

Comparative Observations on the Distribution of Fluorescent Pigments (Porphyrins) in the Chicken and Quail Uteri

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(Table 1; Plate 1)

The mechanism of pigmentation in avian eggs has been given little account of, although fluorescent porphyrin pigments were presented in the coverings of eggs of various kinds of birds (FISCHER and KÖGL, 1923;¹⁾ TURCHINI, 1924;²⁾ FURREG, 1931;³⁾ KLOSE and ALMQUIST, 1937;⁴⁾ TAMURA et al., 1965⁵⁾). TURCHINI (1924)²⁾ reported the occurrence of a porphyrin in the uterus of chicken oviducts, however, his description was neither detailed, nor referred to the process of its deposition to eggs. TAMURA et al. (1965⁵⁾, 1966⁶⁾) reported some relationships between porphyrin pigments in the uterus of the quail oviducts and coloration of the cuticle of their eggs.

Recently, the present writers (1967)⁷⁾ examined porphyrin pigments in the coverings of eggs of the Rhode Island Red, White Leghorn and quail, and found that it was distributed in the cuticles and shells, apart from differences of amounts of the pigments. In order to clarify the process of the pigmentation of such egg-coverings, the writers made a comparative observation amongst the oviducts of the Rhode Island Red, White Leghorn and quail, with special reference to the distribution of fluorescent pigments (porphyrins).

MATERIALS AND METHODS

As shown in Table 1, oviducts of eleven quails, four Rhode Island Reds and seven White Leghorns were used in this investigation.

Examinations were made macroscopically and microscopically both in ultraviolet light for fluorescence and in ordinary light for coloration, and the following procedures were performed.

- 1) Macroscopical examination of coloration of oviducts.
- 2) Macroscopical examination of fluorescence of oviducts.
- 3) Microscopical examination of fluorescence of uterine tissues in frozen sections.
- 4) Microscopical examination of pigment granules of uterine tissues in frozen sections.
- 5) Microscopical examination of pigment granules of uterine tissues in paraffin sections.

Ultraviolet light was generated by a mercury lamp (Olympus) and projected

Table 1. Cases from which Oviducts Were Obtained

Position of Egg Kinds of Birds	Magnum	Magnum to Isthmus	Isthmus	Uterus			Oviduct After Laid
				Membrane- Egg	Shell- Egg	Complete Egg	
Rhode Island Red	No. 181		No. 235	No. 182 No. 208			
White Leghorn	No. 202			No. 207 No. 209 No. 246	No. 236		No. 201 No. 203
Quail	No. 101 No. 103	No. 268		No. 127 No. 128	No. 105 No. 106 No. 107	No. 113 No. 119	No. 118

to materials through two blue filters. Besides one yellow filter was set on an ocular lens in microscopical examination.

For paraffin sections, specimens of the uterine tissues were fixed in fluid of REGAUD's, ZENKER-formalin, LEVI's and 10% neutralized formalin respectively. Following the fixation, the specimens were embedded in paraffin, and sectioned 5-10 μ . Unfixed fresh specimens of the uterine tissues were frozen and sectioned 20-30 μ .

RESULTS

Macroscopical Observations

Brown colorations of various depths were observed on the mucous membrane throughout the uterus of the Rhode Island Red, White Leghorn and quail oviducts. In accordance with these colored regions, red or pink fluorescences were found. The strength of fluorescences was observed in parallel with the depth of their colorations. Colorations and fluorescences of the quail uteri were deeper and stronger than those of the Rhode Island Red and White Leghorn uteri. Especially, the quail uteri belonging to shell formation period presented the most conspicuous coloration of blackish-brown and the strongest fluorescence. Those of the Rhode Island Red uteri were medial and somewhat deeper and stronger than those of the White Leghorn.

In ultraviolet light, the fluorescence was weakened and faded in a short time. In the isthmo-uterine and utero-vaginal junctional regions of every oviduct, the coloration was indefinite, but slight fluorescences were partially observed.

From macroscopical observations, it was apparent that, these colors of the uterine mucous membrane of these birds were revealed by porphyrin pigments on account of their fluorescent characters. Moreover, it was noted that, the differences

in their colors were found between the quail, Rhode Island Red and White Leghorn uteri, and that the coloration was observed, also, in the White Leghorn uteri.

Microscopical Observations of Frozen Sections

Distributions of pigment granules and fluorescences were varied according to the kinds of birds. In the sections of the quail uteri at shell formation period, strong red fluorescences were found evenly in the mucous epithelium and, in the ordinary light, collected yellow or brown pigment granules were found there. The uterine tissues of the quails at the other periods of egg formation, presented weak fluorescences, but the pigment granule was invisible. Neither the fluorescence nor the pigment granule was found in the uterine glands of the quails. The fluorescence faded quickly and disappeared in the ultraviolet light. Thus, quick examination was required, especially in the weakly fluoresced ones, and the photographing of them was all the more difficult.

In the tissues of the Rhode Island Red and White Leghorn uteri, weak fluorescences of pink colors were observed in the mucous epithelium almost evenly. However, distribution of definite pigment granules was not found in such fluoresced epithelium. In these uterine glands, the fluorescence and pigment granule were not observed.

Microscopical Observations of Paraffin Sections

In the quail uterine tissues, various amounts of brown pigment granules were found in the apical ciliated cells of the mucous epithelium. In the sections of the uteri belonging to shell formation, the pigment granules were collected in the apical cytoplasm of the cells. In the uterine glands, the pigment granule was invisible.

In the tissues of two Rhode Island Red uteri, a few pigment granules were observed in the apical cells of the mucous epithelium. The granules were brown, and somewhat larger in diameter than those of the quail tissues. The pigment granule was not found in the uterine glands.

In the tissues of the White Leghorn uteri, the pigment granule was not found in the epithelium. In the uterine glands, the definite pigment granule was not observed, but in one case of them, where slightly colored eggs were laid, a few pigment-like granules were observed in the glands.

In the microscopical observation, the fluorescence was observed in the mucous epithelium of each uterus. However, the pigment granules were readily observed only in some quail uteri belonging to the shell formation period. In the others, the pigment granule was not observed in their frozen sections, and through careful searching of the paraffin sections, a few pigment granules were visible in the epithelium of the quail and Rhode Island Red uteri. In the uterine glands, the fluorescence and pigment granule were invisible.

DISCUSSION

The distribution of porphyrin pigments in the egg-coverings of various kinds

of birds was proved by the investigations of FISCHER and KÖGL (1923)¹⁾ in the shells of eggs of various kinds of birds, of KLOSE and ALMQUIST (1937)⁴⁾ in the shell-membrane of chicken eggs, and of TAMURA et al. (1965)⁵⁾ in the cuticle of quail eggs.

Nevertheless, only a few workers referred partially to the mechanism of pigmentation in their egg-coverings (GIERSBERG, 1921⁸⁾; TURCHINI, 1924²⁾; FURREG, 1931³⁾; WARREN and CONRAD, 1942⁹⁾; WOODARD and MATHER, 1964¹⁰⁾; TAMURA et al., 1965⁵⁾, 1966⁶⁾). Amongst these investigators, TAMURA et al. found that the cuticular pigment of quail eggs originated from the apical ciliated cells of the uterine mucous epithelium by histological as well as spectrophotomecal examinations.

Recently, the present writers (1967)⁷⁾ examined the pigment distribution in each layer of the coverings of quail eggs as conspicuously colored materials, in that of Rhode Island Red eggs as medially, and in that of White Leghorn eggs as scarcely. And they found that the cuticles and shells of these eggs were pigmentated with fluorescent pigments (porphyrins).

In this study, the writers clarified distributions of fluorescent pigments in the uteri of these various kinds of birds, that is; on the mucous membrane of each uterus of the quail, Rhode Island Red and White Leghorn oviducts, there were found evenly distributed fluorescences of red to pink colors and coloration of deep to light brown. Therefore, it was apparent that, porphyrin pigments characteristic of their red fluorescences were distributed, in various densities, in the uteri of these oviducts.

In frozen sections of these uterine tissues, such fluorescences were observed in the mucous epithelium of each material, but not in the uterine glands. Brown pigment granules were visible in frozen sections of some of these materials. However, in the others, they were invisible. Namely, only in the quail uteri at the period of shell formation, the pigment granules were readily observed, and they were visible with careful examination of paraffin sections of the quails at the other periods of egg formation and ones of two Rhode Island Reds. But they were invisible in the uteri of the other Rhode Island Reds and the White Leghorns.

The fixatives used in this study had been reported as effective for preservation of porphyrin pigments (TAMURA et al., 1965⁵⁾). Histochemical staining method of porphyrin pigments has not been demonstrated, and the examination of red fluorescences in ultraviolet light is regarded as only one specific method for it in tissue sections (PEARSE, 1961¹¹⁾). Therefore, as above mentioned, it could be concluded that, although the pigment granules were visible in some materials, but invisible in the others, "porphyrins" were distributed in the mucous epithelium of the uterus of these three kinds of birds, and that the substance was not distributed in the uterine glands for lack of the fluorescence and the pigment granule.

Moreover, these results of the fluorescent pigments were closely related to those of the egg-coverings, that is; they were conspicuous in the quail eggs, medial in the Rhode Island Red eggs, and scarce in the White Leghorn eggs (TAMURA and FUJII, 1967⁷⁾).

For explanations of the fact that, whereas fluorescences were observed in the mucous epithelium in all materials, "pigment granules" were detected in some cases of them, and for that of the relationships between these uterine pigments and the egg-coverings, such detailed examinations as electronmicroscopical observations of the structure of porphyrins are necessary.

KLOSE and ALMQUIST (1937)⁴ found porphyrins in the shell-membrane of the chicken eggs. Previously (1967)⁷, the present writers could neither locate it in the shell-membranes of the Rhode Island Red, White Leghorn and quail eggs, nor could find it in the isthmus of their oviducts in this study.

SUMMARY

Macroscopical and microscopical observations were made on the oviducts of the Rhode Island Red, White Leghorn and quail, with special reference to porphyrin pigments. The results obtained are summarized as follows.

Macroscopically, the mucous membrane of the uterus in each bird was colored in various brown and fluoresced red to pink. The brightness of fluorescences was related with the depth of the colorations, and they were distinct in the quail uteri, showing dark brown coloration and red fluorescence, and were moderate, showing light brown and pinkish fluorescence, in the Rhode Island Red and White Leghorn uteri.

In frozen sections, distinct fluorescence and collected brown pigment granules were observed in the mucous membrane of the uterus in some cases of the quails. In the uteri of the others, weak fluorescences were observed, but the pigment granule was unobservable.

Through the careful searching in the paraffin sections, the pigment granules were revealed in the mucous epithelium of the quail and Rhode Island Red uteri. Neither the fluorescence nor the pigment granule was found in the uterine gland in each material.

From these findings, it was concluded that fluorescent porphyrin pigments were distributed in the mucous epithelium of the uterus in the quail, as well as the Rhode Island Red and the White Leghorn, although "pigment granule" was invisible in the sections of the White Leghorn uteri.

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ニワトリとウズラの卵管子宮部の蛍光色素

田村達堂・藤井俊策

Porphyrin が広く鳥類の卵の外皮に分布することは明らかにされているが、卵管における形成と卵への沈着の機序は明らかでない。別報で著者らは、卵の外皮の本色素の分布を示したが、今回は卵管の本色素の分布を検索した。材料としてウズラ、ニワトリ（ロード・アイランド・レッド、白色レグホーン）を用いて、次の結果を得た。

肉眼的には、各卵管の子宮部粘膜に特異的な褐色の帯色をみとめ、それと一致して赤色～桃色蛍光がみとめられた。これらの性質から、porphyrin 色素が分布していることは明らかである。その色調と蛍光はウズラで著名であるが、ニワトリでは淡く弱い。

子宮部組織の凍結切片の観察では、3種の鳥の各組織で粘膜上皮に限って赤色～桃色蛍光がみとめられるが、ウズラで最も著名である。該上皮には、卵殻形成時のウズラで褐色色素顆粒が容易にみとめられるが、他のものでは検出し難い。何れの卵管でも、子宮腺には色素顆粒はみとめられない。

子宮部のパラフィン切片での詳細な観察では、ウズラとロード・アイランド・レッドで粘膜上皮に色素顆粒がみとめられるが、白色レグホーンではみとめられない。子宮腺には、何れでも色素顆粒がみとめられない。

EXPLANATION OF PLATE

ABBREVIATIONS

- I, isthmus and isthmo-uterine junction
 U, uterus
 V, vagina

Figures 1 to 6 are macroscopical photographs which present the mucous membrane of oviducts, especially of the uteri of quail, Rhode Island Red and White Leghorn.

Fig. 1. Quail, in ordinary light.

Fig. 2. Quail, in ultraviolet light.

Fig. 3. Rhode Island Red, in ordinary light.

Fig. 4. Rhode Island Red, in ultraviolet light.

Fig. 5. White Leghorn, in ordinary light.

Fig. 6. White Leghorn, in ultraviolet light.

Fig. 7. Mucous epithelium of the uterus of quail. Numerous pigment granules are possessed in the apical cytoplasm of apical ciliated cells. REGAUD's fixation, paraffin section. $\times 1,000$.

Fig. 8. Mucous epithelium of the uterus of Rhode Island Red. A few pigment granules are visible in the same cells as quail. REGAUD's fixation, paraffin section. $\times 1,000$.

