A HISTOLOGICAL STUDY OF THE FORMATION OF THE CHALAZAE IN THE HEN'S EGG

by

WAI-LAN HUANG

B. S., College of Agriculture
Sun Yat-Sen University, 1933

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INTRODUCTION

Recently many investigators have reported on the formation of the different portions of the hen's egg, including the chalazae. Lillie (1919) stated that the chalazae appear to be opalescent cords twisted in opposite directions. Asmundson and Burmester (1936) pointed out that the original albumen is secreted in the magnum as an apparently homogeneous gel without evidence of the chalazae, but becomes differentiated into several layers when it reaches the isthmus and uterus. According to Hansen (1933) the chalazae are completed after the formation of the shell membrane hence in the uterus. Almquist and Lorenz (1932) have shown that the chalazae consist primarily of strands of a mucin-like protein which is originally formed and partially retained as the firm albumen.

In the study of the mechanics of chalaza formation, Almquist (1936) found that the normal chalaza at the large end of the egg invariably has a clock-wise twist, while that at the small end has a counter clock-wise twist. He concluded that the orientation of the egg with respect to its poles is small end caudad while traveling through the oviduct and simultaneously rotating in a counter clock-wise direction.

Olsen and Byerly (1936) stated that approximately 85 to 90 per cent of eggs were formed small end caudad. Eggs in the posterior blind sac of the uterus may be turned just before or during the act of laying. The direction of the twisting of the chalazae as observed by Almquist (1936) would necessitate that the small end of the egg always be caudad until after the chalazae formation be completed.

Burmester and Card (1939) removed sections from the chalaziferous region of the infundibulum of 31 hens. The region is located between the funnel proper and the cephalic end of the albumen-secreting region. The latter is characterized by deep folds or grooves of epithelium. The result indicated that the removal of this part of the oviduct had little or no effect on chalaza formation in subsequent egg, thus tending to disprove the view of Richardson (1935) that materials for chalaza formation were secreted in this region.

Tarchanoff (1884) and Conrad and Phillips (1938) introduced artificial ova into the infundibulum. The latter observed that as the egg passed down the oviduct, it became surrounded by normal egg structures, including the chalazae. This would indicate that the ovum itself contributes nothing to the constituents of the egg white.

Conrad and Phillips (1937) studied the formation of

the chalazae and inner thin white in the hen's egg. They analyzed the different fractions of the white of oviducal eggs at various stages. They suggested that the chalazae and inner thin white develop simultaneously from the original albumen, which is secreted by the magnum. Formation of these structures is due to a mechanical segregation of mucin fibers during the late stages of egg formation, according to their interpretation.

In order to test the Conrad and Phillips hypothesis, histological studies were made of both the laid eggs and immature eggs which were obtained from different portions of the oviduct. The distribution and differentiation of the mucin fibers have been followed with the view of determining their relationships to the formation of the chalazae.

MATERIALS AND METHODS

Laid eggs varying in age from one hour to eleven days were examined. Also eleven immature eggs were removed from various levels of the oviduct for study. Their relative positions and estimated time since ovulation were as follows:

No. of eggs	Position	Hrs. since ovulation
2	Anterior magnum	1.0, 2.3
1	Mid-magnum	2.8
4	Posterior magnum and anterior isthmus	2.7, 3.0, 3.5, 4.5
1	Constricted uterus	6.0
3	Uterus	10, 13, 16

All eggs were coagulated by boiling from five to ten minutes. Due to difference in specific gravity of the yolk and the white, the position of the egg while boiling will materially influence the distribution of the layers of the white. For that reason the laid eggs were held in a position with the large end upright while boiling. The oviducal eggs were tied in a section of the oviduct while boiling. The position of the chalazae could be seen in the white of the cooked laid egg, but in the immature eggs their position could only be estimated. This necessitated the sectioning of a large area. In order to follow the changes in the egg white during chalaza formation, portions including the two ends and the intervening material on one side of the egg were prepared for sectioning.

In the preliminary studies on laid eggs, fixation was accomplished by the use of mercuric chloride followed by Gilson's fluid. All immature eggs were fixed in Susa's

fixative. The specimens were dehydrated and cleared in dioxane, and infiltrated and embedded in the usual manner. Sections were cut from 8 to 12 microns in thickness. Although several mucin stains were employed in the early phases of the work, thionine blue was the final choice for staining the mucin fibers.

EXPERIMENTAL STUDIES

Observations

Eggs examined from various positions in the oviduct are described in order of their progress through the duct.

Anterior magnum. Hen No. 3719 had the eggs intercepted one hour after the previous oviposition. The egg was located one-fourth the way through the magnum. The white at this stage is homogenous in the three sections (small end, large end, and the middle section). No granular material is seen between the mucin strands. A thick band of mucin which stains a light blue color is next to the yolk.

Hen No. 3856 had the egg removed from the oviduct 2.3 hours after the previous oviposition. The egg was also located about one-fourth the way through the magnum. The white at this stage of formation stains for the most part a light blue color with thionine blue. This light staining mucin is heavily deposited at the periphery of the folds. A deeper staining material distributed between the strands

of mucin was granular in appearance (Fig. 1). The band of mucin adjacent to the yolk was better defined than the former egg.

Mid-magnum. Hen No. 3722 had the egg removed from the oviduct 2.8 hours after the previous oviposition. The egg was located in the oviduct with the large end at half way mark. The deeper staining pocket of granular material is seen in the inner thin region for the first time and more granular material is also distributed between the striations of mucin than in the earlier stage eggs. This pocket of granular material which is probably the inner thin is only present in the small end of the egg (Fig. 2). The eggs obtained from the anterior magnum (Fig. 1) showed the striated mucin and some distribution of granular materials between the striations but no granular pocket. The egg at this stage is without any indication of chalaza formation. With observation under the higher power of the microscope, there are seen a large number of scattered mucin fibers in the granular pocket. The section taken from the region midway between the two ends is a homogenous mass. There is no evidence of granular pockets in the large end but the later of densely staining mucin next to the yolk is well defined.

Posterior magnum. In hen No. 4771, the egg was re-

EXPLANATION OF PLATE I

Fig. 1. Egg obtained from the anterior magnum. The dark area is the granular materials. The light colored striations are mucin fibers, which are heavily deposited near the folds (Hen No. 3856).

PLATE I

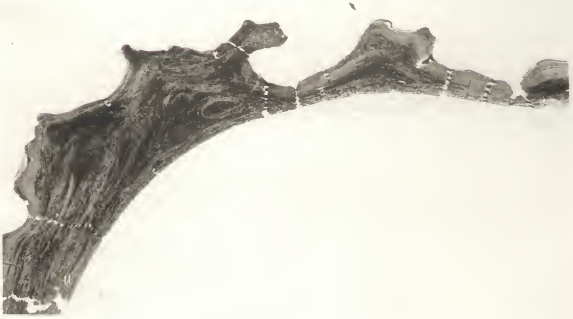

Figure 1

EXPLANATION OF PLATE II

Fig. 2. The small end of an egg obtained from the mid-magnum. The pocket of dark staining granular material is shown in the inner thin region (Hen No. 3722).

PLATE II

Figure 2

moved from the oviduct three hours after the previous oviposition. The egg was located in the magnum with the small end one inch from the magnum-isthmus line. The small end at this stage shows definite concentration and bending of mucin strands in the granular inner thin as the first stage of chalaza formation. One large mucin strand seems to be forming by the collection of the small fibers. The mucin strands at the periphery of the white mass are almost straight, and are definite next to the yolk. The large end of this egg shows a granular pocket in which are clearly seen mucin strands of various sizes when observed under the higher power of the microscope. In the section from the area midway between the ends, granular pockets show at the two ends.

Hen No. 3658 had the egg removed from the oviduct 2.7 hours after the previous egg was laid. The egg was located with the small end one inch from the magnum-isthmus line. Since this egg shows well the early stages of chalaza formation, photographic reproductions of the small end, middle, and large end are recorded in Figures 3, 4, and 5. The mucin fibers in the inner thin white at the small end of the egg have become collected and show twisting. These twists are seen clearly even without the aid of the higher power of the microscope (Fig. 3). The chalaziferous band

EXPLANATION OF PLATE III

Fig. 3. The small end of an egg obtained from the posterior magnum. The mucin fibers in the inner thin have become collected and show some twisting (Hen No. 3658).

Fig. 4. The middle section of an egg white obtained from an egg at the posterior magnum. The chalaziferous layer is compact and the granular inner thin white is definite (Hen No. 3658).

Fig. 5. The large end of an egg obtained from the mid-magnum. The curving of fibers are also shown (Hen No. 3658).

Figure 5

Figure 4

Figure 3

is also sharply shown. In the middle section (Fig. 4) the chalaziferous layer is compact and the granular inner thin is definite. Toward the periphery of the white mass, the network of granular material is less dense. There also is some curving of fibers which are present in the large end (Fig. 5).

Hen No. 4753 had the egg removed from the oviduct 3.5 hours after the previous oviposition. The egg was located with the small end at the line marking the junction of the magnum and isthmus. In the small end, there is some curving of fibers present in the inner thin (Fig. 6) and also some definite fiber concentration in the large end, but no change in the appearance of the middle section.

Hen No. 3642 had the egg removed from the oviduct 4.5 hours after the previous oviposition. The egg was located one-fourth in the magnum and three-fourths in the isthmus. The small end of the egg at this state shows excellent differentiation of the chalaza. A large number of mucin strands are collected to form the chalaza and are communicating with the chalaziferous layer (Fig. 7). Many small fiber strands are attached to the stalk of the chalaza. In the middle section, the mucin strands are quite large in the inner thin, but not so definite as those in the earlier egg. There is yet no evidence of chalaza in the large end.

EXPLANATION OF PLATE IV

Fig. 6. The small end of an egg obtained from the magnum-isthmus line. The curving of fibers is seen in the inner thin white (Hen No. 4753).

PLATE IV

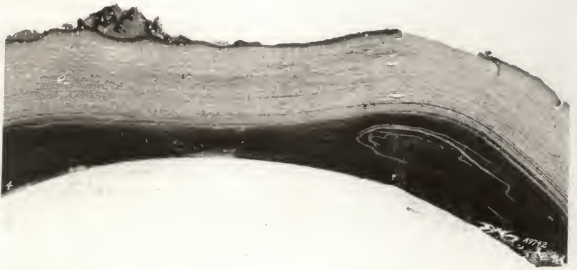

Figure 6

EXPLANATION OF PLATE V

Fig. 7. The small end of an egg obtained from the anterior isthmus. A large amount of fiber strands are collected to form the chalaza and are communicating with the chalaziferous layer (Hen No. 3642).

PLATE V

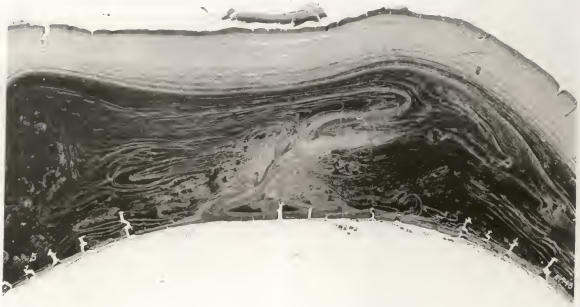

Figure 7

Constricted uterus. Hen No. 3814 had the egg removed from the oviduct six hours after the previous oviposition. The egg was almost through the isthmus with the small end in the constricted uterus. In the small end of the egg, mucin fibers were collected at the point of the future chalaza (Fig. 8). In a comparison of this egg with that of hen 3642 (Fig. 7), it is seen that the position of the egg in the oviduct does not accurately indicate the stage of chalaza formation. Although the egg of hen 3814 was entering the uterus, its chalaza formation was not so advanced as that of an egg (hen 3642) just entering the isthmus. In the large end of the egg (Fig. 9), there is no indication of the chalaza formation.

Uterus. Hen No. 4715 had an egg removed from the uterus 10 hours after the previous oviposition. Various stages of chalaza formation are seen in the different slides made from this hen's egg. In one slide, mucin strands are being pulled toward the inner thin from the firm white. In a second slide the condensed mucin fibers indicate that the chalaza is almost completely formed. In figure 10 the section is not through the stalk of the chalaza nor its point of attachment to the chalaziferous layer. There is yet no definite chalaza nor segregation of fiber strands in the large end. In the middle section definite fiber strands

EXPLANATION OF PLATE VI

Fig. 8. The small end of an egg obtained from the constricted uterus. The mucin fibers are collected at the point of the future chalaza (Hen No. 3814).

Fig. 9. The large end of an egg obtained from the constricted uterus. There is no indication of the chalaza formation (Hen No. 3814).

PLATE VI

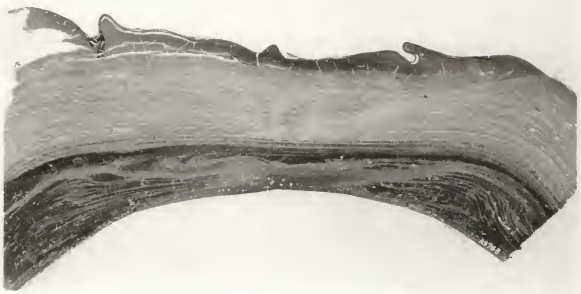

Figure 8

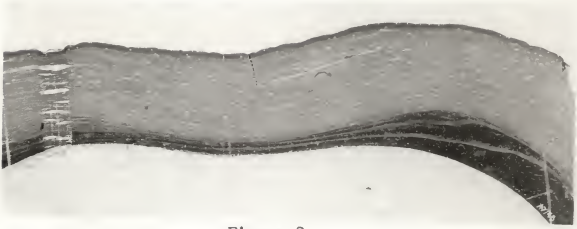

Figure 9

EXPLANATION OF PLATE VII

Fig. 10. The small end of a 10-hour uterine egg. The chalaza is completely formed. Some of the fiber strands in the chalaza are being pulled toward the inner thin from the firm white (Hen No. 4715).

PLATE VII

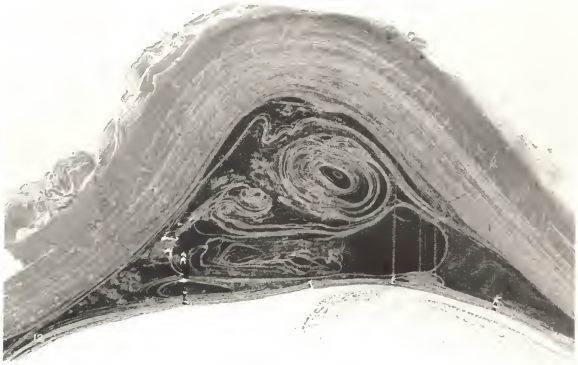

Figure 10

are shown in the inner thin.

Hen No. 3537 had an egg removed from the uterus 13 hours after the previous oviposition. In contrast with the earlier eggs which were obtained from the magnum, isthmus, and the constricted uterus, this egg has no mucin strands in the inner thin egg white of the small end (Fig. 11). It would appear that all of the fibers have been coiled in the chalaza. The section does not show an intimate connection between the chalaza and the firm white nor with the chalaziferous layer. There is no completely formed chalaza in the large end but fiber twists are present (Fig. 12). The compact granular layer is showing in the firm white. In the middle section, large strands can be seen which have been pulled from the firm white into the inner thin layer of egg white. By observation under the higher power microscope, there are six or more definite strands being pulled into the chalaziferous layer.

An egg with hard shell was removed from the uterus of hen No. 3785 sixteen hours after the previous egg had been laid. There are no fiber strands in the inner thin white, except near the chalaza, and none in the outer thin white (Fig. 13). There is yet no chalaza in the large end, but under the low power of the microscope the net-work of mucin fibers appears to be superimposed in the granular material.

EXPLANATION OF PLATE VIII

Fig. 11. The small end of a 13-hour uterine egg. The chalaza is completely formed. There are no fiber strands in the inner thin (Hen No. 3537).

Fig. 12. The large end of a 13-hour uterine egg. There is no well-formed chalaza, but the fiber twisting is present. (Hen No. 3537).

PLATE VIII

Figure 12

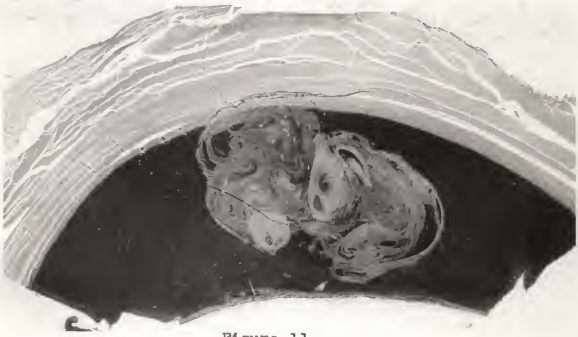

Figure 11

EXPLANATION OF PLATE IX

Fig. 13. The small end of the 16-hour uterine egg. The chalaza is completely formed. There are no fiber strains in the inner thin (Hen No. 3785).

PLATE IX

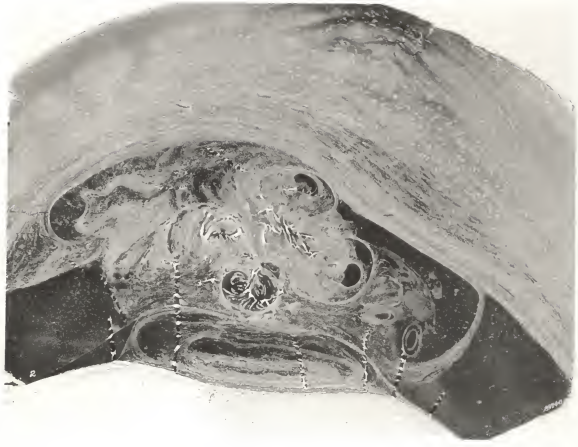

Figure 13

In the middle section most of the fibers seemed to have been removed from the inner thin layer of egg white.

Laid eggs. Eight laid eggs about 1 to 4 hours old and a few 10 and 11 days old were prepared for study. The outer thin layer in a newly-laid egg is granular (Fig. 14), in which some scattered mucin fibers are present. Between this granular layer and the thick white there is a layer containing the small mucin fibers. The thick white layer contains a large number of mucin fiber strands. The inner thin is a granular layer in which a few fibers can be seen in the middle section, but not in the large end nor the small end.

DISCUSSION

Locations of Eggs

Anterior magnum. Egg white in the egg (Hen No. 3719) removed from the oviduct one hour after the previous oviposition, shows only mucin fiber striations and there is no granular material to be seen. In another egg (Hen No.3856) at about the same position in the oviduct, the granular material is distributed between the mucin strands. This granular material stains a dark color with thionine blue whereas the mucin strands are light blue in color. At the periphery of the mass which is in intimate contact with the large folds of the oviduct there is evidence the secretion is not uniform over the entire fold, since the blue stain-

EXPLANATION OF PLATE X

Fig. 14. The small end of the laid egg. This picture shows the completely formed chalaza.

PLATE X

Figure 14

ing mucin is irregularly distributed. This is well shown in figure 1. The mucin also appears as a wide and dense band in next to the yolk, and unquestionably is the chalaziferous layer. The predominance of mucin in secretions at this level is to be expected since it has been shown by Surface (1912) and others that tubular glands which secrete the soluble nitrogen of the egg white, are few in number in the anterior part of the oviduct. Thus, the secretions from this region would be largely mucin.

Egg white at this stage of formation is a homogenous mass with no indication of chalaza formation. The apparent curving of the fibers here is the result of the imprint of the folds on the inside of the duct.

Mid-magnum. The major change at this stage is that the deeper staining of the granular material is becoming concentrated in the inner thin region, and the distribution of the granular material between the mucin strands is increased. It is probable that the secretion of the material which appears granular in the fixed preparations is increased (Fig. 2) in the mid-magnum and that this material gradually passes between the mucin fibers and enters into the inner thin region, thus forming the pocket of inner thin. There is no indication of chalaza formation at this stage, even though there is differentiation of the inner

thin. This observation is not in full agreement with the statement of Conrad and Phillips (1938), that the chalaza and the inner thin white develop simultaneously from the original albumen. With the observation under the higher power of the microscope, a large number of scattered mucin fibers can be seen in the granular pocket. The first evidence of differentiation of the inner thin is seen in the small end of the egg.

Posterior magnum. It is in this series of eggs that the first definite evidence of the chalazae is seen. The egg of Hen No. 4771 of this group was obtained from the extreme posterior magnum and showed definite bending of mucin strands in the granular inner thin of the small end. This was probably the first stage of the chalazae formation. One large mucin strand seems to be forming from the collection of the small fibers. Since the large end of the egg is differentiated later, chalaza formation is less advanced in that region. This end has only a granular pocket at this stage and many mucin strands are visible in this pocket when observed under the higher power of the microscope.

The egg of Hen No. 3658 was found in exactly the same position of that of Hen No. 4771. Chalaza formation is slightly more advanced than in the case of 4771 since

definite twisting of the mucin fibers can be seen in figure 3. Apparently the initial fiber twisting is the precursor of the chalaza and near the junction of the magnum and the isthmus is the point where the fibers become sufficiently segregated to show this phenomenon. The chalaziferous layer also becomes more compact in the middle section of the egg (Fig. 4). This is probably due to the mucin fibers becoming wound about the yolk while the egg is moving through the oviduct and rotating constantly. By this same process the mucin fibers become twisted at the ends of the egg. No satisfactory explanation was found for the fact that the chalaza in the small end of the egg become differentiated much earlier than in the opposite end. The fact that the small end is in advance as the egg moves through the oviduct may bear some relationship to the difference in rate of chalaza formation in the two ends of the egg.

Isthmus. Apparently the chalaza in the small end of the egg, may become definitely formed while the egg is in the isthmus. However, there was considerable variation among hens as to the degree of chalaza formation in this section of the oviduct. No explanation was found for this difference among hens.

Uterus. The chalaza in the small end becomes larger

and more compact. A definite chalaza in the large end was not found in eggs which had been in the oviduct 13 and 16 hours. However, there was some coiling of the fibers in the large end of the egg in these hens (Fig. 12).

Laid egg. There is no difference between the laid egg and the late stage uterine egg, except that in the laid egg chalaza formation had been completed in both ends of the egg. A comparison was made of fresh laid eggs and those which had been held for 10 days at about 60 degrees F. Little difference could be seen in the histological preparations. However, there was virtually no mucin fibers in the inner and outer thin white of the older eggs while some strands of fibers were seen in these two layers of the fresh laid egg.

In general the results of this study tend to support the conclusions of Conrad and Phillips (1938) regarding the mechanics of chalaza formation. The mucin fibers in that portion of the egg white adjacent to the yolk seem to be concentrated in the two ends of the egg and as a band around the yolk forming the chalaziferous layer.

Before the egg reaches the middle of the magnum there is a differentiation of the egg white next to the yolk. At this stage there appears a deep staining granular material which is much like the inner thin white of the laid egg.

It tends to obscure the mucin fibers at this stage, so that the inner thin white seems to be differentiated before the fibers become conspicuously condensed at this region or coiled into the chalazae.

There is considerable difference in the time of chalaza formation in the two ends of the egg. In the small end of the egg, twisting of the fibers is seen about the time the egg leaves the magnum. Not much evidence of twisting of the chalaza of the large end was seen until the egg had been in the uterus about eight or nine hours. Eggs at the same position in the oviduct differed widely in degree of chalaza formation.

Histological evidence was obtained which would indicate that the mucin of the firm egg white contributed to the formation of the chalazae. These strands seemed to be pulled from the inner layers of the thick white by the rotation of the white about the yolk. This same process could explain the concentration of the mucin fibers into the chalaziferous layer about the yolk and also the coiling of the fibers of the inner white into the chalazae.

SUMMARY

1. Egg white obtained from the extreme anterior magnum is homogenous throughout with the mucin arranged in striations extending around the yolk.

2. There is no granular material secreted from the extreme anterior magnum. The white secreted in the posterior half of the magnum seems to carry an additional substance which appears granular and deep staining in the fixed preparations.

3. The inner thin white is formed by the segregation of the granular-appearing material from the rest of the white mass near the yolk.

4. The inner thin layer is well differentiated before there is much evidence of chalaza formation.

5. The differentiation of the chalaza first occurs in the small end of the egg and twisting of these fibers is first seen at about the time the egg enters the isthmus.

6. Differentiation of the chalaza in the large end of the egg does not occur until the egg has been in the uterus a few hours.

7. The chalazae are formed partially by the segregation of the fibers from the inner thin and partially by pulling down the mucin fibers from the firm white layer. This process is accomplished by the rotation of the egg as it moves through the oviduct.

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