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Comparative Observations on the Distribution of Fluorescent Pigments (Porphyrins) in the Egg-Coverings of Chickens and Quail

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(Table 1; Plate 1)

The distribution of porphyrin pigments, which are characteristic of their specific red fluorescences, in egg-coverings of various avian eggs were reported by FISCHER and KÖGL (1923),[1] FURREG (1931),[2] KLOSE and ALMQUIST (1937)[3] and TAMURA et al. (1965).[4] However, the mechanism of pigmentation to eggs in the oviducts, including processes of formation, secretion and deposition of them has not been explained clearly, and only a few workers refered partially to it (GIERSBERG, 1921;[5] TURCHINI, 1924;[6] TAMURA et al., 1965;[4] TAMURA and FUJII, 1966[7]).

In order to clarify the mechanism of pigmentation in the egg-coverings of the domestic fowl, the present writers made an attempt to examine comparatively the distribution of porphyrin pigments in the cuticles, shells and shell-membranes of eggs of the chickens (Rhode Island Red and White Leghorn) and the quail (*Coturnix coturnix japonica*).

MATERIALS AND METHODS

Twenty quail, twenty Rhode Island Red and thirty White Leghorn eggs were used in this investigation. White Leghorn eggs were classified, by colors of egg-surface, into two groups: a group of ten eggs of light brown color and that of ones of white color. These egg-materials were fresh.

Each specimen obtained from egg-coverings by the following treatments was examined in ordinary light for coloration and in ultraviolet light for fluorescence. At first, the egg-surface was observed, then the egg was immersed in 3% aqueous solution of ethylendiamine tetraacetic acid (EDTA), and the cuticle was separated from the shell. The shell-membrane was stripped off from the shell with a pincett. The shells were observed in solid condition, then immersed in 10% aqueous solution of EDTA to decalcify salts. Afterwards, the cuticle, decalcified shell and shell-membrane of every egg were immersed respectively in 1*N* hydrochloric acid, and the color and secondary fluorescence of the specimen as well as the immersing solvent were examined.

Ultraviolet light for the examination of fluorescence was emitted from a mercury lamp (Olympus) and thrown on the material through two blue filters.

Table 1. Observed Colors and Fluorescences in Egg-Coverings

Kind of Egg / Condition / Color and Fluorescence of Each Layer		Quail		Rhode Island Red		White Leghorn			
						Slightly Colored Group		White Group	
		Natural	In 1*N* HCl	Natural	In 1*N* HCl	Natural	In 1*N* HCl	Natural	
Egg-Surface	Color	Dark Brown ǂǂǂ～ǂǂǂǂ		Reddish Brown ǂǂ		Reddish Brown ＋		White －～±	
	Fluorescence	ǂǂǂ		＋～ǂǂ		＋		±～＋	
Cuticle	Color	Dark Brown ǂǂǂ～ǂǂǂǂ	※	Reddish Brown ǂǂ	※	Reddish Brown ＋	※	White to Greenish Brown ±～＋	※
	Fluorescence	ǂǂǂ	ǂǂǂ～ǂǂǂǂ	＋～ǂǂ	ǂǂǂ	＋	＋～ǂǂ	－～±～＋	±～＋
Shell (Decalcified)	Color	Greenish Brown ＋～ǂǂ	※	Greenish Brown ＋～ǂǂ	※	Greenish Brown ＋	※	Greenish Brown ±～＋	※
	Fluorescence	－～±～＋	ǂǂ～ǂǂǂ	＋～ǂǂ	ǂǂǂ	＋	＋～ǂǂ	－～±～＋	±～＋
Shell-Membrane	Color	White to Pinkish White －～±	※※	White to Pinkish White －～±	※※	White to Pinkish White －～±	※※	White to Pinkish White －～±	※※
	Fluorescence	－～±	－～±	－～±	－～±	－～±	－～±	－～±	－～±

※; Colors changed into greenish in 1*N* HCl. ※※; Colors similar to those in natural condition. Signs of － to ǂǂǂǂ show depth of colors and strength of red fluorescences: －; Absent, ±; Indefinite, ＋; Slight, ǂǂ; Weak, ǂǂǂ; Moderate, ǂǂǂǂ; Conspicuous, ǂǂǂǂ; More conspicuous.

RESULTS

Observed colors and fluorescences of the cuticles, shells and shell-membranes of the Rhode Island Red, White Leghorn and quail eggs are presented in Table 1 and Plate 1 (Figs. 1 to 6).

Quail Eggs

Dark brown coloration of the egg-surface was most marked and formed various figures there. The coloration corresponded with the cuticular pigmentation, and the shell-surface, which was revealed by separating the cuticle, was almost white or colored in light greenish-blue. Fluorescences of the egg-surface as well as of the cuticle were strong, but those of the shell-surface were negative or slightly positive.

However, the decalcified egg-shells presented greenish-brown color and medial fluorescence. Both the coloration and the fluorescence were negative or indefinite in the shell-membranes. By immersing in 1*N* hydrochloric acid, the colors of the cuticles and shells were changed into more greenish, and from them stronger fluorescences than in natural condition were emitted. Each solvent immersing the cuticle and the shell showed slight greenish color and fluorescence similar to the specimen. No changes were produced in the shell-membrane in hydrochloric acid.

Rhode Island Red Eggs

The cuticles were thinner in width and somewhat transparent. Medial brown coloration was distributed in the cuticle and shell. Thus, the color of the egg-surface was expressed by a combination of both pigmentations of the cuticle and shell. The decalcified shells were greenish-brown. Fluorescences of the cuticles and shells were pink, and in the latter the primary as well as the secondary fluorescences were somewhat stronger than those of the quail eggs. The shell-membranes were similar to those of the quail eggs.

White Leghorn Eggs

The cuticle was thinner in width and more transparent than that of the Rhode Island Red eggs. In the lightly colored group, fluorescences and colors of the cuticle and shell were similar to those of the Rhode Island Red eggs, but they were lighter and weaker than the latter. The color of the egg-surface was expressed by a combination of colors of the cuticle and shell.

In the group of white eggs, the cuticle was colored in slightly brown, and fluoresced weakly in natural condition and 1*N* hydrochloric acid. The shell in solid condition was white and fluoresced weakly, and the decalcified shell showed very light greenish-brown color and weak fluorescence. The white color of the egg-surface was expressed, through the transparent cuticle, by white color of the shell. The shell-membrane was similar to that of the eggs of the quail and the Rhode Island Red.

DISCUSSION

FISCHER and KÖGL (1923)[1] obtained several porphyrins from the shells of various kinds of avian eggs, and proved that the substance was distributed in the shells of a variety of colored eggs. After that, KLOSE and ALMQUIST (1937)[3] presented it in the shell-membrane of chicken eggs, and TAMURA et al. (1965)[4] in the cuticle of quail eggs. From the description, however, it appears that FISCHER and KÖGL did not divide the cuticles from the shells, so porphyrins extracted from "shells" by them were ones from the cuticles and shells.

In spite of clear elucidation of chemical characters of porphyrin pigments in egg-coverings, the mechanism of pigmentation has not yet been demonstrated definitely. For explanation of it, it was assumed necessary to apprehend the distribu-

tion of the pigments in the cuticle, shell and shell-membrane of the egg-covering respectively. For this purpose, the present writers morphologically studied distributions of porphyrin pigments in each layer of the coverings of eggs of the quail as conspicuously pigmentated materials, the Rhode Island Red as medially, and the White Leghorn as scarcely.

It was worthy of attention in the present results, that the cuticle and shell of every egg of these three kinds of birds were presented with pigmentation of brown to greenish colors and fluorescences of red to pink, though they were very light and weak in the White Leghorn eggs. Strengths of these fluorescences were parallel to depths of the colors, and fluorescences were observed even in indefinitely colored materials. In each shell-membrane, clear coloration as well as fluorescence were not observed. From these findings, it was apparent that porphyrin pigments were existent in the cuticles and shells of egg-coverings of these three kinds of birds.

KLOSE and ALMQUIST (1937)[3] found porphyrins in the shell-membrane of chicken eggs. They could extract it from collected materials of many eggs. The present results as to the shell-membranes were obtained by examination of individual egg. Therefore, the amount of porphyrins in the shell-membrane described by above workers appeared to be more scarce than those of the cuticle and shell of the White Leghorn eggs.

WARREN and CONRAD (1942)[8] studied the time of pigmentation of the shells of turkey and Rhode Island Red eggs, and described that the pigments of these eggs were being deposited throughout the entire period of shell formation. On the other hand, WOODARD and MATHER (1964)[9] observed it in quail eggs, and stated that pigmentation occurred approximately three hours before laid. In the present observation, "white shells" at a glance were pigmentated and fluoresced ones in careful examination. Thus, the writers regard that the pigment deposition in the shells is occurred in early period of shell formation as WARREN and CONRAD reported.

It was the most interesting fact that the amount of the pigments in the egg-coverings, as well as the modes of the distribution of them, were varied according to the kinds of birds. These differences are thought to be depended upon some factors in their oviducts, such as the formation, distribution and deposition of the pigments.

SUMMARY

The distribution of fluorescent pigments in the coverings of the Rhode Island Red, White Leghorn and quail eggs were morphologically examined.

Fluorescences and coloration were observed in the cuticles and shells of these eggs. The strength of fluorescences was in parallel with the depth of colorations. The pigmentation was conspicuous in the quail eggs, moderate in the Rhode Island Red eggs and scarce in the White Leghorn eggs.

Differences in the modes of pigmentation were observed according to the eggs of the different kinds of birds; as to the quail eggs, it was more prominent in the

cuticle than in the shell, as to the Rhode Island Red eggs, almost the same and medial in the above both, and as to the White Leghorn eggs, scarce in the above both.

Pigmentation of the shell-membranes was not clearly observed. It was concluded that, porphyrin pigments characterized by red fluorescences were distributed, at least, in the shells and cuticles of these eggs.

REFERENCES

1) FISCHER, H. und F. KÖGL: Z. physiol. Chem., **131**, 241-261 (1923)
2) FURREG, E.: Biol. Zentr., **51**, 162-173 (1931)
3) KLOSE, A. A. and H. J. ALMQUIST: Poultry Sci., **19**, 173-174 (1937)
4) TAMURA. T., S. FUJII, H. KUNISAKI and M. YAMANE: J. Fac. Fish. Anim. Husb. Hiroshima Univ., **6**, 37-57 (1965)
5) GIERSBERG, H.: Biol. Zentr., **41**, 252-268 (1921)
6) TURCHINI, J.: Cpt. redn. Ass. Anat., **19**, 255-258 (1924)
7) TAMURA, T. and S. FUJII: J. Fac. Fish. Anim. Husb. Hiroshima Univ., **6**. 357-371 (1966)
8) WARREN, D.C. and R.M. CONRAD: Poultry Sci., **21**, 515-520 (1942)
9) WOODARD, A.E. and F.B. MATHER: *Ibid.*, **43**, 1427-1432 (1964)

ニワトリとウズラの卵の外皮における螢光色素 (Porphyrin)

田村達堂・藤井俊策

Porphyrin 色素が広く鳥類卵の外皮に分布することは，化学的には明らかにされているが，それらの外皮各層（クチクラ・卵殻・卵殻膜）における分布は，形態学的には明らかにされていない．また，本色素の卵管での形成ならびに卵への沈着の機序も明らかにされていない．これらの解明の手がかりとして，ウズラとニワトリ（ロード・アイランド・レッド，白色レグホーン）の卵を用い，外皮各層の本色素分布を検索した．

各卵は，クチクラと卵殻に本色素分布を示したが，その量と分布様式には種類によって差異がみとめられた．すなわち：

ウズラ卵では，自然状態で，クチクラにおける色素分布が著明で，卵殻はほぼ白色ないし微青色にすぎない．しかし脱灰することによって，かなりの色素が卵殻に分布していることが示された．

ロード・アイランド・レッド卵では，自然状態で，クチクラと卵殻にそれぞれ中等度の着色がみとめられる．脱灰した卵殻では，その色調はウズラ卵殻より濃い．

白色レグホーン卵では，外皮が極めて淡く着色するか，あるいは白色である．自然状態では，前者はクチクラと卵殻に淡い着色を示し，後者では，クチクラは透明に近く，卵殻は白色である．しかし詳細に検査して，両者ともクチクラと卵殻に，微量ながら螢光色素の分布がみとめられた．

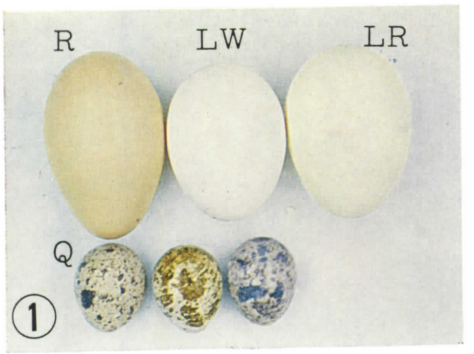
3種の卵の卵殻膜には，各卵についての検査では，色素の分布は明らかでない．

EXPLANATION OF PLATE

ABBREVIATIONS

LR, White Leghorn egg of colored group
LW, White Leghorn egg of white group
Q, quail egg
R, Rhode Island Red egg

Fig. 1. Surface views of Rhode Island Red, White Leghorn and quail eggs. In ordinary light.
Fig. 2. Cuticles separated from eggs. In ordinary light.
Fig. 3. Cuticles in 1*N* HCl. Fluorescence is presented in each material and its solvent. In ultraviolet light.
Fig. 4. Cuticle (1) and decalcified shell (2) of White Leghorn egg (white group) in 1*N* HCl. Slight fluorescence is visible in each material and its solvent. In ultraviolet light.
Fig. 5. Cuticles and decalcified shells. In each of these, pigmentation is observed. In ordinary light.
Fig. 6. Cuticles and decalcified shells. In each of these, fluorescence of red or pink color is presented. In ultraviolet light.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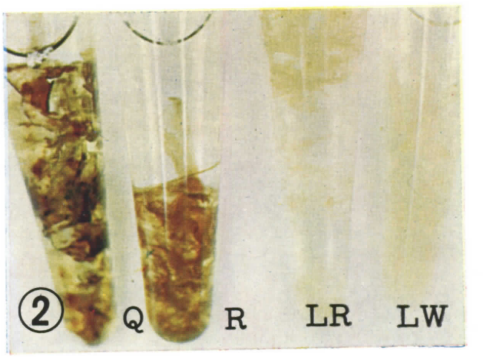
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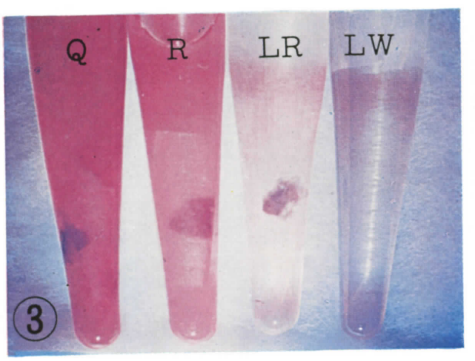
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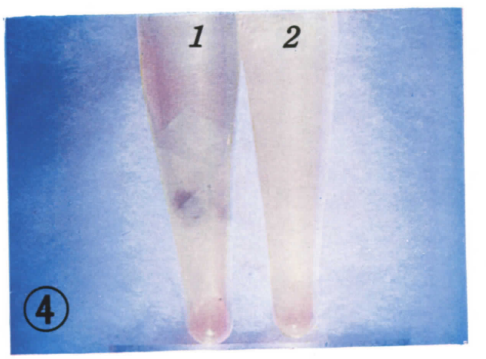
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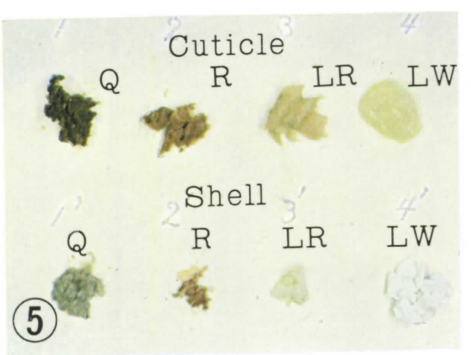
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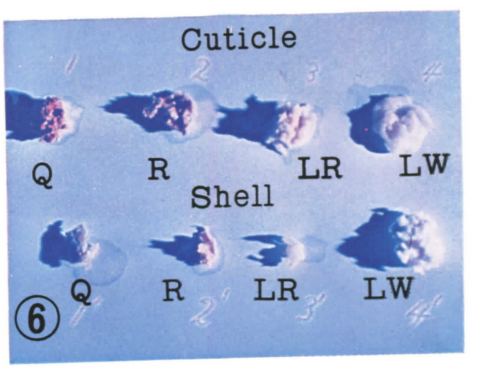
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