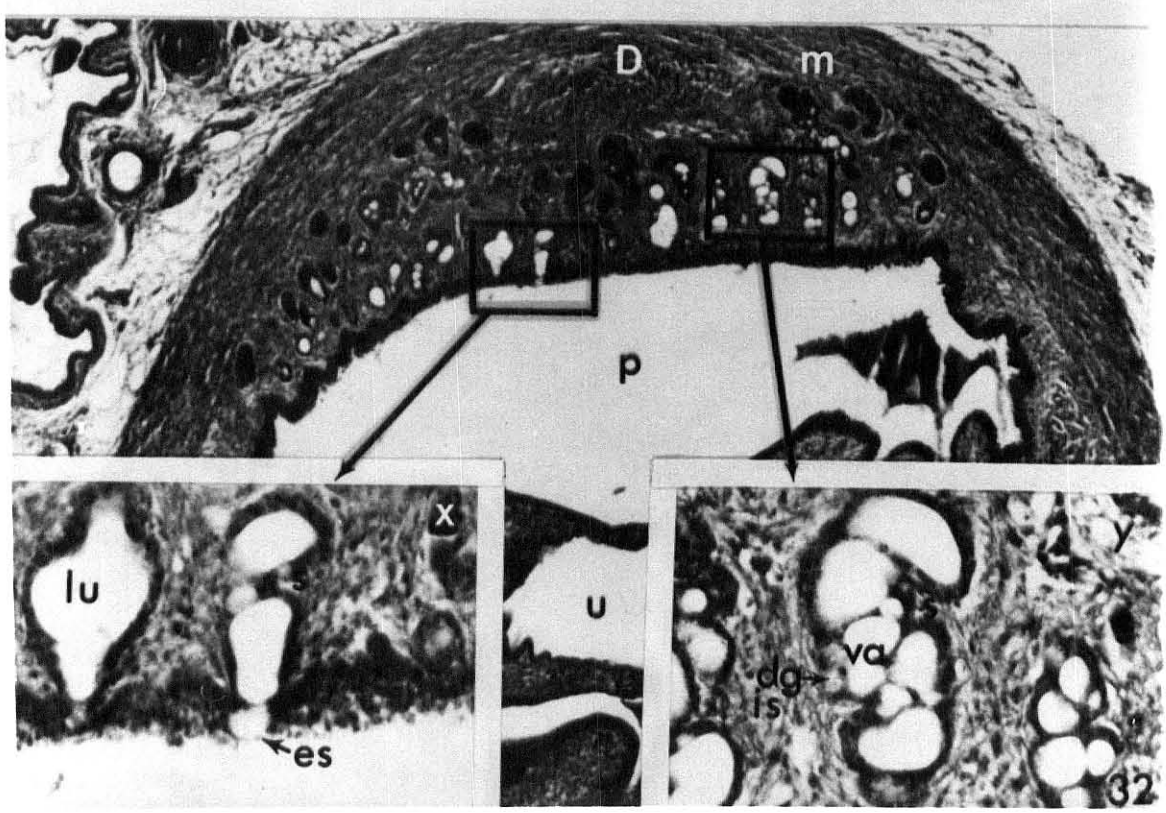
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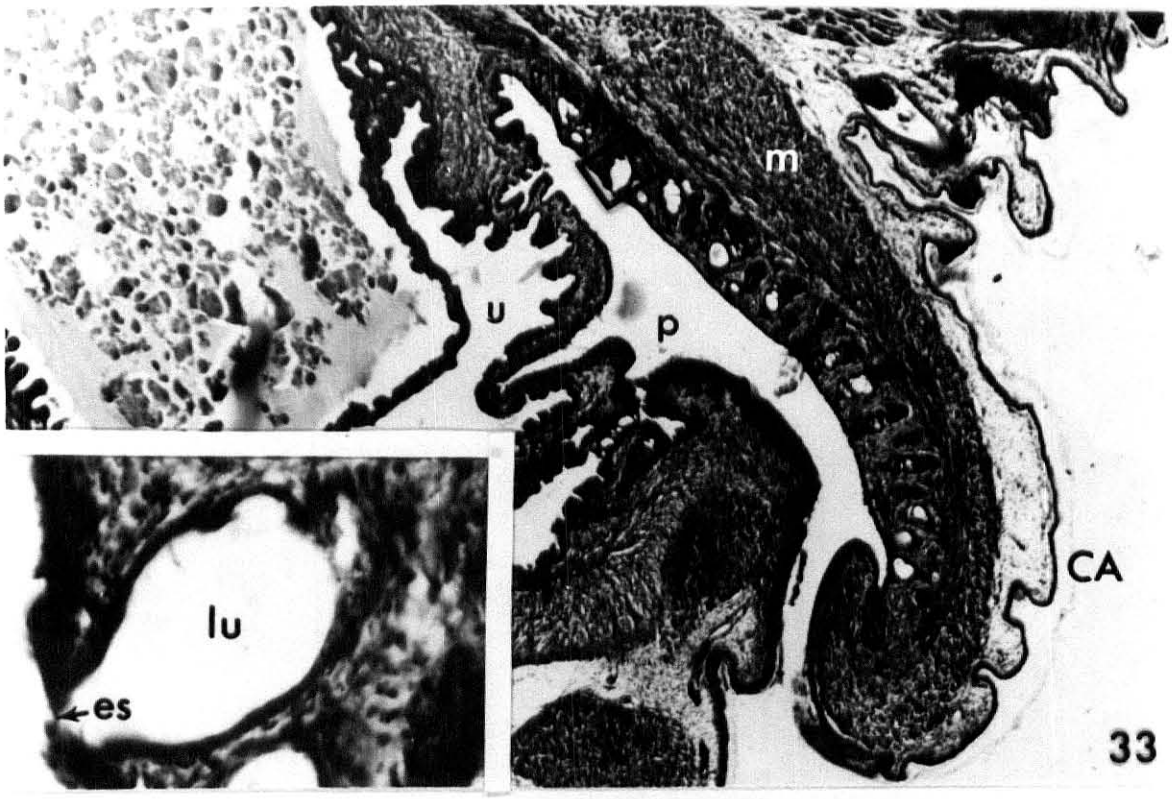
EXPLANATION OF FIGURES

Fig. 33. Sagittal section of 14.5 day Common Coturnix embryo. A lumen is being produced as a result of coalescence of the vacuoles. Epithelial caps are present. (H & E, X57; insert, H & E, X570)

Fig. 34. Transverse section of 14.5 day Common Coturnix embryo. Elastic fibers are present throughout the lamina propria. There is an alignment of connective tissue fibers both in the subglandular and supraglandular regions. (C.T. Stain, X71; insert x, C.T. Stain, X456; insert y, C.T. Stain, X182)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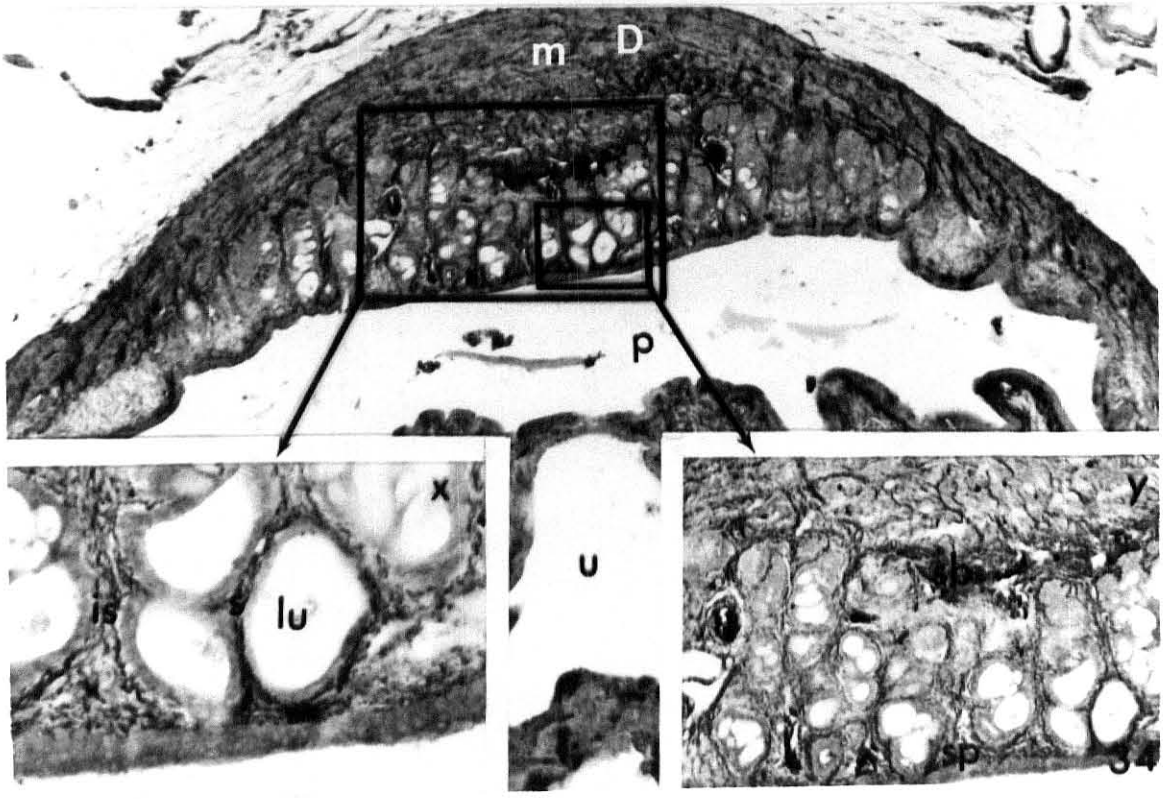
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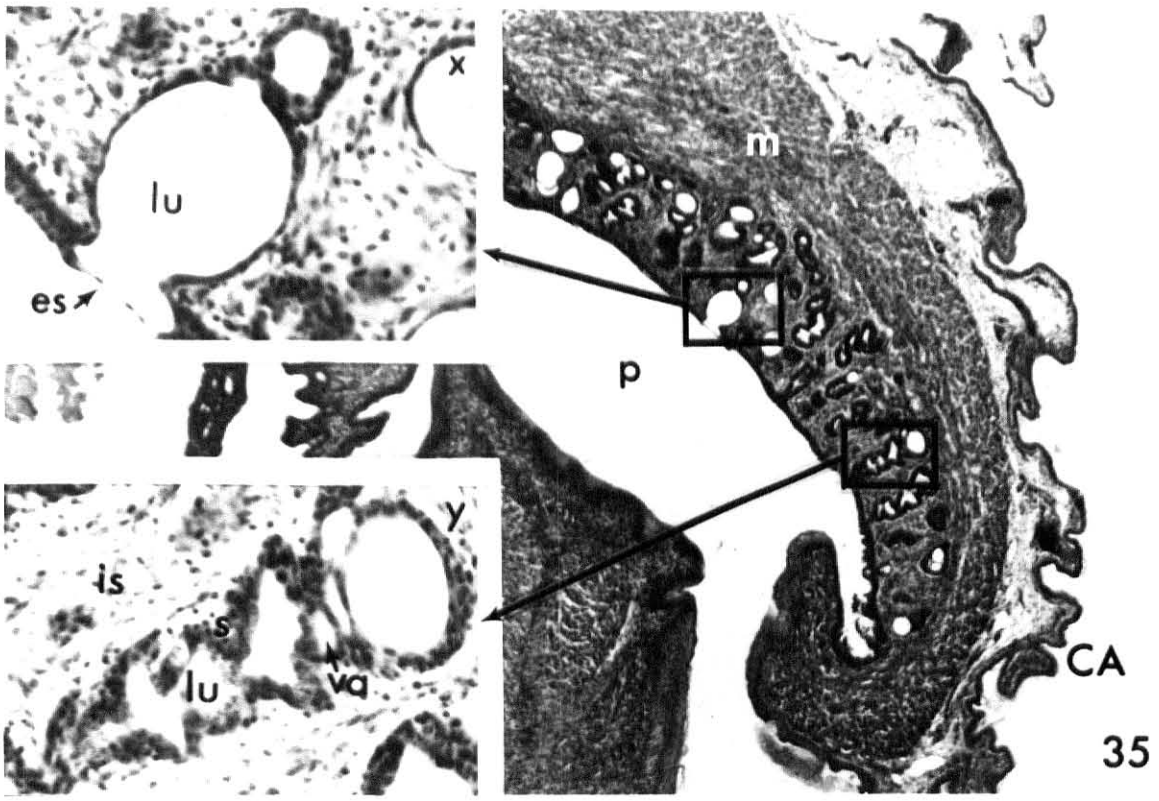
EXPLANATION OF FIGURES

Fig. 35. Median section of 15 day Common Coturnix embryo. Vacuoles have almost completely coalesced to form lumina in the glandular units. Where there is no evidence of cellular degeneration the lumina are lined with either squamous or cuboidal epithelium. Epithelial caps are quite prominent. (H & E, X71; insert x, H & E, X456; insert y, H & E, X456)

Fig. 36. Scanning Electron Micrograph of the dorsal proctodeal surface of 15 day Common Coturnix embryo. Epithelial cap may be seen to have enlarged since 14 days of incubation (compare: fig. 30). (X1150)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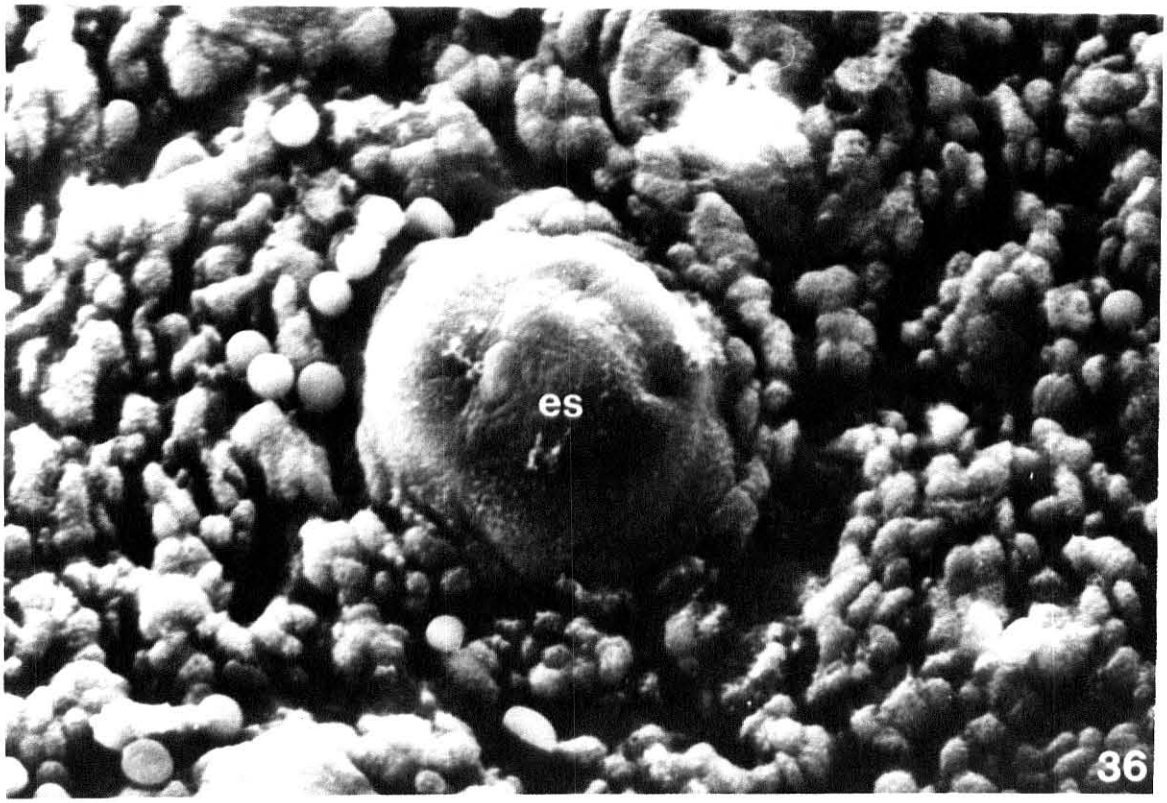
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EXPLANATION OF FIGURES

Fig. 37. Transverse section of 15 day Common Coturnix embryo. Septal extensions are present. As the branches of the glandular units continue to expand the fibers of the reticular lamina are being compressed between the expanding branches. (C.T. Stain, X71; insert x, C.T. Stain, X456; insert y, C.T. Stain, X456)

Fig. 38. Transverse section of 15.5 day Common Coturnix embryo. Lumina are present throughout the glandular units. Epithelial caps are present. (H & E, X91; insert, H & E, X228)

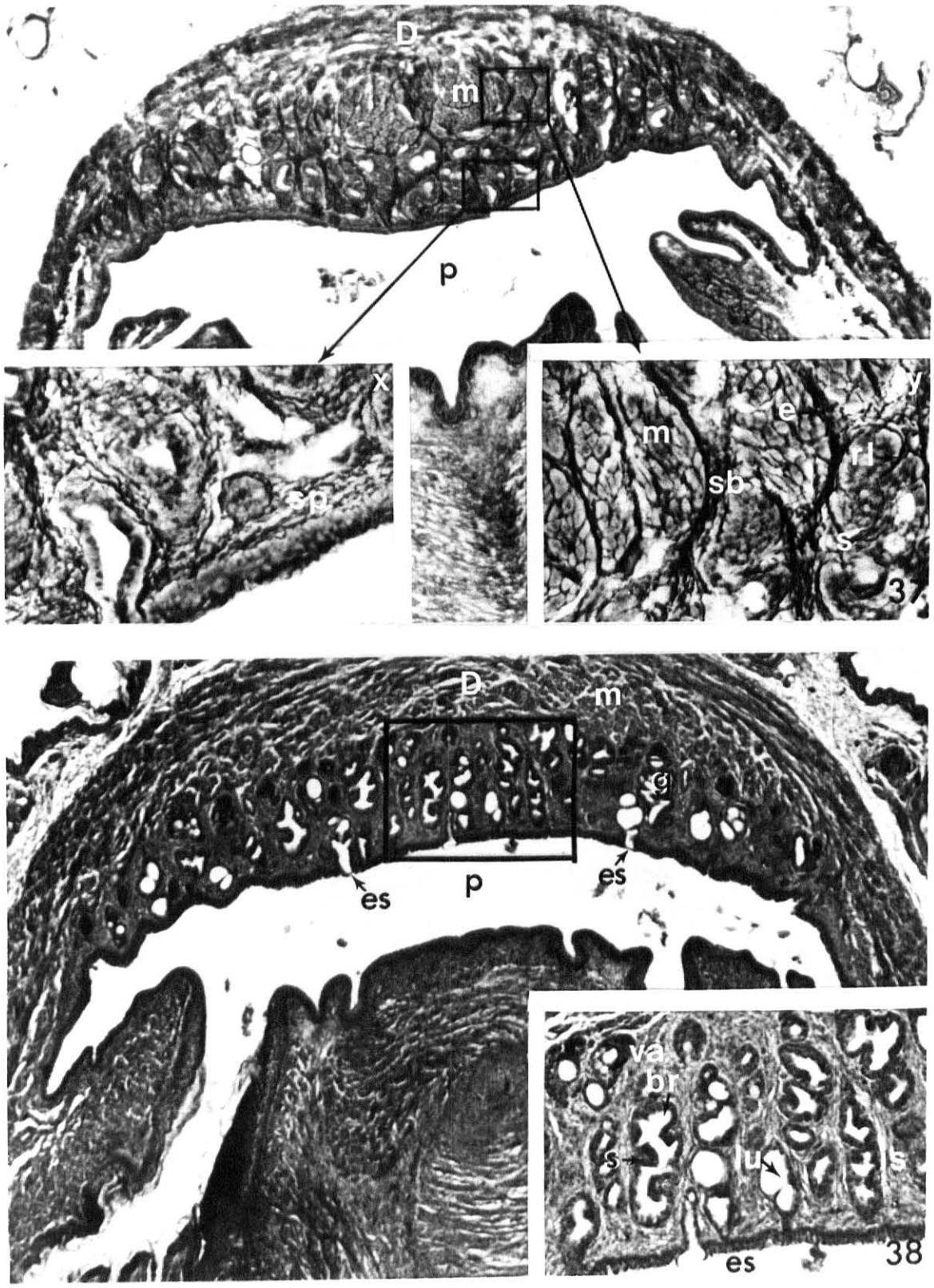
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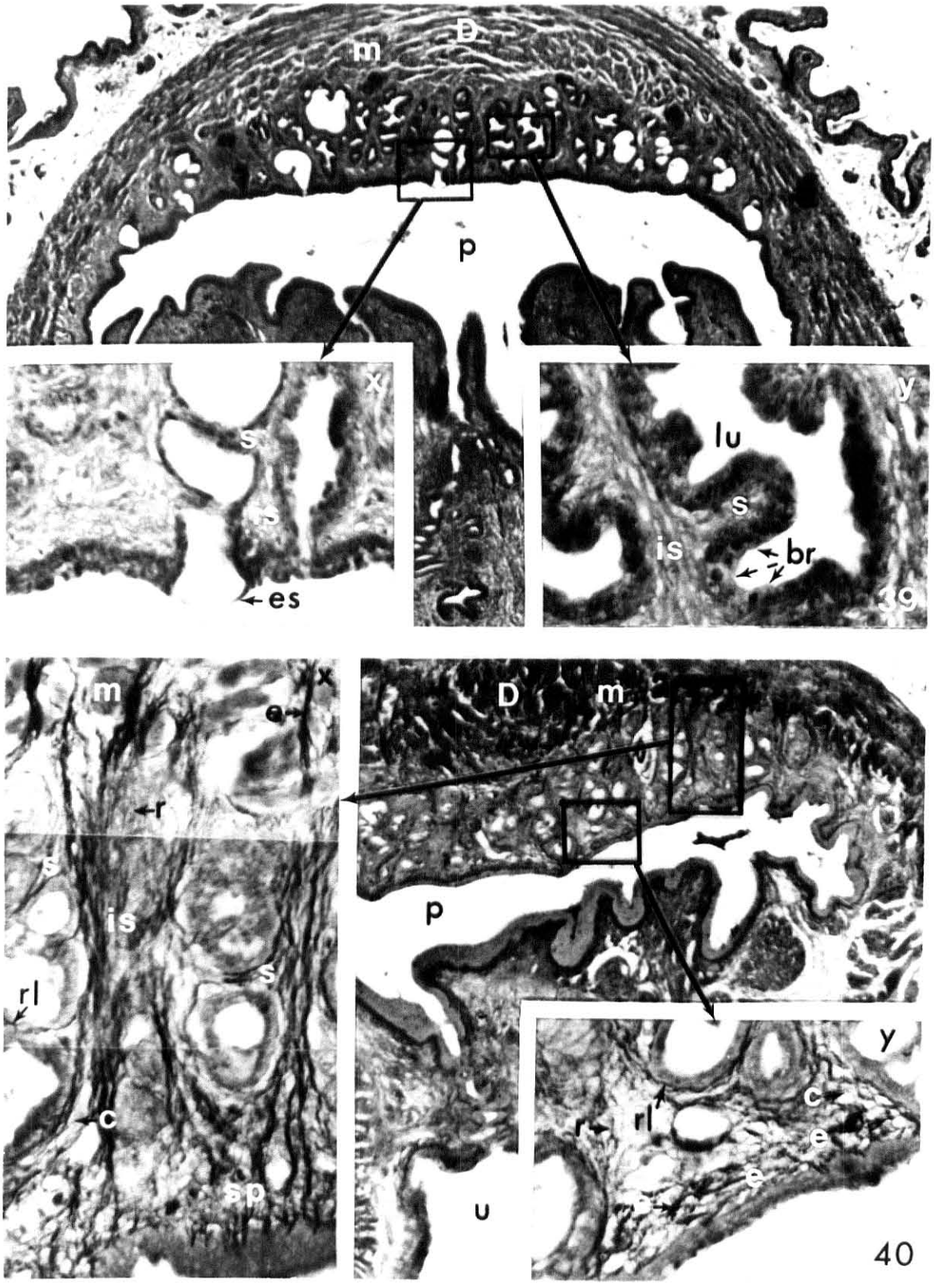
Fig. 39. Transverse section of 15.5 day Common Coturnix embryo. Lumina are present in the glandular units. The glandular units, where vacuolization has stopped, are lined with cuboidal cells. Epithelial caps are still prominent. (H & E, X71; insert x, H & E, X456; insert y, H & E, X730)

Fig. 40. Transverse section of 15.5 day Common Coturnix embryo. Elastic fibers are present throughout the lamina propria, but are not as numerous as the reticular and collagen fibers. There are numerous septal extensions and "trapped" reticular lamina between branches of the glandular units. Note cut ends of elastic fibers produced by an encirclement of the region of the future excretory canal of the unit. (C.T. Stain, X71; insert x, C.T. Stain, X456; insert y, C.T. Stain, X456)



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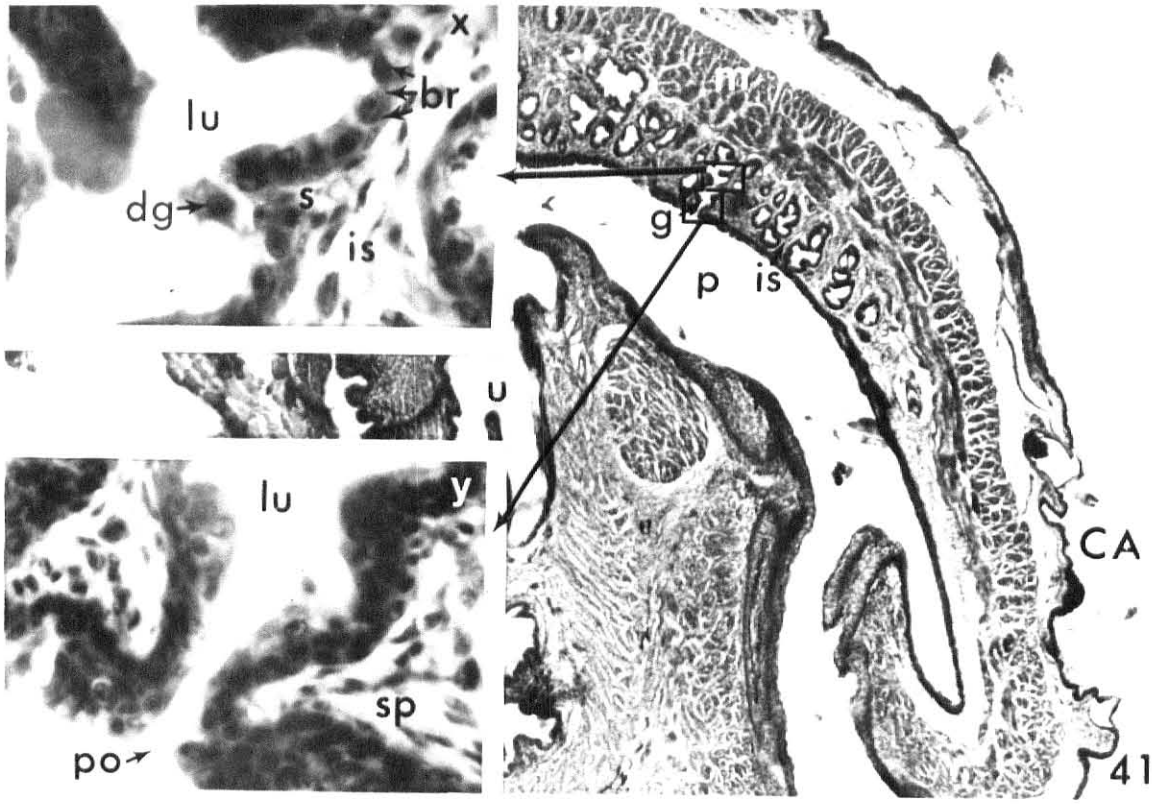
EXPLANATION OF FIGURES

Fig. 41. Sagittal section of 16 day Common Coturnix embryo. Degeneration and vacuolization is nearly complete leaving each glandular unit with a single layer of cuboidal cells. The epithelial caps are no longer present, leaving the lumina of the glandular units open to the proctodeal cavity. (H & E, X57; insert x, H & E, X1140; insert y, H & E X1140)

Fig. 42. Scanning Electron Micrograph of the dorsal proctodeal surface of 16 day Common Coturnix embryo. The epithelia forming the caps appear to have separated leaving the lumina of the glandular units open to the proctodeal cavity; a small papilla is present throughout which the pore of the glandular unit opens. (X5022)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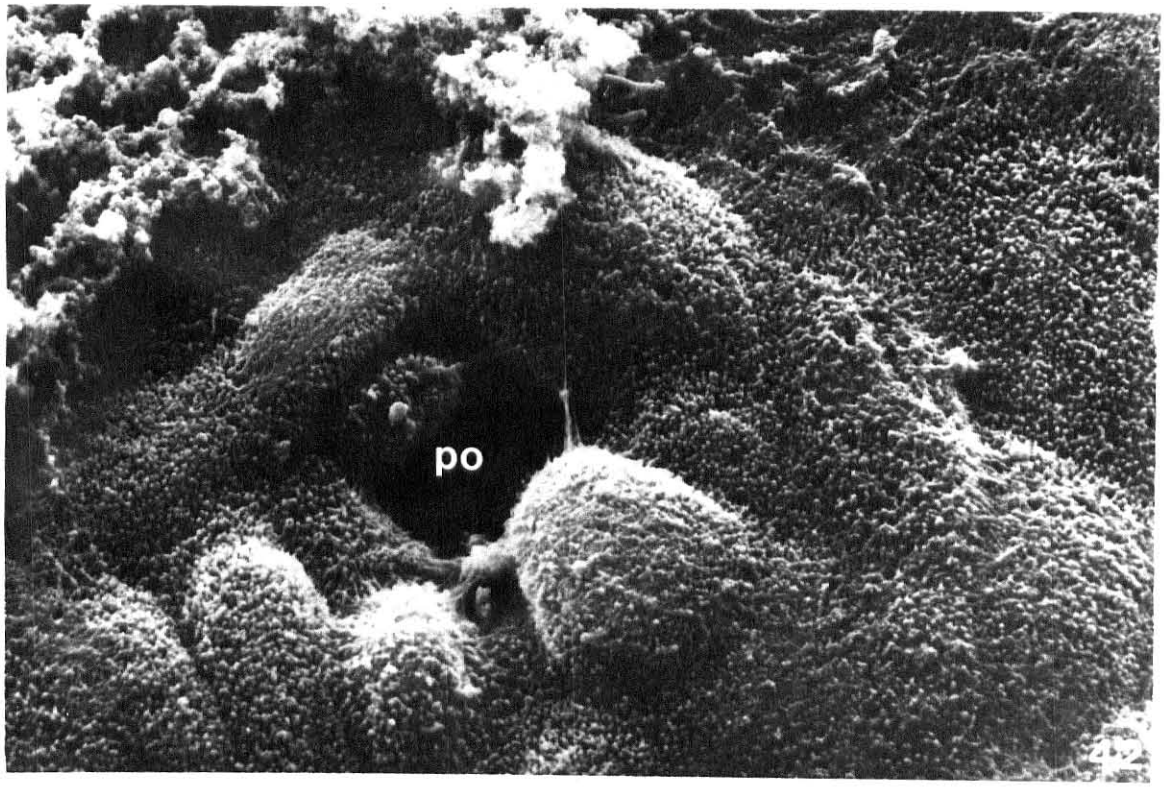
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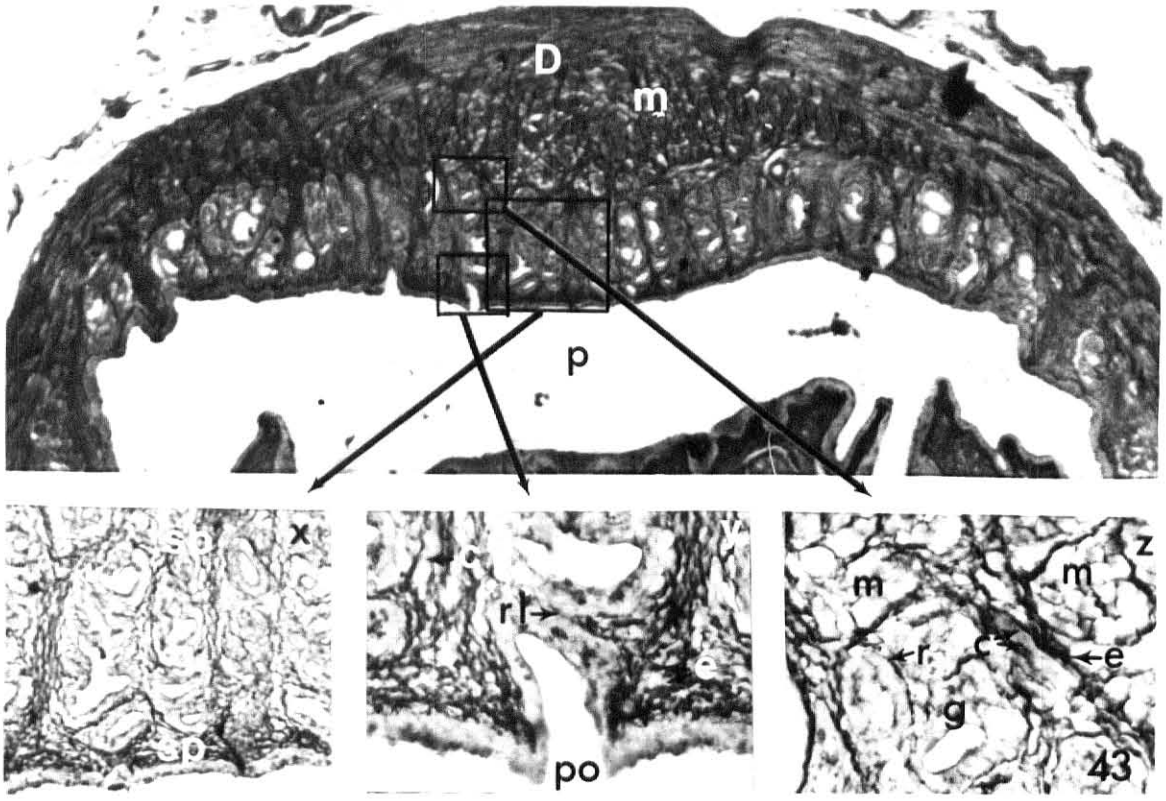
EXPLANATION OF FIGURES

Fig. 43. Transverse section of 16 day Common Coturnix embryo. All three connective tissue fiber types are present throughout the lamina propria. The lamina propria of the supraglandular and subglandular regions are organized parallel to the dorsal proctodeal epithelium. The connective tissue fibers are inseparable from the epimysium and perimysium of the M. sphincter cloacae. A small papilla is present by which the duct of the glandular unit opens into the proctodeal cavity. (C.T. Stain, X71; insert x, C.T. Stain, X182; insert y, C.T. Stain, X456; insert z, C.T. Stain, X456)

Fig. 44. Reconstructed model of glandular unit from 16 day Common Coturnix embryo. A lumen is present throughout the multiple branches of the glandular unit. The pore of the glandular unit is open allowing the lumen of the glandular unit to open into the proctodeal cavity.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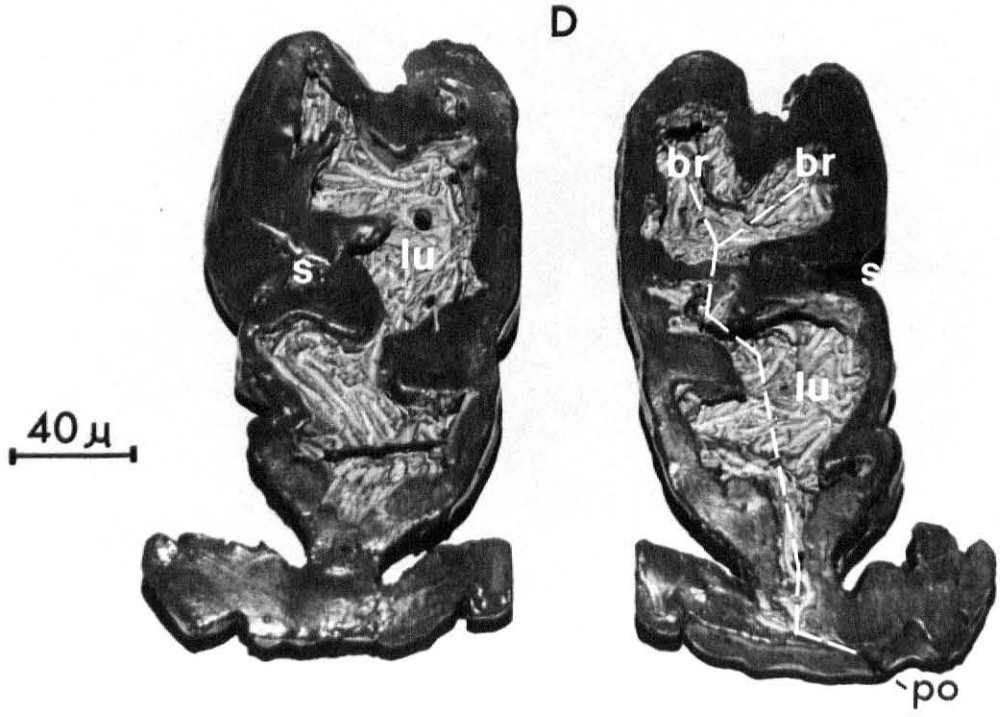
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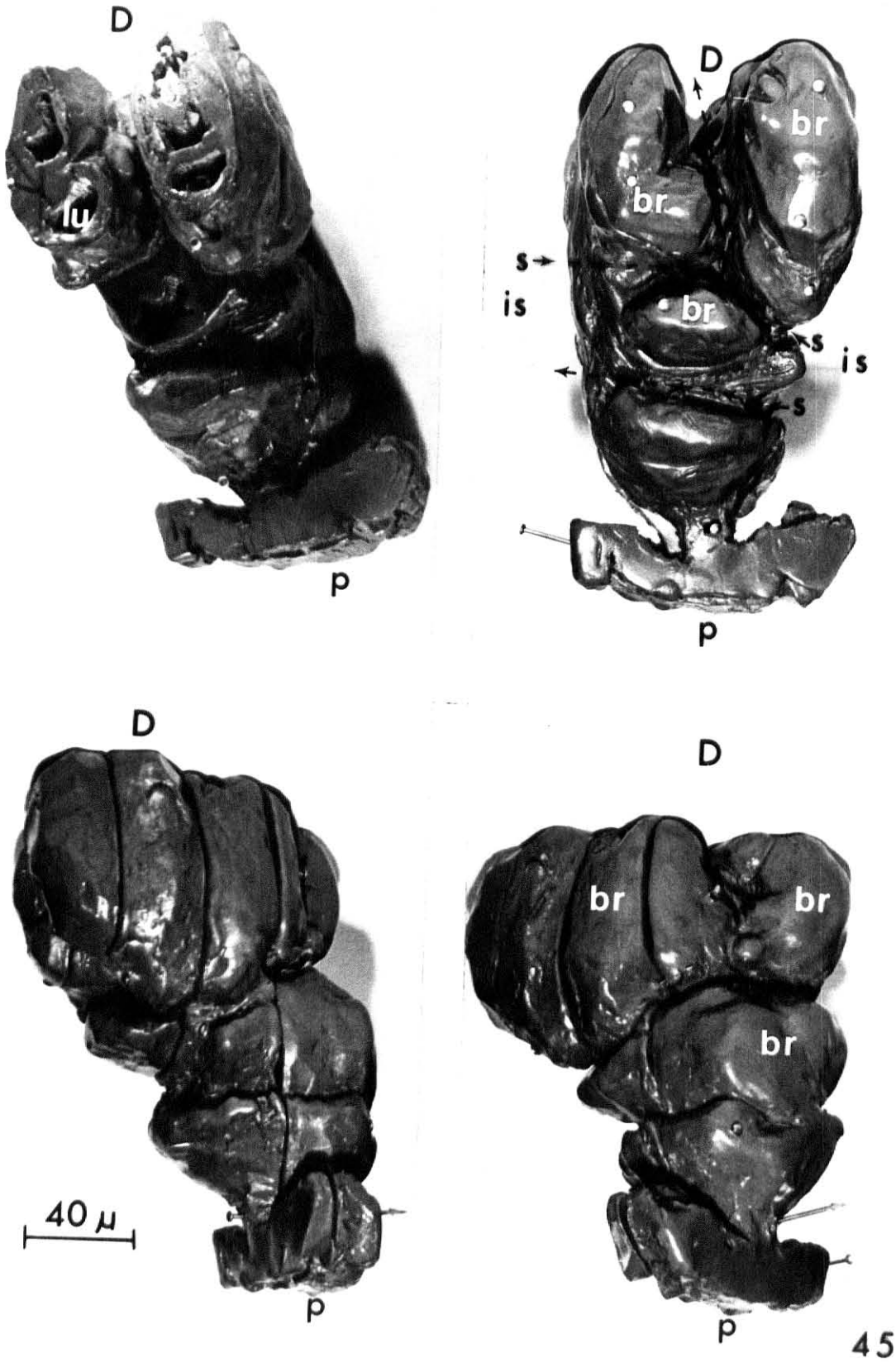
EXPLANATION OF FIGURES

Fig. 45. Reconstructed model of glandular unit from 16 day Common Coturnix embryo. Multiple branches can be identified, giving the glandular unit the appearance of a simple branched alveolar gland.



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THE EMBRYONIC DEVELOPMENT OF THE PROCTODEAL GLAND OF *COTURNIX COTURNIX JAPONICA*

by

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ABSTRACT

The proctodeal gland of the Common Coturnix (Coturnix coturnix japonica) in located within the dorsal wall of the proctodeum; it has been described as an aggregate gland composed of numerous glandular units which secrete, in the sexually active male, a foamy exudate of unknown function.

Two studies which summarize certain aspects of the embryogeny of the proctodeal gland have been published. These papers are contradictory in description of the development. Further, the papers contain little indepth information to foster acceptance of either of the postulated theories on embryonic development.

This study was undertaken to present the detailed embryology of the proctodeal gland from day of first appearance to day of hatching. A total of 57 embryos were collected at 12 hour intervals from day 7 to day 16 (3 embryos/12 hour time period), serially sectioned and stained with either a hematoxylin and eosin stain or a connective tissue stain. Eight embryos from day 11 to day 16 were prepared for study on the Scanning Electron Microscope.

The first indication of proctodeal gland development is in the 9.5 day embryo in which solid epithelial buds (gland primordium) proliferate from the dorsal proctodeal epithelium into the lamina propria. At 11 days incubation the solid epithelial buds have developed into solid convoluted epithelial cords. At 12 days the epithelial cords begin to branch as the result of expansions along their length; concurrently the solid branched epithelial cords (glandular units) show the first evidence of cellular degeneration in the core of the cord. As a result of the cellular degeneration, vacuoles are formed in the glandular units by 12.5 days. By 15 days,

coalescence of the vacuoles has formed a continuous lumen throughout the glandular units. At 16 days the glandular units can be recognized to be individual simple branched alveolar glands.

At about 13 days of incubation epithelial caps begin to develop on the proctodeal surface. These caps consist of a single layer of squamous epithelium which separates the vacuolizing glandular units from the proctodeal cavity. These epithelial caps begin to disappear at 16 days and thus open the lumen of the glandular units to the proctodeal cavity.

The lamina propria associated with the dorsal proctodeal wall is composed of only reticular fibers prior to 10.5 days. Subsequent to this time collagen fibers begin to develop in the lamina propria and extend throughout the lamina propria by 11.5 days. The presence of elastic fibers in the lamina propria was not observed until 11.5 days. The connective tissue fibers of the lamina propria including those associated fibers surrounding the glandular units are interwoven with the epimysium and perimysium of M. sphincter cloacae located dorsal and lateral to the gland.

At 16 days incubation the future secretory cells are cuboidal and present no indication of secretory function.

The proctodeal gland is derived from the ectoderm as an inward growth of epithelium of the dorsal proctodeal wall. The lamina propria which underlies and surrounds first the epithelial buds, then the epithelial cords and finally the glandular units is a derivative of the mesoderm.