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Histological Observations on the Quail Oviduct; with Reference to Pigment (*Porphyrin*) in the Uterus.

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(Tables 1~2; Charts 1~3; Plates 1~4)

The pigments distributed in the egg-coverings including the shell membrane, the shell and the cuticule of various avian egg have been studied for a long time, with reference to the nature, the origin, the formation and mechanism of attaching or deposition of them. Especially, FISCHER & KÖGL (1923)[2] found porphyrins in the shells of various avian eggs and named them ooporphyrin. The porphyrin was demonstrated in the membrane and the cuticule in some kinds of bird and the pigments of coverings have been considered in relation to it. It, however, includs even now some uncleared questions, namely, where the pigments are formed in the oviduct, or where and how the pigments are deposited or attached on the eggs.

The present authors investigated the oviduct of the quail, whose eggs have characteristic figures of deep brown color on the surface, with the object of making clear the origin of the pigment. Consequently, in the uterine mucous epithelium, the authors could prove the pigment, from which the cuticular pigment was considered to be deribed, on the basis of histological observations and some chemical characters.

MATERIALS AND METHODS

1. Total of 25 uteri or oviducts and about 300 eggs of Japanese quail (*Coturnix coturnix japonica*) were made use of throughout this study. 10 uteri were used for observations of macroscopical findings, fresh-frozen sections, solubility in various reagent solutions and absorption spectra of pigment, and the remaining 15 uteri for histological observations. Most of eggs were used for examinations of characters and absorption spectra.
2. For observations of fluorescences, ultraviolet light which was emitted from an Olympus Ultrahigh Voltage Mercury Lamp (200V, 7.2A) attached with two filters, of the BG 1 and 2, was applied to materials. Moreover, microscopically, the F3Y filter was attached onto the ocular lens.
3. Methods for examinations of characters of the cuticular pigment and the uterine pigment are latterly described in their relevant sections.
4. Absorption spectra were measured with a Shimazu Bausch & Lomb Spectronic 20 Colorimeter in the visible range (wave length of 350 to 625 mμ).

5. For histological observations, throughout total materials, the uteri were fixed in ZENKER-formol solution and REGAUD's solution, and in addition, some solution of ZENKER's, LEVI's, neutral formalin, calcium formalin, CARNOY's and BOUIN's were used. The tissues of the uterus were embedded in paraffin and sectioned at 4 to 6 μ thick. For examinations of quantity of the pigment, sections were observed in unstained condition. In staining methods, mainly periodic acid SCHIFF's procedure (PAS-hematoxylin) was availed, and in addition, each staining method of hematoxylin and eosin, HEIDENHAIN's iron hematoxylin and HEIDENHAIN's azan was used.

RESULTS

Presence of Pigment in the Oviduct

As shown in Fig. 1, the oviduct of the quail in laying state could be divided into five divisions, as well as in the domestic fowl, the infudinbulum, the ampulla or magnum, the isthmus, the uterus and the vagina. On outside aspect of the oviduct, distinct deep dark brown coloration was presented in the uterine region (Fig. 1), and more marked coloration was observed on the inner surface of the uterus dissected out (Fig. 2). Such coloration was not shown in the immature.

Under ultraviolet light, the colored portion fluoresced strong red or pink (Figs. 3 and 4).

The quail uterus has sharp demarcations between the isthmus and the vagina, and leaf-like folds develope on the circular ridges in the uterus (Fig. 5). Apparently, the brown coloration and red fluorescence were limitedly observed in the uterus. On the leaf-like folds, the side surface and the basal portion presented darker coloration and more distinct fluorescence. Anywhere the fluorescence was witnessed in the immature oviduct.

Among the uteri in laying state, those containing an egg with calcified white shell showed the deepest coloration and the strongest fluorescence, while those containing an egg covered with figured cuticule showed paler coloration and weak fluorescence.

Uterine liquid obtained from the lumen of the uterus containing an egg with calcified white shell by absorption into absorbent cotton was faint brownish in color and fluoresced distinct pink.

Microscopically, fresh-frozen sections of the uterus with a calcified egg showed coloration of yellow or brown in the mucous epithelium evenly (Fig. 6), and under ultraviolet light, the epithelium fluoresced strong red (Fig. 7). The fluorescence was disappeared quickly in terms of the emittion of ultraviolet light. Portional differences with the coloration and the fluorescence were not observed in the epithelium.

In the sections of the uterus with a figured egg, the coloration and fluorescence were not shown evenly, but only at a few portions, and fluorescence was weak. In sections of the uteri without egg, the coloration and fluorescence were variably in cases, but presented almost similar features with the case of a figured egg.

Colored Figures on the Surface of the Quail Egg

Various figured eggs of brown color of the Japanese quail are presented in Fig. 8. Under ultraviolet light, the red fluorescence was emitted in the colored portions. Also the paler portions emitted the fluorescence, so that differences with the fluorescence were scarcely observed between these two portions.

By immersing the egg in diluted trichloroacetic acid, the cuticule was stripped off as pieces of membrane. Microscopically, the colored portions were observed as the close gathering in rich volume of pigment granules of brown color, and the paler portions as the scatering in minute volume (Fig. 9).

When stripped the cuticule with a razor from the shell surface, the deeply colored portions were thick, while the paler portions thin in those membranes. The surfaces of the shell stripped off the cuticule were white or faint greenish blue tint and did not fluoresce at any portions. From this, the superficial colored figures of the quail egg were represented by the cuticular pigment which had fluorescent character. The pigment investigated by present authors in the egg was this cuticular one.

Solubility of the Pigments in Uterus and Cuticule for Various Solutions

The solubility of the uterine and the cuticular pigments for the various reagent solutions was tested. As the materials, the cuticule was stripped off with a razor, and the uterus was dissected into pieces of the tissue. These materials were immersed in various reagent solutions, then observed mainly in the secondary fluorescences of the filtered solutions. Additionally, the frozen slices of the uterus on the slide glass mounted the same solutions were observed under microscope.

Table 1 presents the secondary fluorescences in these extracted solutions.

Both pigments were well dissolved in metalic acids and the solutions showed redish colors and fluoresced strong pink to red.

In these metalic acids singly, however, the pigments were not perfectly dissolved, and only HCl-methanol (1:19) dissolved almost completely the pigments. The solution presented greenish red in color and the fluorescence was strongest among any other solutions. In organic acids, trichloroacetic acid extracted fluorescent material accompanying only faint pale green color. Moreover, in alkaline solutions, NaOH and KOH solutions presented faint fluorescence but almost no color and, in organic solvents, methanol fluoresced.

From these tests, it is characterized that the uterine and the cuticular pigments were fairly well dissolved in metalic acid solutions, especially to perfection in HCl-methanol, and emitted fluorescence in these solutions.

Table 1. Solubility of Fluorescent Pigments of the Uterus and Cuticule

Solvents	Intensity of Fluorescence of Solutions	
	Cuticule	Uterus
HCl (5-10%)	++++	+++
H_2SO_4 (5-10%)	+++	+
HNO_3 (10%)	+++	+
HCl-Methanol (1:19)	+++++	+++
CCl_3COOH (5-10%)	+++	++
CH_3COOH (10%)	−	−
H_2O_2 (5%)	−	−
NaOH (10%)	+	±
NH_4OH (10%)	±	−
KOH (10%)	−	±
Methanol	+	++
Aceton	−	±
Chloroform	−	±
Benzol	−	−
Ether	−	±
Carnoy's Solution	+	+

Remarks : − ; non fluorescent, ± ;very weak or non,
+ ; weak, ++ ; moderate, +++ to +++++ ; strong

Absorption Spectra of the Pigments

Each of the uterine and the cuticular pigments was consumptive as a porphyrin based upon solubility for metalic acid solutions, primary and secondary fluorescences and brown or yellow color of them. So that the authors attempted to confirm porphyrin about these two kinds of pigments through examing absorption spectra.

The uterine and the cuticular pigments were extracted into HCl-methanol following the porphyrin-methylester method of FISCHER & KÖGL (1923)[2]. The solutions were red in color and fluoresced in both materials. Then, absorption maxima were measured in HCl-methanol solutions and in chloroform solutions, in which the precipitates produced by adding lN-NaOH to the HCl-methanol solutions of the pigments were dissolved. In addition, the solution obtained by rinsing quickly the uterine lumen with HCl-methanol was measured as uterine liquid. These absorption spectra are presented in Charts 1 and 2.

In each measured solution, distinct SORET band, which is characteristic to porphyrin and has absorption maximum near 400 mμ wave length, was presented and these spectra showed similar absorption maxima each other.

In Table 2, their absorption maxima are compared with those of ooporphyrin extracted from the shells of various avian eggs and so named by FISCHERE & KÖGL (1923).[2]

Chart 1. Absorption spectra of methyl-esterified pigments of cuticule, uterine tissue and uterine liquid (in HCl-methanol solution)

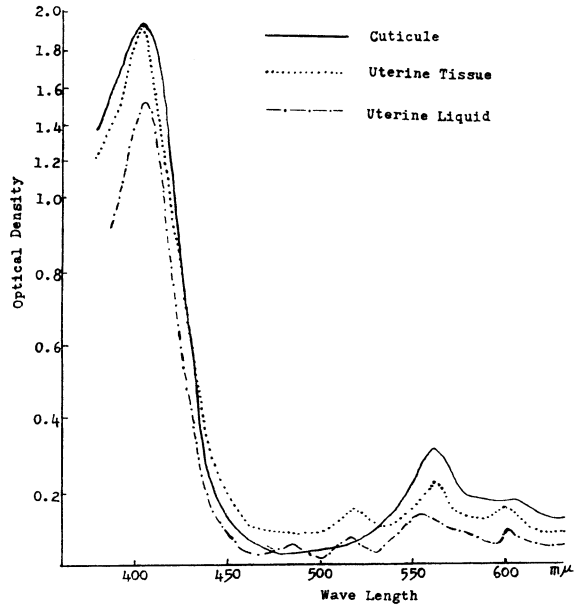

Chart 2. Absorption spectra of methyl-esterified pigments of cuticule and uterine tissue (in chloroform solution).

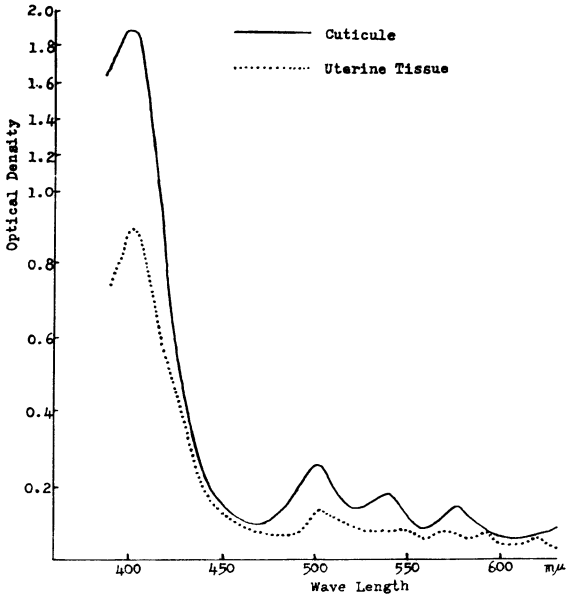

Table 2. Absorption Maxima of Pigments in 3 Parts (mμ)

Solution of Extraciton	HCl-Methanol				HCl-Methanol				Diluted HCl	
Measured Solution	HCl-Methanol				Chloroform				Diluted HCl	
FISCHER & KÖGL			II	I	IV	III	II	I	II	I
Ooporphyrin			557-560,	602-605,	505-508,	539-540,	574-579,	628-630,	558,	602,
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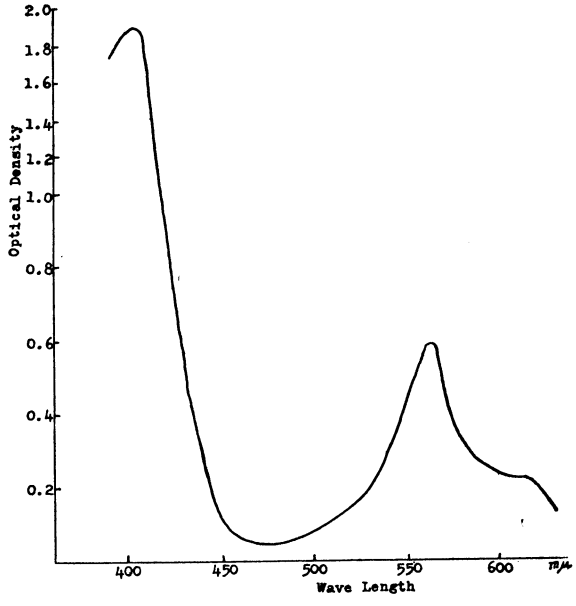
Cuticule	400-405,		560,		400-405,	500,	540,	575,	405,	560,
Uterine Tissue	400-405,	520,	560,	600,	400-405,	505,	(570),	(590), (620)	——	
Uterine Liquid	400-405,	485, 520,	550,	600,	——				——	

Note: ——; could not be extracted to measure

In many points, the absorption spectra of the pigments in HCl-methanol and chloroform solutions are similar to the spectra of it.

Moreover, though free porphyrin is given from the diluted HCl-solution of refined cristalline porphyrin (FISCHER & KÖGL, 1923),[2] the present authors measured the absorption maxima of the cuticular pigment solution in 2N-HCl, in which cuticular pigment was well dissolved. These data are presented in Chart 3 and compared with ooporphyrin of FISCHER & KÖGL in Table 2.

Chart 3. Absorption spectrum of cuticular pigment (in diluted HCl).



From this series of examinations of absorption spectra, the present authors concluded that the uterine and the cuticular pigments showing fluorescent characters are porphyrins may be taken as similar to ooporphyrin.

Histological Observations on the Uterine Mucous Epithelium

As above-mentioned, the present authors found the fluorescent pigment in the uterine epithelium and made clear the characters of the pigments of the cuticule and the uterus as a porphyrin in each of them. In addtion to these findings, the uterine epithelial cells were observed with reference to the pigment.

For these purposes, influences of fixation to the pigment were tested in advance. Two solutions of REGAEUD's and ZENKER-formol well left the pigment, but, in contrast to them, CARNOY's and BOUIN's solutions made the pigment invisible of the mucous

epithelium. Neutral formalin, BAKER's calcium formalin, ZENKER's and LEVI's solutions, presented moderate result for remaining the pigment, respectively. Therefore, the histological findings were obtained mainly from the preparates fixed with ZENKER-formol and REGAUD's solutions fixing even minute volume of the pigment.

In laying quail, two kinds of cell type are distinguished according to the position of the nuclei, the cilia and the forms of the cells, in the mucous epithelium as well as in the domestic fowl described by RICHARDSON (1935),[8] that is, the ciliated apical cell and the non-ciliated basal cell (Fig. 10).

The tissue, from the uterus with a calcified white egg (3 materials), commonly presented a layer of yellow or brown pigment granules in the epithelium near the superficial area (Fig. 11), as well as in the frozen sections. In high magnifications (Figs. 12 and 13), these pigment granules were gathered at the apical ends of the apical cells. In the sections stained with PAS procedure, the PAS positive granules were observed in the same cells, in which the granules occupied the supranuclear region and some of them stretched to the region of the pigment granular accumulations (Fig. 18). These PAS positive granules were also stained with iron hematoxylin and eosin. In each material of this group, appearances of the PAS positive granules were similar, namely, most of the apical cells filled the granules in the supranuclear regions and in some cells the granules were seen infranuclear regions. Otherwise, the pigment granules were not observed in the infranuclear regions. Moreover, sometimes, an empty region was seen between the two accumulations of the PAS positive granules and the pigment (Fig. 19). This region may be considered to be the GOLGI negative. In this present study, the authors have not confirmed the secreting or releasing features of these granules in the apical cells.

In the uterus with a figured egg (1 material), only preparates with REGAUD's solution disclosed the pigment granules in a few apical cells in a minute quantity, and the PAS positive granules in the apical cells were seen in some of the preparates in a smaller quantity than in the tissues of a white calcified egg (Figs. 14 and 20).

In the sections of uteri without egg (5 materials), variable findings as to the pigment and the PAS positive granules were observed by individuals. In two cases, the pigment granules were few and the apical cells containing the PAS positive granules were few also (Figs. 15 and 21). In other two cases, a middle volume of the pigment granules were noticeable in the supranuclear region in many apical cells (Figs. 16 and 22). Also the PAS positive granules were observable in the supranuclear regions of the cells. In the other one residual case, the pigments were presented in large quantity and they formed the superficial layer at many portions, and the PAS positive granules were contained in the apical cells in various volume (Figs. 16 and 23).

In immature and resting state (5 and 1 materials), neither pigment nor the PAS positive granule were seen.

Otherwise, the observations on the non-ciliated basal cells in each preparate did not offer clear and remarkable findings on the PAS positive reaction.

It is most interesting, from these findings, that, with egg-formation cycle in the oviduct of the quail, changes in volumes of the pigment granules, and that the ap-

pearances of the PAS positive ones were parallel to the pigment.

DISCUSSION

Figures of various forms and colors on the surface of the many kinds of avian eggs have been interested for a long time. Although some workers have reported on such subjects as where the pigments are formed, and as where and how the pigments deposited or attached in the egg-coverings, even now these subjects have contained some uncleared problems.

Thus, the present study was attempted to make clear the problem on the formation of such pigment in the oviduct of the quail, from which the eggs characterized in the typical superficial colors of the coverings are laid.

Each of the regions from the ovarium to the end of the oviduct was indicated as the portion of the pigment formation by some workers; that is, WICKMANN (1880)[13] pointed out the ovarium, GIERSBERG (1921)[5] the infundibulum and the magnum, ASMUNDSON (1931)[1] the isthmus, TURCHINI (1924)[12] the uterus and SURFACE (1912)[10] the vagina as the portion of it, respectively.

In the present investigation on the oviduct of the Japanese quail, only the uterus was colored in brown and fluoresced pink to red, and, moreover, it was proved that the coloration and the fluorescence were revealed by the fluorescent and brown pigment granules in the apical cells of the uterine mucous epithelium, through the observation of fresh-frozen sections as well as paraffin embedded sections of the uterine tissue.

On the other hand, the colored figures of the surface in the egg of the Japanese quail were, in agreement with the findings of VON NATHSIUS (1893)[11] of the European quail, observed to be represented by the fluorescent cuticular pigment, and the shell was not to fluoresce in spite of its faint bluish or greenish difuse coloration. Thereupon, the former belongs to the superficial, while the latter to the ground pigment, and the present investigation deals with the former.

Comparative examinations in color, primary and secondary fluorescences, solubility of the uterine and the cuticular pigments and uterine liquid were introduced as similar characters, especially such as the solubility and the secondary distinct fluorescence in various metalic acid solutions, and the characters peculiar to the porphyrin.

The porphyrin in the avian egg was found, for the first time, in the shells of various avian eggs by FISCHER & KÖGL (1923)[2], who named them ooporphyrin, thereafter in the cuticule by FURREG (1931)[3] and in the shell membrane by KLOSE & ALMQUIST (1937)[6].

Moreover, the present authors confirmed that both of the uterine and the cuticular pigment were porphyrin through examinations of absorption spectra of them, and presented those of ooporphyrin-methylester described by FISCHER & KÖGL (1923)[2]. From these similarities in their characters of the uterine and the cuticular pigment, it may be recognised that the cuticular pigment is deribed from the pigment in the uterine epithelial cells.

In the literiature on the subject of the pigment-formation in the oviduct, only

TURCHINI(1924)[12] mentioned secretion of porphyrin in the ciliated cells in the uterus, his report, however, is insufficient both in the description and the morphological evidences.

As to the description on porphyrin in the epithelial cells of the organs of other kind, the glandular epithelial cells of the HARDERIAN gland in the rat was observed by GRAFFLIN (1942)[4].

On the other hand, the histological or histochemical method aiming at the demonstration of the porphyrin has not been found even up to the present time, and the fluorescence of it in fresh condition has been described as only sharp method of it (PEARSE, 1961)[7]. Therefore, the selection of fixative for the histological observation on the pigment is of highest importance. The authors' results in the uterine pigment, in agreement with the description of GRAFFLIN (1942)[4] in the HARDERIAN gland, denoted REGAUD's solution and ZENKER-formol as the best fixative for the purpose.

In the present study, the histological observation of the uterine epithelium was performed mainly about the changes, related to the egg-formation cycle, of the features of the pigment and the PAS positive granules in the apical cells.

Most remarkable and interesting was the extreme differences of the pigment granules in the apical cells between two kinds of state of the uterus, with a white calcified egg and with a figured egg. Namely, in the former condition the pigment granules accumulated near the free surface of the cells showing a superficeal layer of the pigment accumulation in the epithelium, while in the latter only a few pigment granules were seen dispersed in the cell without showing the layer. These fingings may be easily explained by interposing between each condition, of coloration of the egg, the former was considered as the accumulation of the pigment and the latter as the phase released the pigment from the cells.

Also, in this connection, the uterus without containing an egg showed various volumes of the pigment in the cells, while may be due to the time after laying. Thus, the features of the pigment granules in the apical cells in the uterine epithelium were thought as the changes related with the egg-formation cycle. Although the present investigation could not clarify the figures of the secretion and release of the pigment, the fluorescent pigment in the uterine epithelial cell may be changed into the cuticular pigment on the basis of the histological and the chemical findings.

Only GIERSBERG (1923)[5] mentioned the conception abou tthe pigment formation in the oviduct of halks and others, and so his description has been cited by ROMANOFF & ROMANOFF (1949),[9] and FISCHER & KÖGL (1923)[2] respectively. He was the opinion that the pigments of egg-coverings are formed in the infundibulum and the magnum, in which the red coupscls infiltrated from the subepithelial capillaries are changed into the pigment through being intaken into wandering cells. Despite his interesting opinion, his description did not deal with morphological evidences. The present authors' findings, at least in the quail, may not accept GIERSBERG's explanation.

Moreover, the PAS positive material appeared with distinct appearances in the ciliated apical cells, against indistinctness in the basal cells. Especially, in the uterus containing a white calcified egg, the apical cells contained the PAS positive granules of large volume in the supranuclear region and the pigment accumulation near the surface

in the cells, while, in the uterus containing a figured egg, the cell contained little volume of the PAS positive granules and the pigment granules. Thus, although also about those PAS positive granules secretion and releasing feature were not made clear, the features of the PAS positive granules may be safely assumed to be related to the cuticular formation.

SUMMARY

The present study was undertaken in an effort to clarify the portion where the pigment of the egg-covering are formed in the Japanese quail oviduct. Results obtained and conclusion reached may be summarized as follows:

1. The mucous membrane of the uterus in laying state colors in dark brown and fluoresces strongly red color. In addition, also the uterine liquid fluoresces red color. Microscopically, the coloration and the fluorescence of the uterus are represented by the yellow or brown pigment granules in the apical cells of the mucous epithelium.
2. The colored figures in the surface of the egg are represented by the pigment embedded in the cuticule, cuticular pigment. This pigment is granular, brown and fluorescent in red. In the present study, this pigment was dealt. The shell surface of the egg are white or faint greenish or bluish, and non-fluorescent.
3. The pigment of the cuticule and the uterine epithelium show such similarities as yellow or brown color, granular nature, solubility in metalic acid solutions, primary and secondary fluorescence in red color, and these characters are peculiar to porphyrin.
4. Absorption maxima of the pigments in the uterus and in the cuticule were measured in methylester-form and SORET band is presented in each spectrum, so that these two pigments are porphyrins. Moreover, these spectra contain some of the absorption maxima corresponded with those of ooporphyrin described by FISCHER & KÖGL.
5. The utrine pigment is fixed well in REGAUD'S solution and ZENKER-formol and disappeared in CARNOY'S or BOUIN'S solution.
6. The uterine pigment changes in the volume of the granules in the apical cells related to the egg-formation cycle; that is, in the uterus containing a calcified white egg the granules are accumulated in the cells and in the uterus containing a colored egg, the granules are little in each of these cells, and in post-layed state, the volume of the granules are variable. Accordingly, this pigment granule may be considered as secreted in the cells and released to cuticular pigment in the uterine lumen.
7. In the apical cells, also, the PAS positive granules are observable. These granules present feautres in their volumes showing parallel to the pigment granules. Therefore, the granules, too, may be taked as secred and released with the pigment granule, and thought to be related with the cuticular formation.

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うずらの卵管の組織学的観察，とくに子宮部の色素 (*porphyrin*) について

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鳥類の卵の卵表の色素形成については，いまだ不明確な点が多いが，うずらを用いて色素形成に関する形態学的観察をおこなった．

うずら卵管では肉眼的に，子宮に限って著明な濃褐色の着色と赤色螢光がみとめられ，これは凍結切片上その粘膜上皮に存する．これは産卵時期により変化し，白色卵殻の卵を子宮に含むときもっとも著明であるが，着色した卵を含むときもっとも弱い．一方，卵表の色彩はクチクラ色素により発現され，この色素は赤色螢光を発する．子宮およびクチクラの色素は共に鉱酸によく溶解し，強い2次螢光を発する．両者の主としてメチルエステルの吸収スペクトルを求めた結果，それぞれ SORET 帯を有し，*porphyrin* であることを示し，かつ FISHER & KÖGL の *ooporphyrin* と類似のスペクトルを示した．

組織学的には，色素は子宮粘膜上皮の繊毛細胞 (apical cell) に認められる．この色素顆粒は産卵時期に伴って著変し，肉眼的ならびに凍結切片上の所見と一致する．従って本細胞からクチクラ色素が分泌されると考えられる．

また本細胞には PAS 反応陽性の粗大顆粒が認められ，色素顆粒の増減とほぼ平行した関係を示す．これは，クチクラ形成と関係があると考えられる．

EXPLANATION OF FIGURES

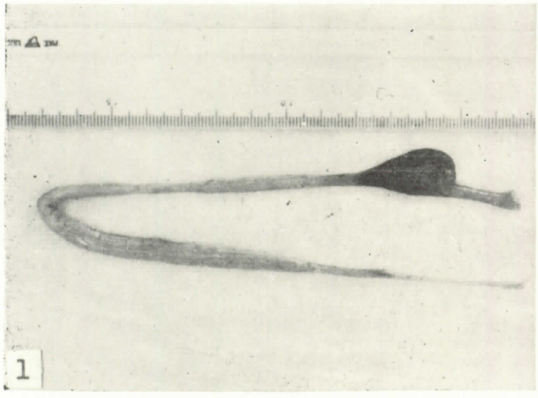
Plate 1

Fig. 1. Oviduct of the quail in laying state. Brownish dark coloration is distinctly shown in the uterus. × 1/2.2

Fig. 2. The oviduct dissected out to show the inner surface. × 1/2.5

Figs. 3. and 4. Photographs of the oviducts under ultraviolet light. Intense fluorescence of red color (dark in photograph) is shown corresponded to the coloration in the uterus × 1/2.2 and × 1/2.5

Fig. 5. The mucous surface of the uterus. Demarcations of the uterus between the isthmus (I) and the utero-vaginal region (V) are sharp and circular ridges on which leaf-like folds arrange closely are developed in the uterus (U). × 1.6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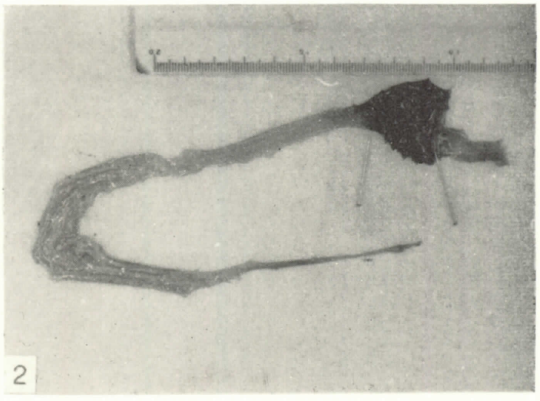
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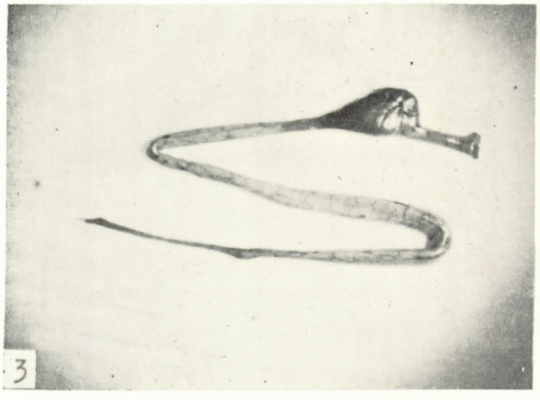
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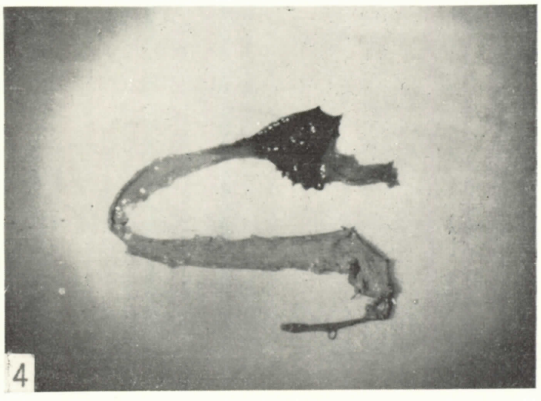
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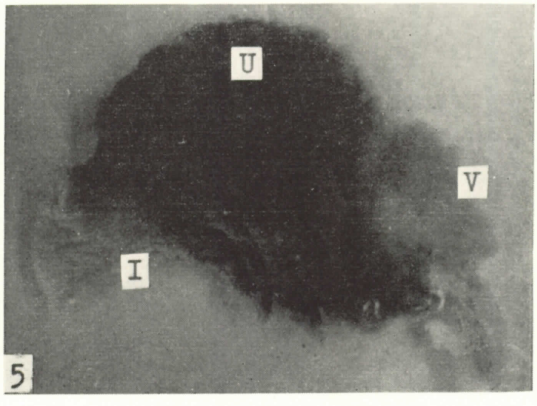
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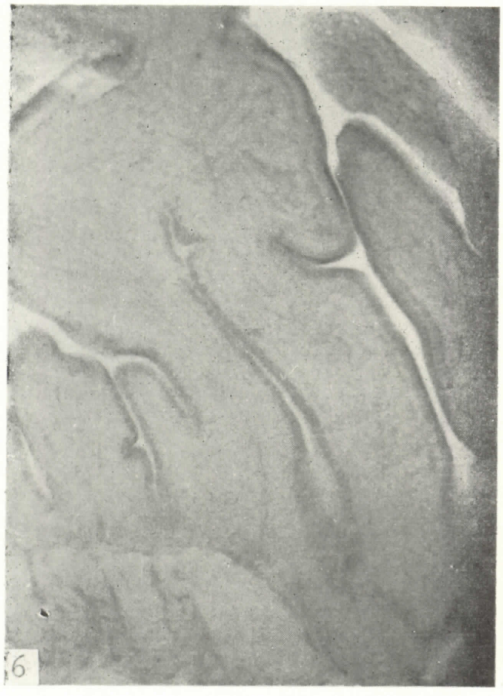
Plate 2

Fig. 6. Frozen section of leaf-like folds of the uterus in laying state. Brown coloration is seen in the mucous epithelium. × 100

Fig. 7. Photograph under ultraviolet light of the leaf-like folds. The mucous epithelium fluoreses red (dark in photograph). ×100

Fig. 8. Various figures of brown color on the surface of the Japanese quail eggs. White eggs of upper right were removed from uterus before coloration. × 1/2.5

Fig. 9. A piece of membrane of the cuticule. The brown pigment granules are distributed in both deeply colored portion (left) closely and pale portion (right) dispersely. × 100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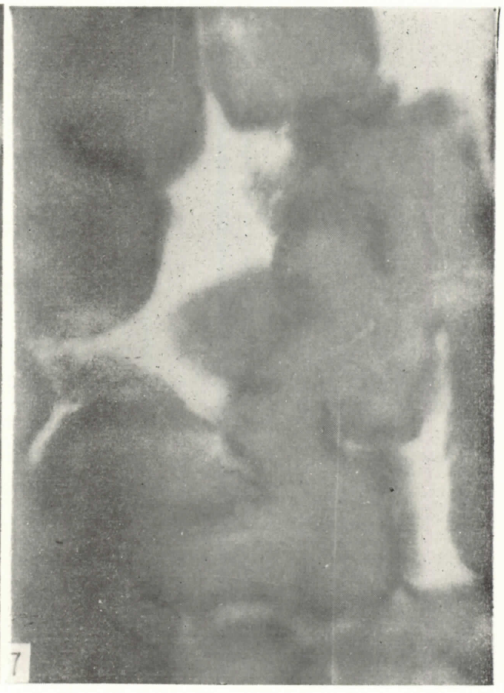
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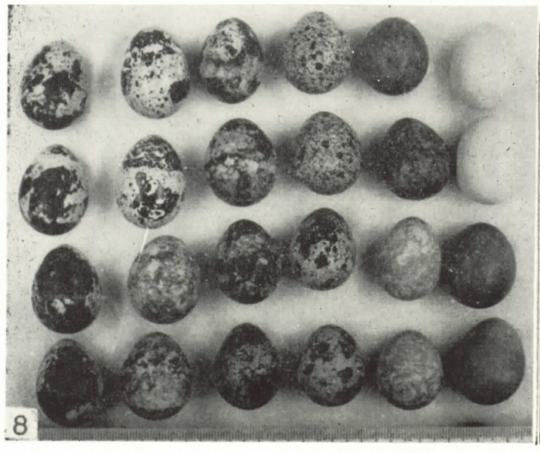
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Plate 3

Fig. 10. The mucous epithelium of the uterus. Apical cells (A) with apically positted nuclei and cilia and basal cells (B) with basally positted nuclei without cilia are distinguished in the epithelial cells. Hematoxylin-eosin stain. × 1,000

Figures 11 to 17 are photographs of unstained paraffin sections of uteri showing various laying states.

Fig. 11. Egg with white calcified shell in the uterus. Yellow to brown coloration is observed evenly in the superficial region of the epithelium as forming a superficial layer. × 100

Fig. 12. Middle magnification of Fig. 11. × 400

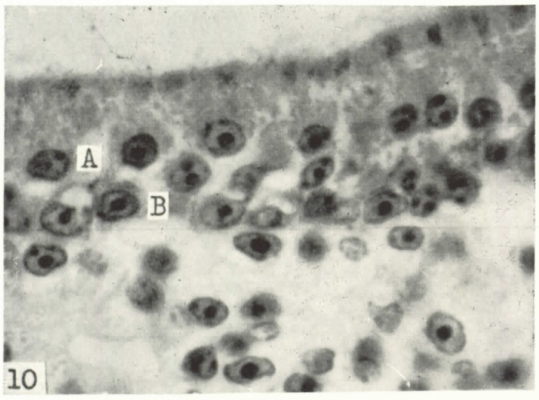
Fig. 13. High magnification of Fig. 11. The coloration of epithelium is recognized as dense accumulations of brown pigment granules in the tips of apical cells. Few granules are seen in inner portions of the cells. × 1,000

Fig. 14. Egg with figured cuticule in the uterus. Only a few pigment granules are seen in the apical cells dispersely (arrow). Marked difference between this feature and Fig. 13 is observed. × 1, 000

Fig. 15. No egg in the uterus. Only a few granules are seen (arrow). × 1,000

Fig. 16. No egg in the uterus. Pigment granules of a small quantity are seen throughout the epithelium in the supranuclear region (arrow). × 1,000

Fig. 17. No egg in the uterus. Pigment granules of a large volume are seen in the free surface of the apical cells. This features are observed throughout the epithelium. × 1,000



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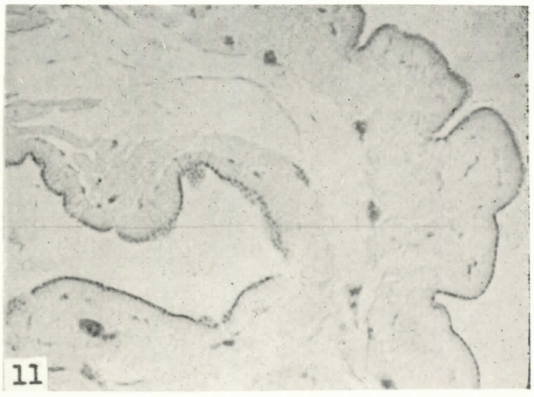
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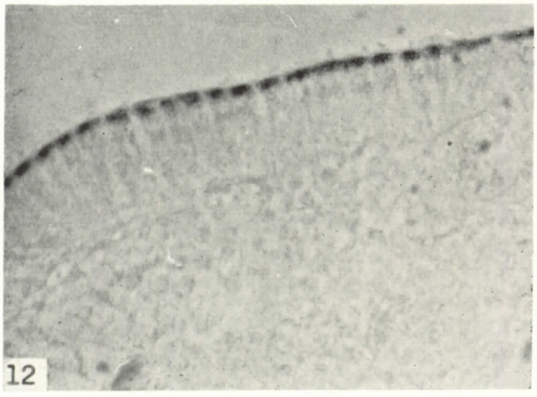
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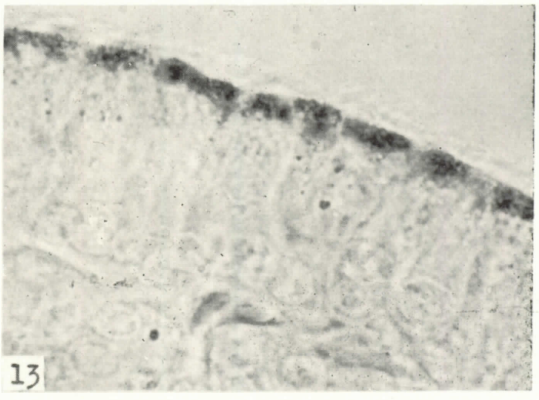
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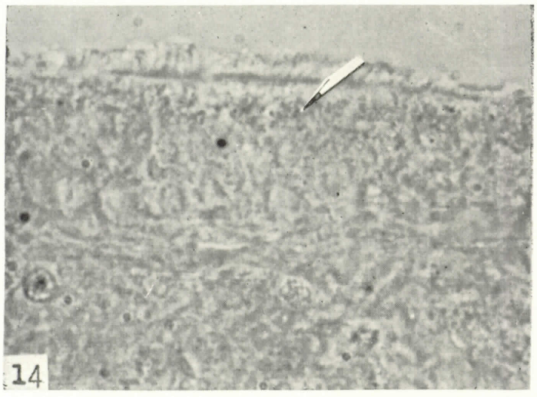
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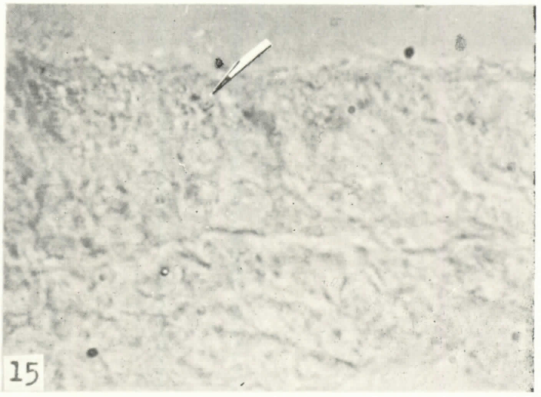
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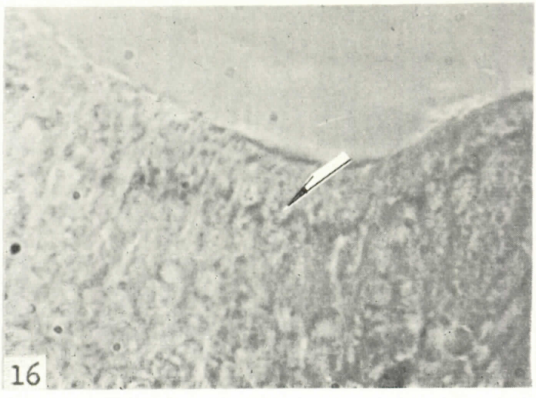
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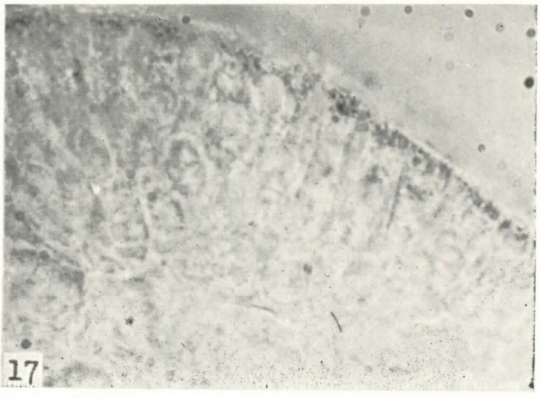
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Plate 4

Figures 18 to 23 are photographs of sections stained with PAS procedure of uteri showing various laying states.

Fig. 18. Egg with white calcified shell in the uterus. PAS positive large granules are seen mainly in supranuclear regions of the apical cells (PA) and the pigment granules in the free surface of the cells (PG). The basal cells containing PAS positive granules are few. × 1,000

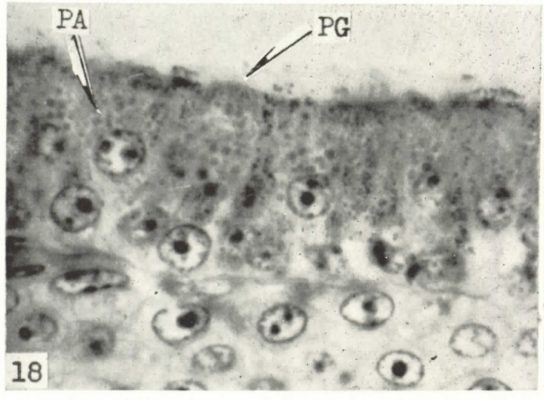
Fig. 19. Egg with white calcified shell in the uterus. Spaces between both accumutations of pigment granules and the PAS positive granules are seen and may be thought as the GOLGI negative. × 1,000

Fig. 20. Egg with figured cuticule in the uterus. PAS positive granules are not seen in the apical cells showing fine vacuolar plasm. The basal cells containing PAS postitve granules, smaller than in the apical cells, are many. × 1,000

Fig. 21. No egg in the uterus. PAS positive granules are seen in neither the apical cells nor tne basal. × 1,000

Fig. 22. No egg in the uterus. A few PAS positive granules are seen in the apical cells in the supranuclear regions. × 1,000

Fig. 23. No egg in the uterus. Much volume of PAS positive granules are seen in both apical and basal cells. The pigment granules accumulate in the free surface of the apical cells. × 1,000



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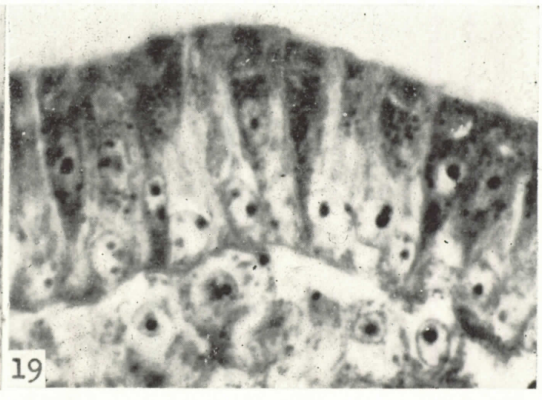
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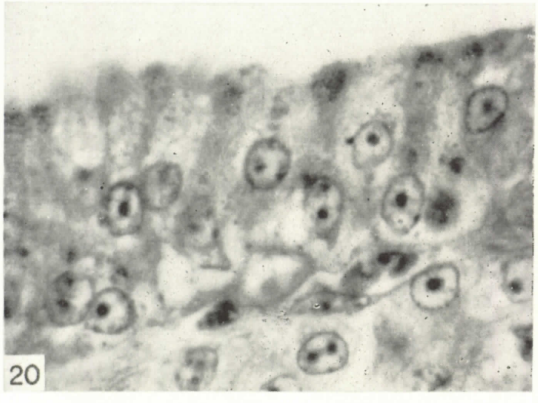
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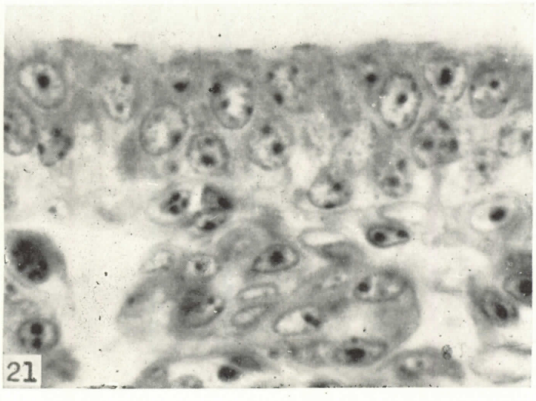
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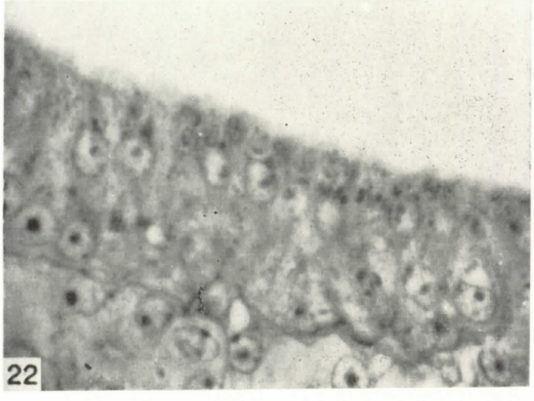
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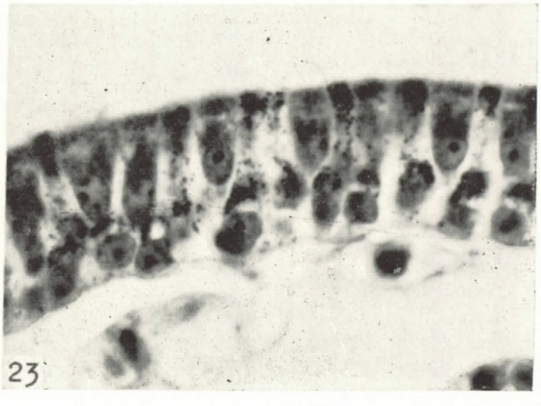
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