J. Fac. Fish. Anim. Husb.
Hiroshima Univ. (1970), 9: 139~150

Scanning Electron Microscopy of Shell-Membrane Formation in Hen's Eggs

Shunsaku FUJII, Tatsudo TAMURA, and Toshikazu OKAMOTO

Department of Animal Husbandry, Faculty of Fisheries and Animal Husbandry, Hiroshima University, Fukuyama

(Figs. 1-16)

It is generally known that the egg shell of the avian egg is composed structurally of a shell membrane, a calcium layer, and a cuticle from inside to outside, and that functionally the shell membrane is formed in the isthmus, and the calcium layer and the cuticle are in the uterus, while the egg descends the oviduct. Innumerable reports have been published on the structure and morphological formation mechanism of these constituents of the egg shell. Studies on the shell membrane have also been made by a number of investigators, including NATHUSIUS (1868),[1] STEWART (1935),[2] MORAN and HALE (1936),[3] ROMANOFF and ROMANOFF (1949),[4] and SIMONS and WIERTZ (1963).[5] Many of them, however, have been carried out on the completed shell after oviposition. Little has been known about the structure and morphological formation mechanism of the shell membrane while the egg is developing. In addition, these previous studies were made only by the conventional light microscope.

In one of their preceding studies, the authors[6,7] observed the structure of the completed egg shell after oviposition. In another, they examined the calcification process of the egg shell by using a scanning electron microscope. As a result, this instrument was very useful for morphological observation of such a hard tissue as the egg shell and such a fibrous tissue as the shell membrane. In the present study, the shell-membrane formation of the chicken egg was scrutinized mainly by scanning electron microscopy.

MATERIALS AND METHODS

Eggs of White Leghorn hens were used in this study. Observations were made on eggs at varying stages of shell-membrane formation. When the shell membrane is formed in the isthmus of the oviduct, eggs were removed from the isthmus and its neighboring position. Eggs were collected from many carcasses just after death at a poultry eviscerating plant. In more detail, eggs were removed from the following five parts: the beginning, upper, middle, and lower portion of the isthmus and the uterus. They were fixed in 10% formalin solution as whole eggs. After fixation for a few hours, they were broken, and the white and yolk dripped off. Then the inside of the shell membrane was washed carefully with physiological saline solution to remove any adhering albumen. The shell membrane was refixed fully in the same fixative. The fixed membrane was cut into small pieces, which were washed with water and used as specimens.

The specimens were treated further in the following manner according to the kind of examination. For scanning electron microscopy, some specimens were mounted on a cover glass. They were dried rapidly by an air-dryer, or dehydrated by passing through a series of graded acetone. Then they were coated with gold, and examined by a scanning electron microscope, type JSM-2 (Japan Electron Optics Laboratory, Ltd.), at an accelerating voltage of 25 KV. For light microscopical observation, other specimens were stained by Bielschowsky's silver impregnation method for connective tissue and used in a form of membranous preparation.

RESULTS AND DISCUSSION

As is well known, the completed shell membrane is a fibrous membrane enveloping the egg contents. It consists of two layers, an inner and an outer shell membrane. The present observations were done in the course of development of the shell membrane.

An egg collected from the beginning part of the isthmus is enclosed by such a thin and transparent membrane that it is hardly distinguished macroscopically. In general, the forward end of an egg which has reached the isthmus has a thicker membrane than the backward end remaining in the albumen-secreting portion. When stained by the silver impregnation method, the shell membrane at this stage was exhibited as a sparse meshwork of fine fibers (Fig. 1). Each fiber was about 0.5μ in diameter, and appeared feeble. The interstices among fibers were filled with fine granular material.

Scanning electron microscopy revealed that the shell membrane had such a structure as shown in Figs. 2–5. Figs. 2 and 3 present the inner surface of the membrane, and Figs. 4 and 5 the outer surface of it. The inner surface of the membrane seems to be covered by a thin mucin-like sheet. The appearance of fibers, therefore, was not clearly recognized, although traces of fibers were indicated vaguely beneath the sheet. The sheet developed into the inner layer of the inner shell membrane. On the contrary, a sparse meshwork of fibers was revealed on the outer surface of the membrane (Figs. 4 and 5). The fibers were considerably deformed in shape due to drying by the time when samples were prepared. They were about 0.5μ in diameter. The interstices among fibers were cemented with organic concretions.

RICHARDSON (1935),[8] who studied the secretory phenomenon of the oviduct histologically, reported that the formation of the shell membrane was initiated at the beginning part of the isthmus, and that the precursor of the membrane was first secreted from the isthmian glands as granules. Then they became fiber-like strands of viscous material, from which fibers were derived. Judging from the opinion of RICHARDSON, it may well be that the picture shown in the above figures indicates the early stage of the shell-membrane formation. In other words, the formation of the shell membrane started by accumulation of fine fibers and organic cement material.

An egg collected from the upper part of the isthmus was enveloped by a somewhat thick membrane. The membrane was easily distinguished macroscopically. The membrane at this stage was exhibited by the silver impregnation method, as shown in Fig. 6. The membrane was interwoven rather densely by fibers of the same size as before. By scanning electron microscopy, the mucin-like sheet observed in Figs. 2 and 3 showed an increase in thickness, containing fine fibers. With the advance in the shell-formation, fibers increased in amount and a meshwork of fibers became denser and denser.

An egg collected from the middle portion of the isthmus was enveloped by a thicker membrane. The shell membrane at this stage is shown in Fig. 7 after being stained by the silver method. The shell membrane was composed of more densely arranged fibers than before. It is noted in Fig. 7 that relatively large fibers have appeared scatteringly on the already formed meshwork of fine fibers. The fibers are round in shape and $1-2\mu$ in diameter, running straight and parallel to one another. Fibers of this type increased progressively in amount to form a meshwork eventually. The meshwork of fibers is the so-called the inner shell membrane.

An egg collected from the end portion of the isthmus had a fully formed thick shell membrane. The picture of the shell membrane at this stage is given in Figs. 9–14. As shown in Fig. 9, the shell membrane is composed of three layers clearly different in structure and arrangement of fibers from one another. The specimen shown in this figure was prepared by tearing the membrane. The innermost layer, which is at the bottom of the figure, is lined by a characteristic coat, that has the appearance of an amorphous membrane consisting of mucin-like substance. Careful observation of the coat in Fig. 10 reveals the presence of a small amount of fine fibers embedded in a mucin-like matrix. Fibers are distinguishable especially on a cross plane of the membrane. This coat is homologus with the mucin-like sheet observed in Figs. 2 and 3. It has not been mentioned by any previous investigators. The authors call it the inner layer of the inner shell membrane.

The middle part shown in Fig. 9 is the so-called inner shell membrane which has been pointed out by some previous workers. The authors propose to call it the outer layer of the inner shell membrane, since the layer is clearly distinguished in structure from the inner layer mentioned above. The meshwork of fibers in the outer layer was constructed by relatively large fibers (Fig. 11). These fibers were round in shape and branched a little. Each fiber was about 2μ in diameter. When stained by the silver impregnation method, the outer layer of the inner shell membrane was proved to have such structure as illustrated in Fig. 8. In this preparation, the inner layer was torn away. The most striking feature of the fibers of the outer layer is that small granular concretions adhere to the circumference of each fiber. Althogh these concretions seem to be an artifact at a glance, they are also present around silver-impregnated fibers (Fig. 8). SIMONS and WIERTZ (1963)[5] reported that electron-microscopically each fiber of the inner shell membrane was wrapped by a mucin-like envelope around the keratin core. Accordingly, these concretions probably have some relation to mucin.

MORAN and HALE (1936)[3] divided the inner shell membrane into two layers. In the present study, the authors divided it into two layers similarly, an inner layer and an outer layer. This division of the inner shell membrane, however, is not always the same as that of Moran and Hale, which was based on the appearance of a meshwork of fibers. As mentioned above, the authors divided the inner shell membrane from a viewpoint of developmental process. SIMONS and WIERTZ (1963)[5] observed the inner layer of the inner shell membrane.

The upper part shown in Fig. 9 is the inner surface of the outer shell membrane. The fibers of this layer are largely different in diameter and in the direction of running from those of the inner shell membrane. They were somewhat flat in shape and 2–3μ in width. They have formed a coarse meshwork, with fibers combined into knots (Fig. 12). On the contrary, the outer surface of the outer shell membrane adjoining directly to the calcium layer was composed of a coarse meshwork of fibers (Figs. 13 and 14). Here, fibers were extensively flattened to be ribbon-like and 3–4μ in width. They were interwoven with one another to make knots.

MORAN and HALE (1936)[3] reported that the outer shell membrane consisted of three distinct layers. The layer adjoining directly to the shell was composed of tangled coarse fibers of keratin (2–5μ diameter). The other two layers contained finer fibers (0.5μ diameter) than the first layer. In the present study, the authors could not differentiate the three layers described by MORAN and HALE. However, when the fibers of the inner surface (Fig. 12) are compared with those of the outer surface (Fig. 13), there is a clear difference between two the surfaces of the outer shell membrane in the appearance and the width of fibers. The fibers of the outer shell membrane may probably be transmitted successively from inside to outside.

An egg collected from the uterus was enclosed in a fully formed shell membrane, which looked entirely like a completed shell membrane after oviposition. The shell membrane of the completed egg has been examined in a previous study (Fig. 15).[6] All the eggs in the uterus have started shell formation (Fig.16). The shell formation progressed by deposition of calcium on the already accomplished shell membrane. No morphological changes occurred in the shell membrane in the process of the shell formation in the uterus. Details in the shell formation have been described in a previous paper.[7]

From above results, it is clear that the formation of the shell membrane is accomplished in the end portion of the isthmus just before eggs descend to the uterus.

SUMMARY

A morphological study on the shell-membrane of the chicken egg was performed in the course of developement by the use of a scanning electron microscope and the silver impregnation method.

The shell membrane began to form at the beginning of the isthmus by the for-

mation of a coarse meshwork of fine fibers. Fine fibers were cemented with one another by mucin-like material to form a meshwork which became the inner layer of the inner shell membrane. Then relatively large fibers were added to the already formed meshwork of fibers of the inner layer in the middle portion of the isthmus. They developed into the outer layer of the inner shell membrane. Very large fibers were added to the inner shell membrane in the end portion of the isthmus. They developed into the outer shell membrane. The formation of the shell membrane was accomplished just before eggs descended to the uterus.

The shell membrane of the fully formed egg was divided into three layers, the inner and the outer layer of the inner shell membrane and the single layer of the outer shell membrane. The inner layer of the inner shell membrane is a thin layer adjoining directly to albumen. It is a mucin-like layer containing fine fibers. The outer layer of the inner shell membrane is formed by a meshwork of fibers about 2μ in diameter. These fibers are characterized by the presence of granular concretions around each fiber. The outer shell membrane is formed by a coarse meshwork of fibers 3–4μ in width. These fibers are combined with one another in such manner as to form knots.

REFERENCES

1) NATHUSIUS, W. V.: *Z. wiss. Zool.*, **19**, 322 (1868). (quoted from Romanoff's The Avian Egg).
2) STEWART, G. F.: *Poult. Sci.*, **14**, 24-32 (1935).
3) MORAN, T. and HALE, H. P.: *J. exp. Biol.*, **13**, 35-40 (1936).
4) ROMANOFF, A. L. and ROMANOFF, A. J.: The Avian Egg, John Wiley and Sons, New York (1949).
5) SIMONS, P. C. M. and WIERTZ, G.: *Z. Zellforsch.*, **59**, 557-567 (1963).
6) FUJII, S. and TAMURA, T.: *J. Fac. Fish. Anim. Husb. Hiroshima Univ.*, **8**, 85-98 (1969). (in Japanese with English summary).
7) ————: *Ibid.*, **9**, 65-81 (1970).
8) RICHARDSON, K. C.: *Trans. Roy. Soc. (London)*, **225** (B), 149-195 (1935).

鶏卵の卵殻膜形成の走査電子鏡検

藤井俊策・田村達堂・岡本敏一

鶏卵の卵殻膜の形成機構が，主として走査電顕によって，一部は銀染色によって形態的に観察された.

卵殻膜の形成は，卵管の峡部起始部において，先ず細線維とこの間隙を埋めるムチン様基質の構成によって始まった．この線維網が内卵殻膜の内層となった．峡部中央部で，内層の上にやや太い線維が加わって，内卵殻膜の外層が形成された．峡部末端部で，更に太い線維が加わって，外卵殻膜が形成された.

卵殻膜は，卵管峡部で形成を終わった。卵管子宮部では，既に完成した卵殻膜の上に，卵殻形成が進行した.

完成した卵の卵殻膜は，内側から外側に向かって，内卵殻膜内層，内卵殻膜外層，外卵殻膜の３層に大別された.

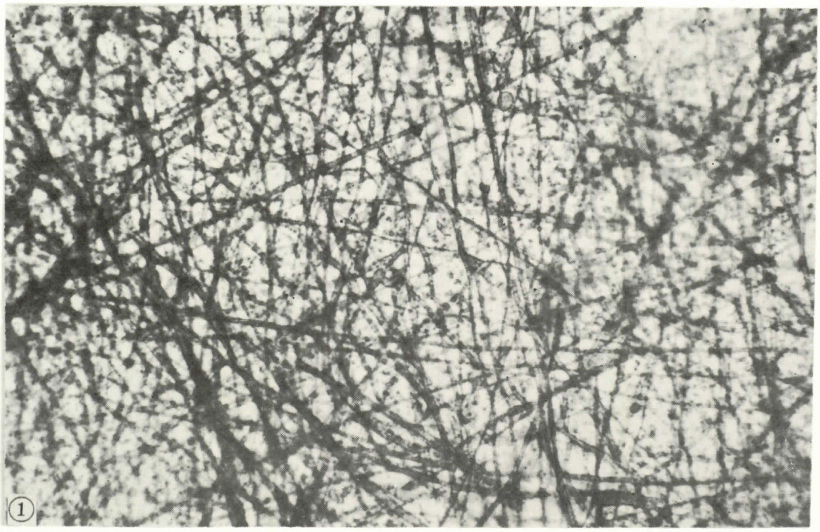

Fig. 1. The shell membrane of an egg collected from the beginning of the isthmus. The shell membrane is formed by a coarse meshwork of fine fibers. Silver impregnation. ×400.

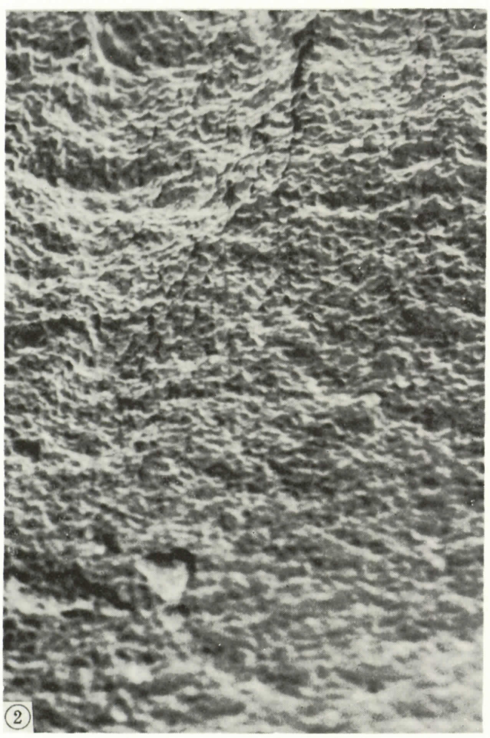

Fig. 2. The inner surface of the shell membrane of an egg collected from the beggining of the isthmus. The inner surface is covered by a mucin-like sheet. Scanning electron microscopy. ×1,000.

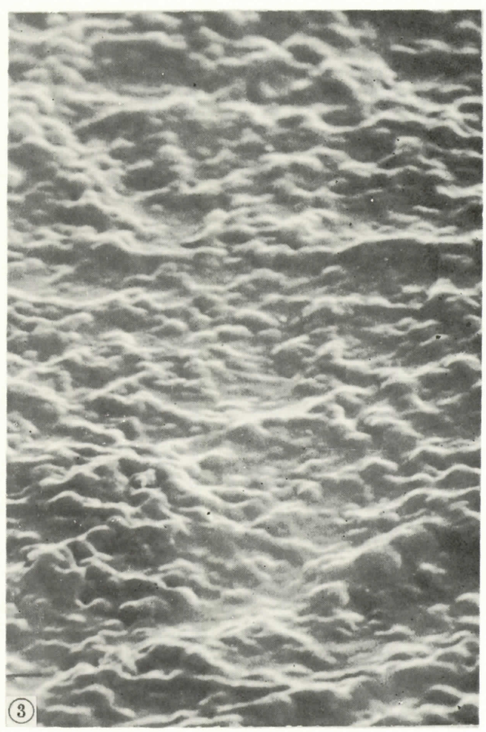

Fig. 3. A high-power magnification of Fig. 2. ×3,000.

Fig. 4. The outer surface of the shell membrane of the same egg as shown in Fig. 2. Note a meshwork of fibers. Scanning electron microscopy. ×1,000.

Fig. 5. A high-power magnification of Fig. 4. ×3,000.

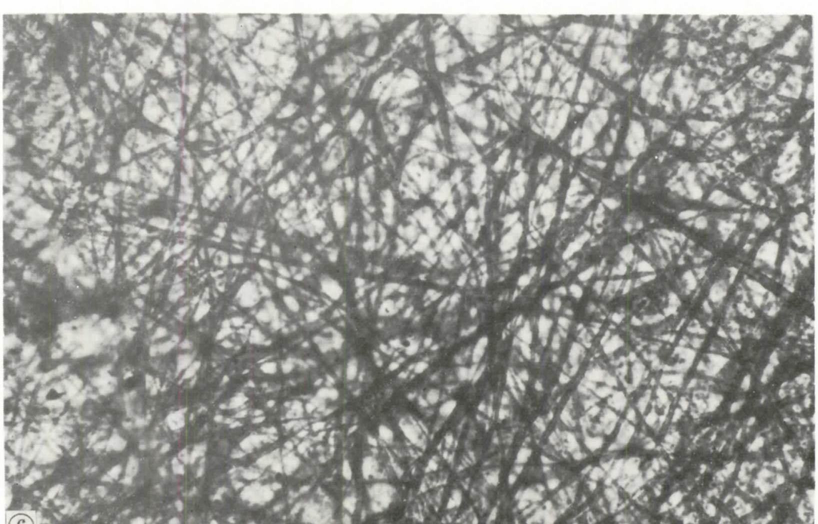

Fig. 6. The shell membrane of an egg collected from the upper portion of the isthmus. The shell membrane is formed by a relatively dense meshwork of fibers. Silver impregnation. ×400.

Fig. 7. The shell membrane of an egg collected from the middle portion of the isthmus. Relatively large fibers have appeared on the already formed meshwork of fine fibers. Silver impregnation. ×400.

Fig. 8. Part of the fibers of the outer layer of the inner shell membrane. The inner layer of the inner shell membrane has been torn away. Note granular concretions adhering to the circumference of each fiber. Silver impregnation. ×400.

Fig. 9. The shell membrane of an egg collected from the end portion of the isthmus. Three layers of the shell membrane are clearly differentiated. Scanning electron microscopy. ×300.

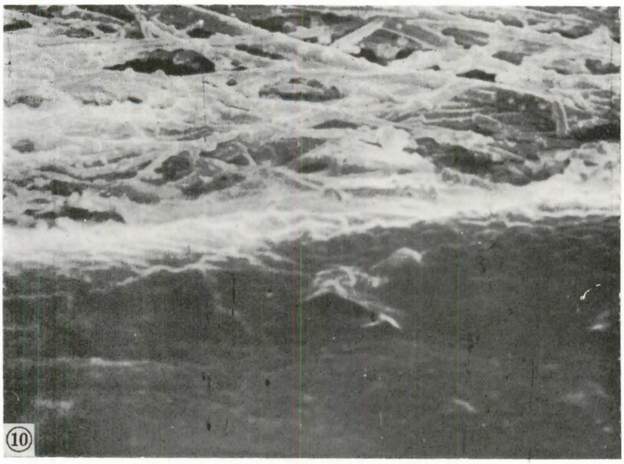

Fig. 10. The inner layer of the inner shell membrane. Fine fibers are embedded in the organic matrix. Scanning electron microscopy. ×1,000.

Fig. 11. The outer layer of the inner shell membrane. This layer is formed by a dense meshwork of fibers. Note granular concretions adhering to the circumference of each fiber. Scanning electron microscopy. ×1,000.

Fig. 12. The inner surface of the outer shell membrane. Scanning electron microscopy. ×1,000.

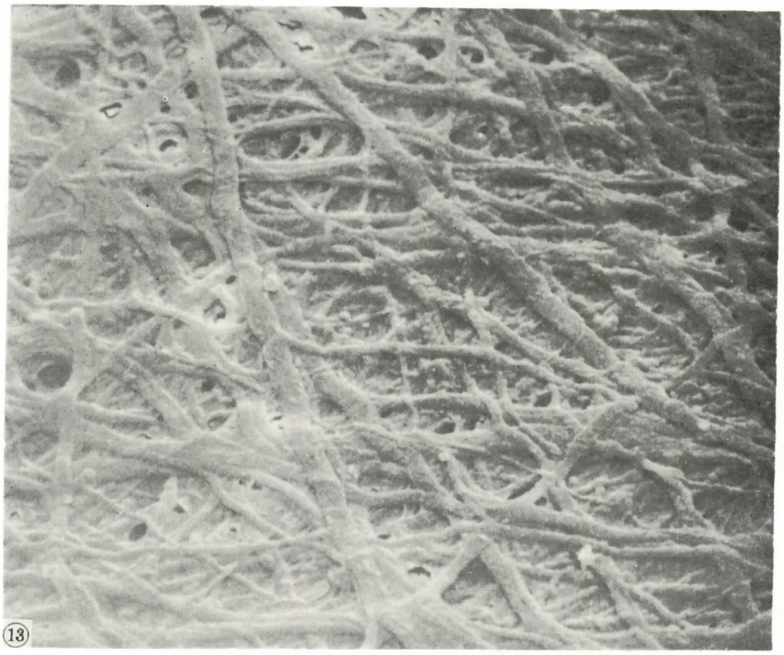

Fig. 13. The outer surface of the egg shell of an egg collected from the end portion of the isthmus. This membrane is formed by a coarse meshwork of large fibers. Scanning electron microscopy. ×1,000.

Fig. 14. A high-power magnification of Fig. 13. ×3,000.

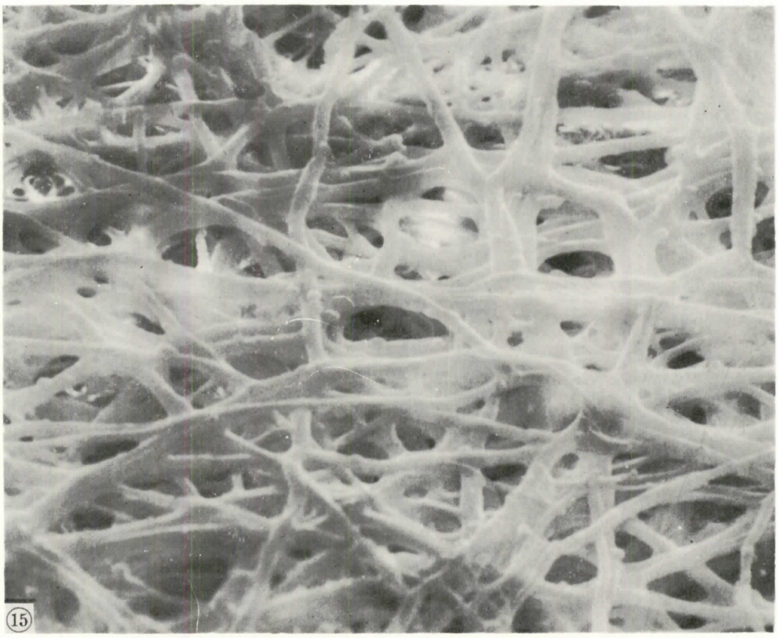

Fig. 15. The outer surface of the outer shell membrane obtained from a completely formed egg after oviposition. Fibers are combined with one another in such manner as to form knots. Scanning electron microscopy. ×1,000.

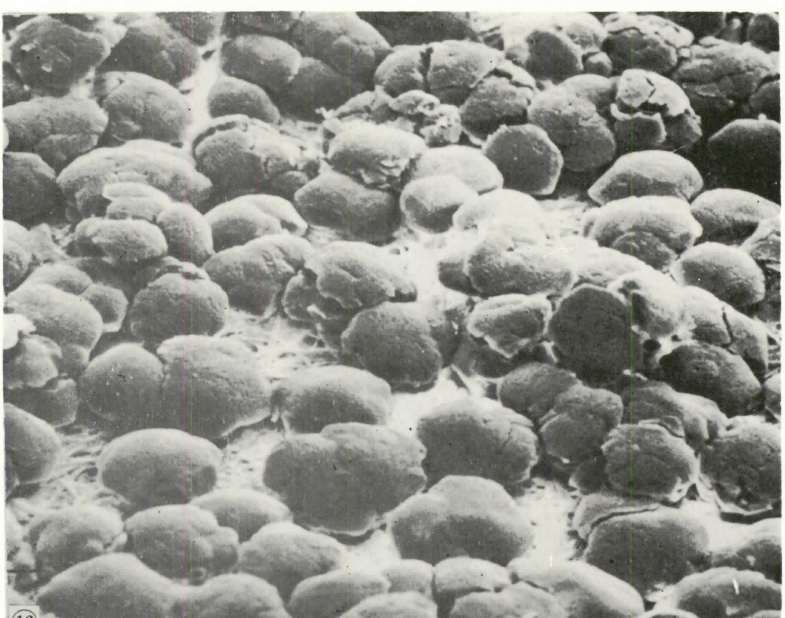

Fig. 16. The outer view of an egg collected from the uterus. Note the deposition of calcium on the surface of the fully formed shell membrane. Scanning electron microscopy. ×300.