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Histological Studies on Formation of Egg-Covering in Quail Oviduct

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(Figs. 1–12; Plates 1–2)

On the whole, function of each region of the oviduct in a series of avian egg formation have been rather well explained. The stratification and structure of the covering or shell of their eggs, as white and yolk, have been demonstrated in detail (STEWART, 1935[1]; SIMKISS and TYLER, 1957[2]).

Egg-covering is a regular but elaborated structure. After having passed through a complicated process, the covering can be completed in the oviduct.

RICHARDSON (1935)[3] made specified examinations of the mucous epithelium and glands in the isthmus, isthmouterine region and uterus of hen's oviduct, and he discussed functions of the secretory cells in relation to the formation of the covering. Recently JOHNSTON *et al.* (1963)[4] studied the ultrastructures of hen's uterus and its functions.

The author reported previously (1965[5], 1966–a[6], 1966–b[7]) on the characteristic histological features of quail isthmus and uterus and their functions. The present investigation has been performed to define the histological characters of quail egg-coverings.

MATERIALS AND METHODS

10 quail oviducts at various stages of egg formation and 16 quail eggs were examined in this study. Small pieces of the isthmus, isthmouterine region and uterus were fixed in 10% neutrailzed formol, embedded in paraffin wax and sectioned crossly or longitudinally at 7 μ in thickness. Egg-coverings ("egg-shells") were cut off with scissors from eggs and fixed in 10% neutralized formol for 7 days. Then they were decalcified in 10% EDTA solution for three to five days. Small pieces of decalcified coverings were embedded in paraffin wax and sectioned crossly at 10 to 15 μ in thickness.

Sections of oviducts and coverings were stained by methods of hematoxylin-eosin, azan, periodic acid SCHIFF (MCMANUS, 1948[8]), alcian blue (MOWRY, 1956[9]), alcian blue-periodic acid SCHIFF (MOWRY and WINKLER, 1956[10]), metachromasia with azure A buffered at pH 5.0 (SPICER and DUVENCI, 1964[11]), mercury-bromphenol blue (BONGAG, 1955[12]) and acetone sudan black (BERENBAUM, 1952[13]).

Additionally each layer of cuticle, shell matrix and shell membrane was separated as a membrane from decalcified covering and stained, without embedding and sectioning, directly.

RESULTS

Mucous cells of the isthmal epithelium of quail oviducts were goblet type, not so

high, but filled with a mucous secretion (Fig. 1). The secretion was stained strongly redish pink by periodic acid SCHIFF (PAS) reaction (Fig. 1), but not affected by alcian blue, nor azure A (pH 5.0). No secretory material was observed in ciliated cells of the epithelium (Fig. 1). The mucous cells and ciliated cells lined alternately in the epithelium (Fig. 1). The folds covered with the epithelium were large, and in their corium the isthmal glands spread (Fig. 1). The secretory cells of the glands contained many larger granules well stained by PAS reaction (Fig. 1).

These histological features were observed throughout the isthmus except for the posterior region of it. In this region, the typical figures of the isthmus were altered. It was recognized as the isthmouterine region as described in hen's oviducts (BRADLEY, 1928[14]; RICHARDSON, 1935[3]).

The isthmouterine region was not distinguished macroscopically, but only histologically from the isthmus as well as the uterus.

In the anterior portion of the region, instead of the mucous cells of the isthmal type, some higher mucous cells filled with mucous substance appeared (Fig. 2). The mucous cells reacted as the ones of the isthmal type (Fig. 2). Moreover, it presented characteristically strong stainabilities both by mercury-bromphenol blue (Fig. 5) and acetone sudan black (Fig. 6). These cells were termed as the isthmouterine mucous cells. The ciliated cells in this portion were similar to those of the isthmus. In the corium, some of the glands were filled with larger secretion granules, but most of them with smaller ones (Fig. 2).

In the middle portion of the isthmouterine region, both isthmouterine mucous cells and uterine mucous cells were observed. The uterine mucous cells were slender. Their secretion material showed weakly positive by PAS reaction, alcian blue (Figs. 3 and 4) and azure A stains. The ciliated cells were similar to the anterior portion (Fig. 3). Any stainable secretion was not observed in most of the glands (Fig. 3). They were considered as uterine glands (Fig. 4). Only a few glands with smaller secretory granules were observed among the uterine glands. The glands containing smaller granules in the anterior and middle portion were thought to be functionally varied isthmal glands.

In the posterior portion of the isthmouterine region, the mucous cells were almost uterine type mixed with a few of the isthmouterine type, and the glands were uterine.

In the uterus, the epithelium was composed of the uterine mucous cells mentioned above and ciliated cells (Fig. 4). The ciliated cells contained granules only stained by PAS reaction and another brown or yellow pigment granules (Fig. 4). The uterine glands in the corium were as mentioned above (Fig. 4). Some detailed descriptiptions on the uterus have been published by the author (1966–a[6]).

Stratification of the decalcified covering or integument of quail eggs was constructed with inner and outer shell membranes, mammillary layer, spongy layer or shell matrix and cuticle (Fig. 7). The fundamental structure of each layer was corresponded with one of the hen's eggs (STEWART, 1935[1]; SIMKISS and TYLER, 1957[2]).

The inner shell membrane composed of fine fibers was thinner but compact (Fig. 7). The fibers were stained pink by PAS reaction. The outer shell membrane was thicker. The fibers were stained by PAS test, larger in caliber and they formed a coarser network (Figs. 7 and 8). The membranes were not stained by alcian blue (Figs. 7, 8 and 9) nor azure A.

Mammillae were attached to both outer membrane and shell matrix (Figs. 7 and 8).

Each mammilla was divided into two portions, a inner central core and an outer superficial portion (Figs. 7 and 8).

The central core was like a compact and firm structure. It was deeply stained by PAS reaction, but not by alcian blue (Figs. 7 and 8). Covering the core, a superficial structure of fine fibers was distinguishable (Figs. 7 and 8). Whereas the structure seemed to be continued to the spongy layer, its fibrous arrangement was more compact than the latter. The author described it as a superficial portion of mammilla. The superficial portion was stained by PAS reaction as well as alcian blue, and reacted for alcian blue by these double stains (Figs. 7 and 8). The mammillae were attached so firmly, especially the central cores, that many knob-like mammillae were observed on the separated shell membrane (Fig. 9).

The organic shell matrix or spongy layer was composed of fine fibers and stained as the superficial portion of the mammilla (Figs. 7 and 10).

The cuticle of quail eggs was also thicker (Fig. 7) compared to the hen's eggs (ROMANOFF and ROMANOFF, 1949[15]), it was more easily separated from eggs as a membrane. The cuticle was structureless and stainble by PAS test, not by alcian blue (Fig. 7) Brown pigment granules were distributed in it.

In addition to these findings, the central core of the mammilla was characteristically strongly stained by mercury-bromphenol bleu (Fig. 11) and acetone sudan black (Fig. 12) stains.

From the above mentioned stainabilities of the oviducal secretions and the components of egg-covering, the following histochemical properties were deduced. Neutral mucopolysaccharides are present in the secretions of isthmal and isthmouterine mucous cells, uterine ciliated cells, isthmal glands, and in the materials of inner and outer shell membranes, central core of mammilla, and cuticle. Acid mucopolysaccharides are present in the secretion of uterine mucous cells, in the materials of the superficial portion of mammilla and shell matrix. Lipids combined with protein are rich in the isthmouterine mucous cells and in the central core of the mammilla.

DISCUSSION

The author reported previously the histological characters of quail oviducts (1965[5]), 1966-a[6]), 1966-b[7]), and discussed, on the basis of secretory cycles of the uterine epithelium and of histochemical features of secretions of epithelium and glands of the isthmus and uterus, certain corelations between the formation of egg-coverings and the oviducal secretions. He assumed that the shell membranes derive from the isthmal glands, the organic shell matrix from the uterine mucous cells, the inorganic shell materials from the uterine glands, and the cuticle from the uterine ciliated cells. However, he has not examined the structures and characteristics of quail egg-covering. The present report deals with them and some additional findings of the oviducts.

The structures of quail egg-coverings are basically similar to those of hen's eggs, and the stratification of the inner and outer shell membranes, mammillary layer, shell matrix and cuticle is apparent.

Descriptions about the mammillae remain somewhat indefinite except for the core (STEWART, 1935[1]; ROMANOFF and ROMANOFF, 1949[15]; SIMKISS and TYLER, 1957[2]). The mammilla was divided into central core and superficial portion by this author.

Von NATHUSISUS (quoted by ROMANOFF and ROMANOFF, 1949[15]) described that

the cuticle of the quail egg is the thickest and hen's one the thinnest amongst various kinds of bird-eggs. So the present author has used the quail egg-coverings as the most useful material for investigation of the cuticle.

Staining methods used in this study are mostly routine ones for mucopolysaccharides and proteins. In these reactions, it appeared that the inner and outer shell membranes, central core of the mammilla and the cuticle are rich in neutral mucopolysaccharides, and contrarily, that the superficial portion of the mammilla and the shell matrix contain acid mucopolysaccharides, and that the cetral core is characterized by containing lipid accompanied by protein.

SIMKISS and TYLER (1957)[2] examined histochemically the shell matrix and mammillae of hen's eggs. They found out that the shell matrix as well as the mammillae are acid mucopolysaccharide-protein complexes, and that the mucopolysaccharide in the former is a polymerized one, but in the latter is unpolymerized. In quail eggs, the shell matrix as well as the superficial portion are regular to reactions of alcian blue and azure A (pH 5.0), however, the central core does not react for such tests.

As described by SIMKISS and TYLER (1957)[2], the central core of the quail egg is also deeply stained by mercury-bromphenol blue and acetone sudan black stains. The latter is useful for bound lipids (PEARSE, 1961[16]), and such stainabliities are thought as caused by high concentration of lipoproteins in the structure.

In this study, the isthmouterine region of the quail oviducts was mainly examined. This region was described by BRADLEY (1928)[14] and RICHARDSON (1935)[3]. Though each of them noticed especially the glands of the region, they did not refer to the mucous cells in the epithelium described by the present author. The mucous cells are characteristic for their shapes and reactions to lipid and protein stains. Through their characteristics, the cells are different from the isthmal type as well as the uterine type, so they should be termed as isthmouterine mucous cells. Cells filled with such lipoprotein are not found in the sithmus nor uterus either.

It is confirmed nowadays, that, while the egg was transported from the anterior isthmus to the posterior uterus, the egg-covering is formed from the inner shell membrane to the outermost cuticle. As mentioned above, the present author has brought forth some assumptive interrelations between the secreted materials from the oviduct and the constituents of the egg-covering (1966-a[6], 1966-b[7]). He considers that the findings in this paper are susceptible to some explanations of the mechanism of formation of egg-coverings in birds. The whole discussion through the histological results obtained previous and present studies on the oviducts and egg-coverings of the quail suggest following interesting relationships between the oviduct and the egg: the shell membranes are derived from the isthmal mucous cells; the central core from the isthmouterine mucous cells; the superficial portion and the shell matrix from the uterine mucous cells; the inorganic substances from the uterine glands, and the cuticle from the uterine ciliated cells.

SUMMARY

To clarify the formation of egg-coverings in birds, the isthmus, isthmouterine region and uterus of quail oviducts and their egg-coverings were investigated histologically. The obtained results are as follows.

1. Mucous cells proper to the isthmouterine region are termed as isthmouterine

mucous cells. Their features are different from the isthmal and uterine mucous cells. The isthmouterine mucous cells are characterized by high stainability for lipoprotein reaction.

2. Stratification of decalcified quail egg-coverings and structures of each layer of them are similar to those of hen's eggs. The mammilla is divided into central core and superficial portion.

3. Neutral mucopolysaccharides are present in the shell membranes, central core of the mammilla and cuticle; acid mucopolysaccharides in the superficial portion of the mammilla and shell matrix.

4. The central core contains a certain amount of concentrated lipoprotein.

5. The author discussed the interelationship between the oviducal secretions and the constituents of the egg-coverings, on the basis of findings reported in previous and present papers.

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ウズラ卵管における卵外皮形成の組織学的研究

田　村　達　堂

鳥類卵の卵外皮形成機構を明らかにする目的で，ウズラ卵管の峡部，峡子宮部，子宮部，および脱灰したウズラ卵卵外皮の組織学研究を行なった．

1. 峡子宮部には固有の粘液細胞が存在する．本細胞には脂蛋白が濃密に存在する．2. ウズラ卵卵外皮はニワトリのそれと類似の層形成ならびに各層の構造を有する．卵殻乳頭は中心核と周辺部とに分けられる．3. 中性粘液多糖類が卵殻膜，卵殻乳頭中心核，クチクラに，酸性粘液多糖類が卵殻乳頭，卵殻乳頭，卵殻基質にみとめられる．4. 卵殻乳頭中心核には脂蛋白も豊富である．5. 既報[6)7)]および本論文の成績に基づいて，卵管の分必物と卵外皮構成物質との相互関係を検討した．

EXPLANATION OF FIGURES

Plate 1

Figures 1 to 6 show quail oviducts.

Fig. 1. Isthmus. PAS reaction. Secretions of the isthmal mucous cells and isthmal glands are stained deeply. ×1,000.

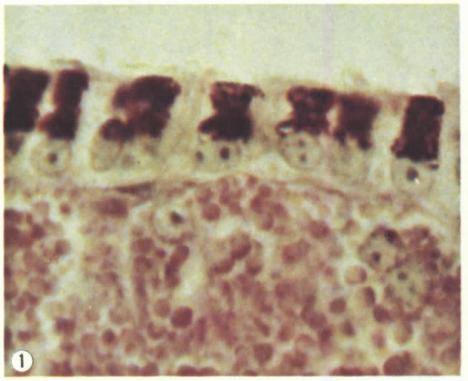
Fig. 2. Anterior portion of the isthmouterine region. PAS reaction. The mucous cells are higher than in the isthmus and stained strongly. Secretion granules in the glands are smaller. The mucous cells are termed as isthmouterine mucous cells. ×400.

Fig. 3. Posterior portion of the isthmouterine region. Alcian blue-periodic acid SCHIFF double stains. In the epithelium mucous cells of isthmouterine type (I) and uterine type (U) are mixed. Glands are parts of the uterine glands. ×400.

Fig. 4. Uterus. Alcian blue-periodic acid SCHIFF. The uterine mucous cells (M) contain alcian blue-positive mucin. The uterine ciliated cells (C) contain PAS-positive granules near the nucleus and pigment gralules (P) in the apical cytoplasm. In the uterine glands no secretion granule is observed. ×1,000.

Fig. 5. Anterior portion of the isthmouterine region. Mercurybromphenol blue stain. The isthmouterine mucous cells are deeply stained. ×1,000.

Fig. 6. Anterior portion of the isthmouterine region. Acetone sudan black stain. Secretions in the mucous cells are reacted intensely. ×1,000.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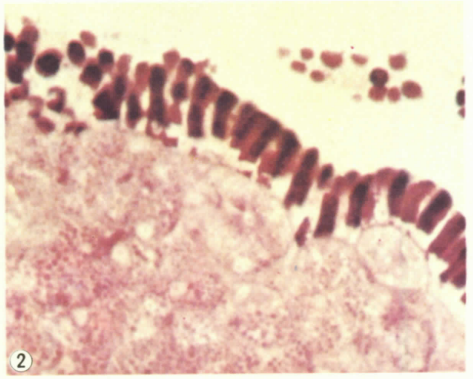
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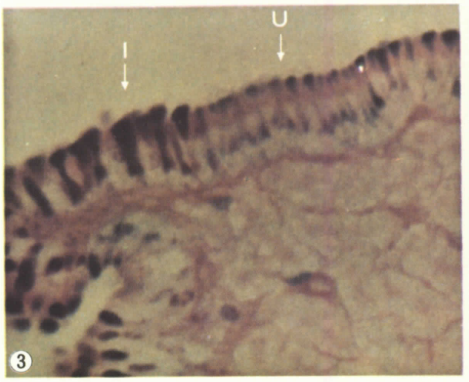
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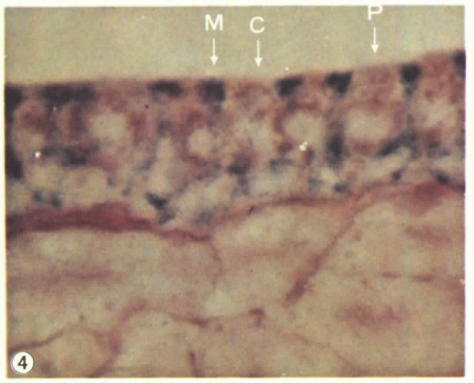
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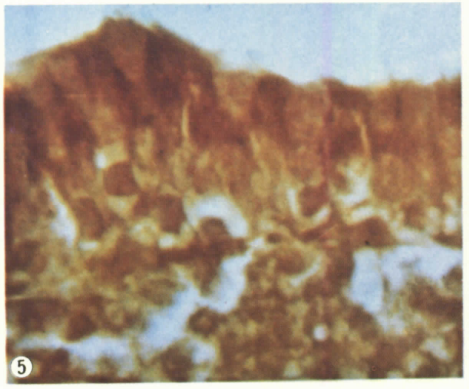
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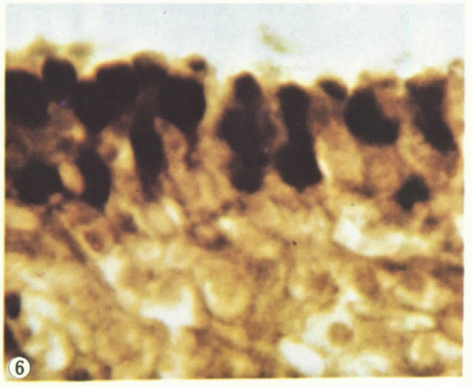
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Plate 2

Figures 7 to 12 show quail egg-coverings.

Fig. 7. A sectioned covering. Alcian blue-periodic acid SCHIFF double stains. An egg-covering is constructed by inner shell membrane (SMi), outer shell membrane (SMo), mammillary layer (Ma), spongy layer or shell matrix (S) and cuticle (Cu). ×150.

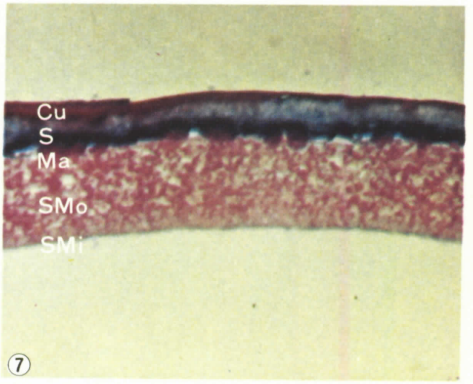
Fig. 8. An enlarged figure of Fig. 1. A mammilla is divided into central core (Cc) and superficial portion (Sp). The central core reacts to PAS, the superficial portial portion to alcian blue. Fibrous arrangement of the superficial portion is more compact than that of shell matrix (S). Central cores are embedded in the outer shell membrane and attached firmly to the fibers. Cu, cuticle. ×1,000.

Fig. 9. A separated shell membrane. Alcian blue-periodic acid SCHIFF. The membrane is stained pink entirely. Bluish spots on the membrane are superficial portions of mammillae. Somewhat deeply stained structures are central cores. ×100.

Fig. 10. A separated shell matrix. Azure A (pH 5.0). The shell matrix is stained metachromatically. Unstained portion is cuticle. ×100.

Fig. 11. A sectioned covering. Mercury-bromphenol blue stain. Central cores (Cc) are deeply stained. ×150.

Fig. 12. A sectioned covering. Acetone sudan black stain for lipoprotein. Central cores (Cc) are strikingly stained. ×150.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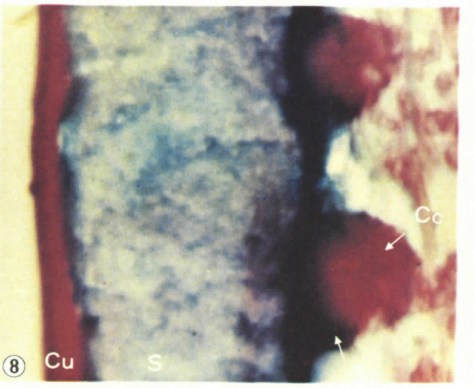
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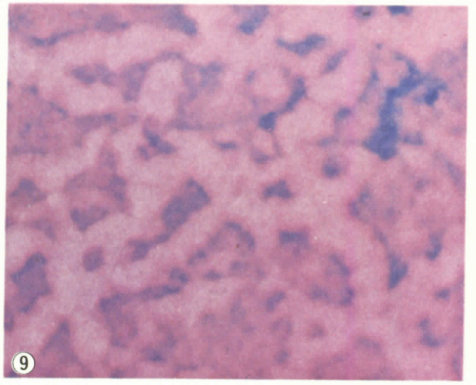
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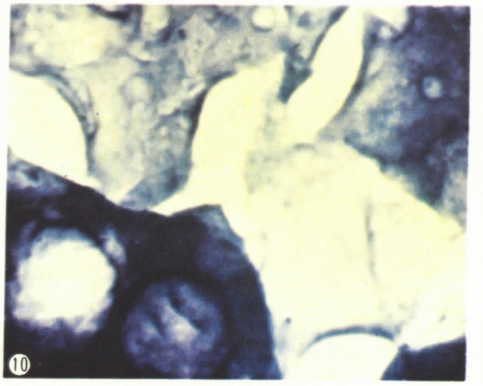
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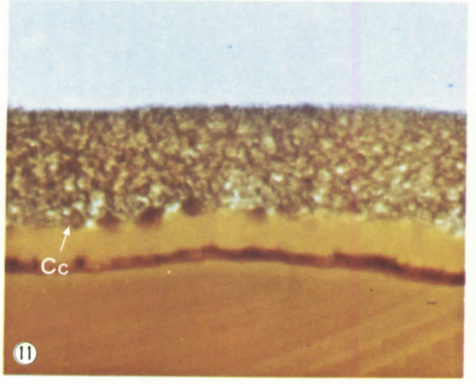
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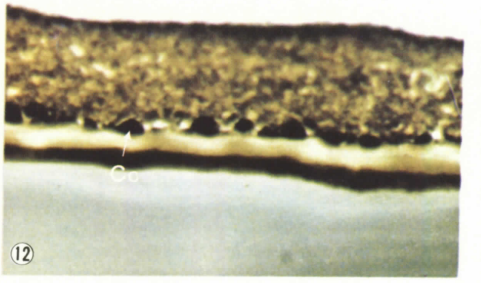
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