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Light and Scanning Electron Microscopical Studies on the Structure of the Vitelline Membrane of the Hen's Egg

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(Plates 1-4)

In the hen's egg, the vitelline membrane is a translucent membrane separating the yolk from the white. It not only holds the yolk mechanically, but also plays an important role at the time of fertilization when the sperm penetrats into the yolk, and at the time of hatching when the embryo comes out. Moreover, its mechanical strength and elasticity decide the marketing grade of the egg. For these reasons, several research workers have examined the structure and function of the vitelline membrane. McNALLY (1943)[1] and ROMANOFF and ROMANOFF (1949)[2] made a detailed literature review on the morphological structure of this membrane as clarified by light microscopy. Recently, BELLAIRS *et al,* (1963)[3] examined the ultrastructure of the vitelline membrane of the hen's egg by electron microscopy. BAIN and HALL (1969)[4] and JENSEN (1969)[5] also examined ultrastructural changes in the vitelline membrane during the stages of development, storage, and hatching of the egg. The optical and electron microscope are efficient in observing the plane structure of the membrane. It is, however, hardly possible to elucidate the three-dimensional structure of the membrane with their help alone.

The present studies were carried out to clarify the three-dimensional structure of the vitelline membrane of the hen's egg by means of a scanning electron microscope. For comparison the whole mount preparation of the membrane was examined by a new method of light microscopy devised by the present authors.

MATERIALS AND METHODS

Specimens of the vitelline membrane were collected from newly laid eggs of White Leghorn hens for this examination. The egg shell was cracked in two along the equatorial line. Then the white was poured out by transferring the whole yolk repeatedly from one half of the cracked shell to the other half. The thick white adhering to the vitelline membrane was detached from this membrane by blunt forceps. The whole yolk was then put into a Petri dish filled with distilled water. The vitelline membrane was cut in two or more by sharp scissors to use as specimens. Furthermore, the dissected membrane was washed carefully in distilled

water to remove the yolk which clung to the inside of the membrane. It was immersed in a 2% lithium carbonate solution at 37°C for a few hours. By this treatment, it became transparent probably due to the removal of such soluble substances as mucin.

After gentle washing in distilled water, each specimen was fixed in a 10% formalin solution. The fixed specimen was subjected to the following steps. For light microscopy, it was mounted on a glass slide avoiding with great care to make wrinkles. It was stained with a 0.2% aniline blue solution to which had been added acetic acid at the rate of 2%, or with a 1% alcian blue solution to which had been added acetic acid at the rate of 0.5%. For scanning electron microscopy, a specimen of the vitelline membrane was spread out on a cover glass, which was dipped in a 50% acetone solution under semi-drying conditions. Then it was dehydrated through a series of graded acetone, held in each grade for 5 mintutes. After having been dried in the incubator, it was coated with gold and examined under a scanning electron microscope, type JSM 2 (Japan Electron Optic Laboratory, Ltd.), at an accelerating voltage of 25 KV.

RESULTS AND DISCUSSION

It has been recognized before by light microscopy that the vitelline membrane of the hen's egg is composed of a fibrillar network. It was not revealed clearly by light microscopy, however, how these fibrils are arranged to form the membrane. The membrane is so thin and fragile that it is difficult to cut tangentially through its surface. Even if prepared, the tangential section of the vitelline membrane will fail to exhibit the three-dimensional arrangement of the fibrils. Furthermore, the whole mount preparation of the membrane was hardly stained by any usual staining method. For these reasons, there are large differences in appearance of the vitelline membrane observed under the light microscope by previous authors.

The figures presented by MORAN and HALE (1936)[6] vaguely exhibited the structure of the vitelline membrane which was a network of fibrils consisting of three layers. DORAN and MUELLER (1961)[7] indicated a membrane divided into two tinctorially characteristic layers; that is, an inner layer composed of collagenous material and an outer layer of mucin substance. BELLAIRS *et al.* (1963)[3] pointed out that it was difficult to observe the fibrils of the vitelline membrane in any whole mount specimen, unless a phase contrast microscope was used. According to them, the whole mount specimen was not clearly stained by collagen staining or by elastic fiber staining, and when stained, it was colored diffusely and uniformly.

In fact, the present authors tried to stain the whole mount preparation by ordinary staining only to find that no staining method was successful. This difficulty of staining the membrane under wet conditions, in spite of the very thin and apparently stainable character of this membrane, is considered to be due to the presence of the mucin substance which resists all staining. Accordingly, the mucin

was removed from the membrane by immersing the specimen in a lithium carbonate solution. By this treatment, the whole mount specimen became stainable with ordinary stains as mentioned below. Some structural changes, however, may have been induced in the membrane.

Light microscopy

When a macerated specimen was stained with aniline blue or alcian blue, a fibrillar structure visualized in the vitelline membrane, as shown in Figs. 1–6. Figs. 1 and 2 show the inner surface of the vitelline membrane. As is clear in these figures, the inner surface of the membrane is composed of a network of fibrils. Individual fibrils are slender in shape. Fibrils run parallel to the surface of the yolk. The network of fibrils was a little coarse in arrangement in some parts of the inner surface, particularly along the equatorial line (Fig. 1), wherease it was dense at the polar ends(Fig. 2). Preparations stained with alcian blue presented clearer figures of the vitelline membrane than those with aniline blue (Figs. 3 and 4). As shown in these figures, fibrils run in such manner as to draw an arc, gathering in bundles in places (Fig. 3). There was a more prominent circularly interlaced fibrillar network in the portion along the equatorial line than in the regions of the polar ends. This pattern of fibrillar network is characteristic of the inner layer of the vitelline membrane.

In the preparations stained with alcian blue, the fibrils themselves were not stained at all, but an underlying layer attached to the inner layer was stained with the dye. As a result, the fibrils were embossed distinctly. The layer stained with alcian blue was considered to be a continuous membrane which was observed ultrastructurally By BELLAIRS *et al.* (1963)[3] for the first time. According to them, the continuous membrane is a thin granular layer located between the outer and the inner layer of the vitelline membrane. In addition, BAIN and HALL (1969)[4] found out that the continuous membrane was usually attached to the inner layer, which was separated from the membrane in a wet state.

The outer surface of the vitelline membrane was presented as an interlaced network of fibrils (Figs. 5 and 6). As shown in these figures, the fibrils of the outer surface are fine and look feeble. They formed a network coarse in arrangement in the portion along the equatorial line (Fig. 5), and dense at the polar ends (Fig. 6). The fibrils gathered in thick bundles or in wide sheets near the chalaza. These bundles of fibrils originated from the equatorial line and stretched in the direction of the polar ends. They were transmitted into the chalaza. This definite layer on the outer surface is though to be a chalaziferous layer which has been described by ROMANOFF and ROMANOFF (1949)[2]. The actual fibrils of the outer layer, however, could not be observed by the present method, since they were too fine to be visualized by light microscopy.

Scanning electron microscopy

Scanning electron microscopy distinctly revealed the fine fibrillar network of the vitelline membrane (Figs. 7–10). Figs. 7 and 8 show the inner surface of the membrane. As is clear from them, the inner surface is formed by a typical network of fibrils. The network is constructed by fibrils of varying size. These fibrils are flat in shape and appear a little solid, fused with one another to form a knot. From this knot fibrils stretch in a radial direction, giving off small branches. The branches anastomose with one another in a three-dimensional direction. The small fibrils were 0.2–0.7μ in width and the fused large ones 1–2μ in width. Spaces between fibrils are remarkable and approximately 10μ in width. In Fig. 8, numerous small granules are noticed to adhere to the surface of fibrils. They may have been produced by an artifact. Such three-dimensional architecture of fibrils as this formed the network of the inner layer of the vitelline membrane. There is a considerable difference between the structural appearance of fibrillar network in Figs. 1 and 8. This is because Fig. 1 reveals only fibrils large enough to be visualized by light microscopy.

On the other hand, the outer surface of the membrane was formed by a dense lattice-work of fine fibrils, as shown in Figs. 9 and 10. At a glance, one can see the difference in the structural pattern of fibrillar network from the inner surface. This difference suggests that the vitelline membrane may be provided with at least two types of layers. The fibrils of the outer surface are very fine and appear feeble and fragile. Individual fibrils are interlaced with one another in a longitudinal and a transverse direction, without forming any knot of fused fibrils. They were uniform in size and about 0.07μ in width. Spaces between fibrils were narrow. They are probably filled with gel-like substances during the living state of the membrane.

Careful observation of Fig. 10 shows that relatively large fibrils run scatteringly on the mesh of fine fibrils. Those large fibrils are apparently the bundles of fibrils joining themselves to fine fibrils (Fig. 11). They are few in number in the equatorial portion of the outer surface and abundant at the polar ends. These bundles of fibrils seem to accord with the fibrils which are shown in Figs. 5 and 6. They run straight in the direction of the chalaza and form a thick membranous layer containing large bundles of fibrils (Fig. 12). This layer is the so-called chalaziferous layer.

Romanoff and Romanoff (1949)[2] explained that the chalaziferous layer was a matted, fibrillar capsule of a thin layer of dense albumen arround the vitelline membrane. Bellairs *et al.* (1963)[3] and Bain and Hall (1969)[4] hardly referred to the chalaziferous layer in their descriptions. According to Fromm (1964),[8] the layer becomes brittle and is broken into flackes on account of dehydration, which makes it difficult to distinguish this layer by optical or electron microscopy. In the presnet study, however, it was possible to observe the structure of the chalaziferous layer, although the appearance of the layer was not so clear (Fig. 12). This layer

showed a vague structure, since it contained fibrils which were presumably delicate in nature and gel-like substances which had been condensed by drying at the time of preparation of the specimen.

The chalaza is illustrated in Fig. 13. It was a condensed mass of gel-like substances. ROMANOFF and ROMANOFF (1949)[2] stated that the chalaza was composed of numerous fine, mucin-like fibrils, which are firmly attached to the surface of the yolk. According to FROMM (1967)[9], it is a gel-like amorphous structure containing mucin-like substances. In the present scanning electron microscopy, there were no distinguishable fine mucin-like fibrils, but condensed homogeneous masses in the chalaza. The presence of mucin-like fibrils might possibly be a reason for such a vague appearance of the chalaza as this.

BELLAIRS *et al.* (1963)[3] investigated the fine structure of the vitelline membrane by electron microscopy, and found that this membrane was composed mainly of two types of layers, an inner and an outer. The inner layer was a network of solid cylindrical fibrils 0.2–0.6μ in width. The outer layer was composed of some sublayers, each of which was a lattice-work of fibrils 150Å in diameter. There was a continuous membrane 500–1,000Å thick between the two layers. The inner layer was abowt 2.7μ in thickness and the outer layer 3.0–8.5μ. JENSEN (1969)[5] examined ultrastructural changes in the vitelline membrane during the stage of embryonic development. BAIN and HALL (1969)[4] observed these changes in the same membrane during the stages of formation, development, and storage of the egg. As a result, the three authors lent support to the conclusions drawn by BELLAIRS *et al.*[3]

As mentioned above, the present authors studied the three-dimensional structure of the vitelline membrane by scanning electron microscopy. The results obtained also lend support to the diagram of the vitelline membrane given by BELLAIRS *et al,*[3] In the present investigation, however, it was impossible to distinguish a continuous membrane between the two layers by scanning electron microscopy, since no observation was made on any cross section of the vitelline membrane. The presence of such continuous membrane was seen as that of a membrane stained positively with alcian blue, under the light microscope.

SUMMARY

The three-dimensional strucuture of the vitelline membrane of the hen's egg was observed by optical and scanning electron microscopy. The general structure of this membrane was exhibited even by light microscopy when a mucin-like substance had been removed from the membrane by immersion in lithium carbonate solution.

The vitelline membrane consisted principally of two layers, an inner and an outer layer. The inner layer was a network of flat, solid fibrils 0.2–2.0μ in width. These fibrils were fused with one another in such manner as to form knots. The

outer layer was a network of fine, feeble fibrils about 0.07μ in diameter. These fibrils were interlaced with one another in longitudinal and transverse directions and anastomosed a little. There was a chalaziferous layer on the surface of the outer layer. It was composed of bundles or sheets of fibrils. The chalaza was revealed as a condensed amorphous mass of gel-like substances.

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鶏卵の卵黄膜の光顕的・走査電顕的研究

藤井　俊策・田村　達堂・岡本　敏一

鶏卵の卵黄膜の立体的構造が，光顕と走査電顕によって観察された．卵黄膜は，生の薄膜標本では光顕的に観察が困難なものであるが，これを炭酸リチウム液に浸漬すると，粘液物質が除去され，普通の染色で充分観察されるようになった．この方法によって調べた卵黄膜の光顕と走査電子鏡検像が示された．

卵黄膜は内と外の2層の線維膜構造であった．内層はテープ状の薄い線維の交織から成る線維膜であり，外層は細線維から成る線維網であった．外層の表面には，太い線維束と粘液物質から成るカラザ層が存在していた．カラザ層は多量の粘液物質を加えながらカラザに移行した．カラザは走査電顕では，ゲル物質の凝塊として示された．

EXPLANATION OF PLATES

Fig. 1. The inner surface of the vitelline membrane near the equatorial line of the yolk. The membrane was stained with aniline blue solution on a whole mount specimen. A coarse network of fibrils was formed. x1,000.

Fig. 2. The inner surface of the vitelline membrane near the polar end of the yolk. This specimen was prepared in the same manner as that shown in Fig. 1. A dense network of fibrils was formed. x1,000.

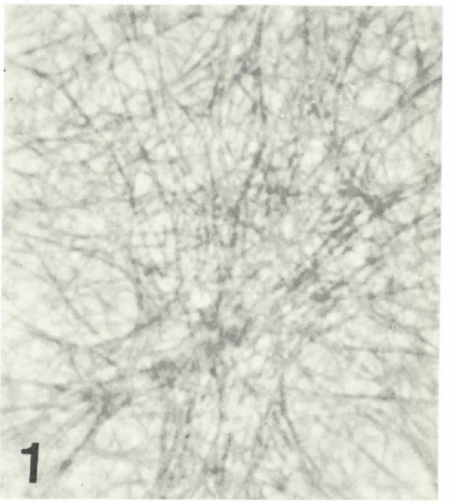
Fig. 3. The inner surface of the vitelline membrane near the equatorial line of the yolk. The membrane was stained with alcian blue solution on a whole mount specimen. A circularform network was formed. x400.

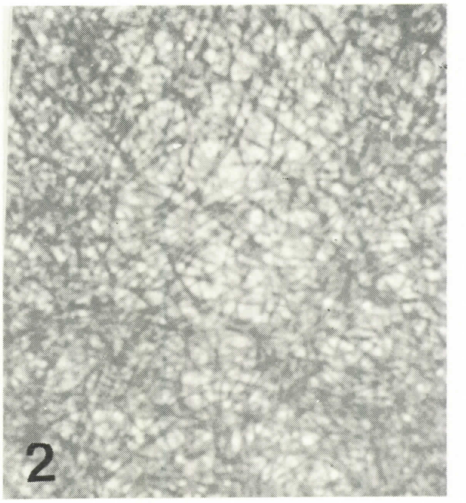
Fig. 4. A high-power magnification of Fig. 3. x1,000.

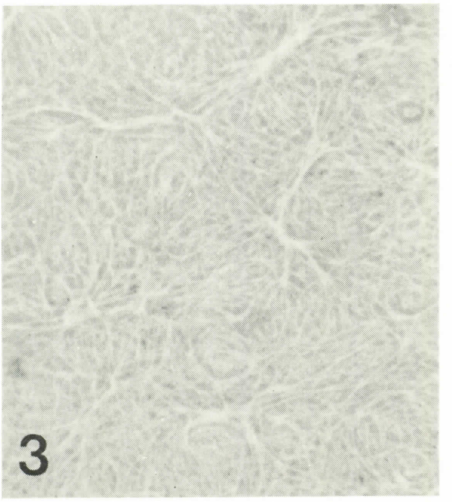
Fig. 5. The outer surface of the vitelline membrane near the equatorial line of the yolk. This specimen was prepared in the same manner as that shown in Fig. 1. A coarse network was formed by fine, feeble fibrils. x1,000.

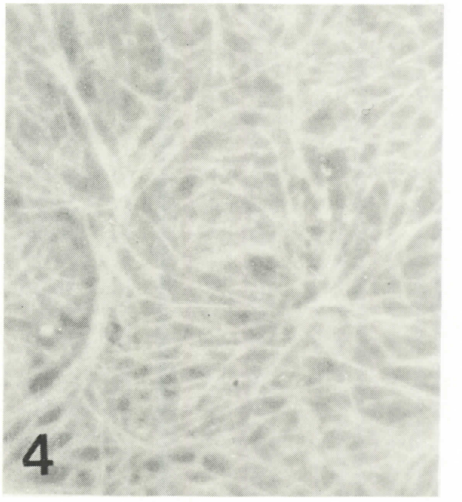
Fig. 6. The outer surface of the vitelline membrane near the polar end of the yolk. This specimen was prepared in the same manner as that shown in Fig. 1. A relatively dense network was formed. Some fibrils were torn off partially during the process of preparation of the specimen, x1,000.

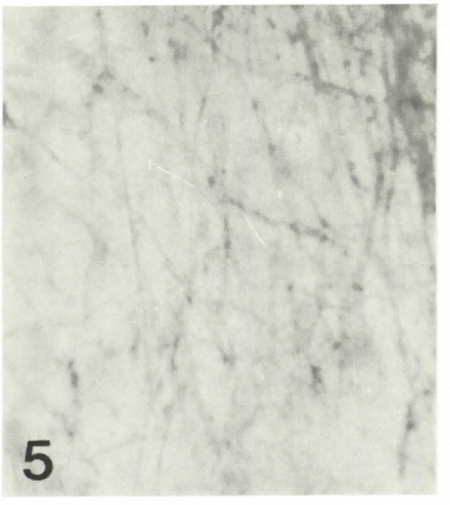
Plate 1.











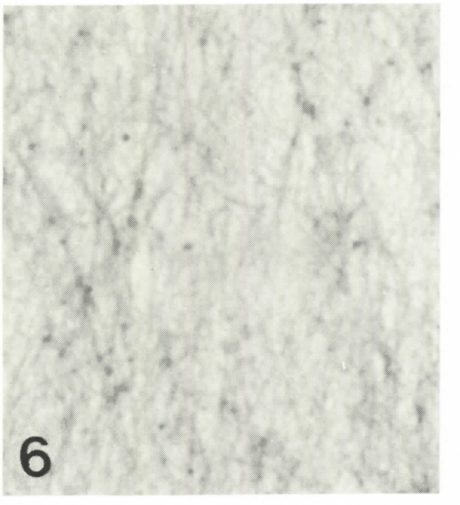
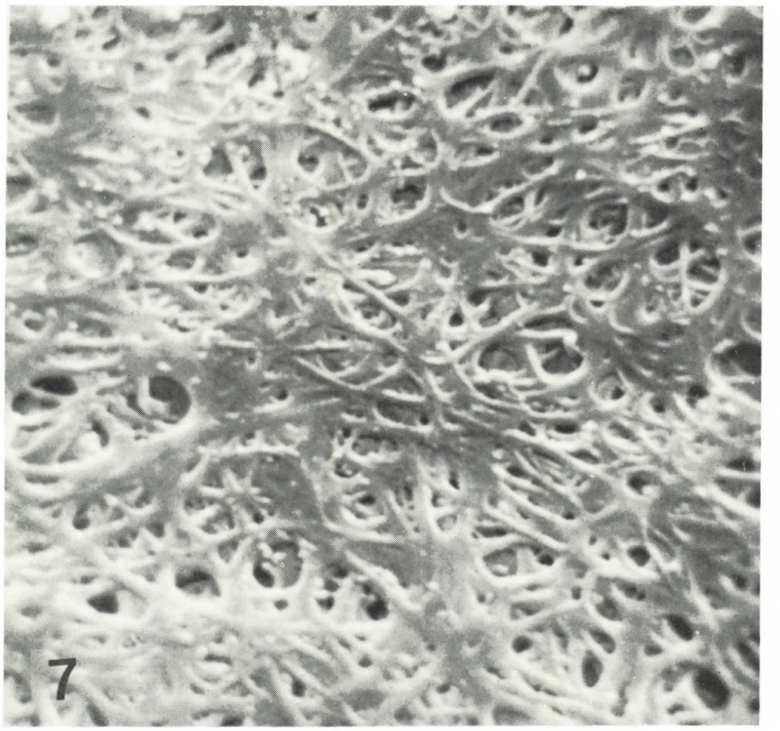


Fig. 7. The inner surface of the vitelline membrane demonstrated by scanning electron microscopy. A network of fibrils is shown distinctly. x3,000.

Fig. 8. A high-power magnification of Fig. 7. x9,000.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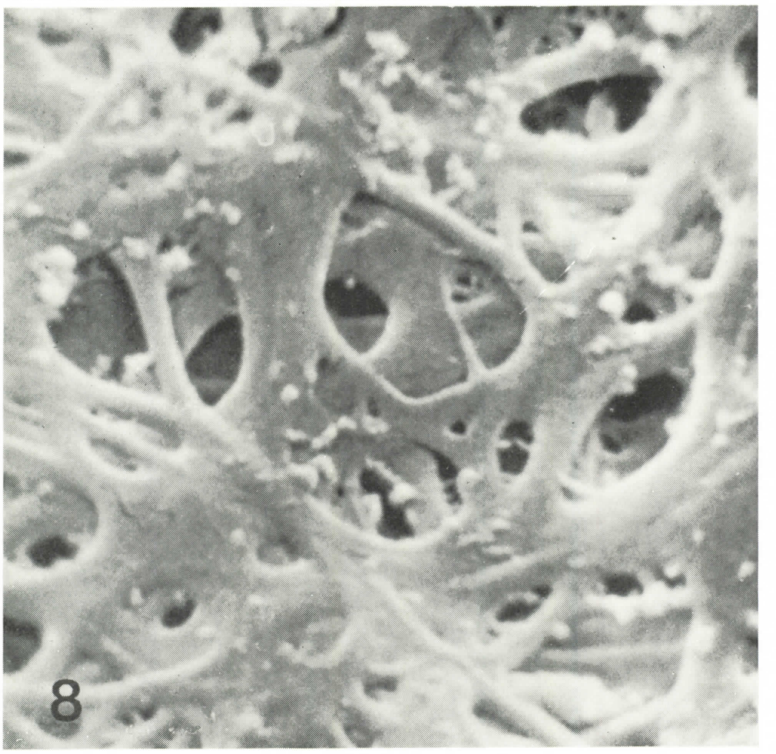
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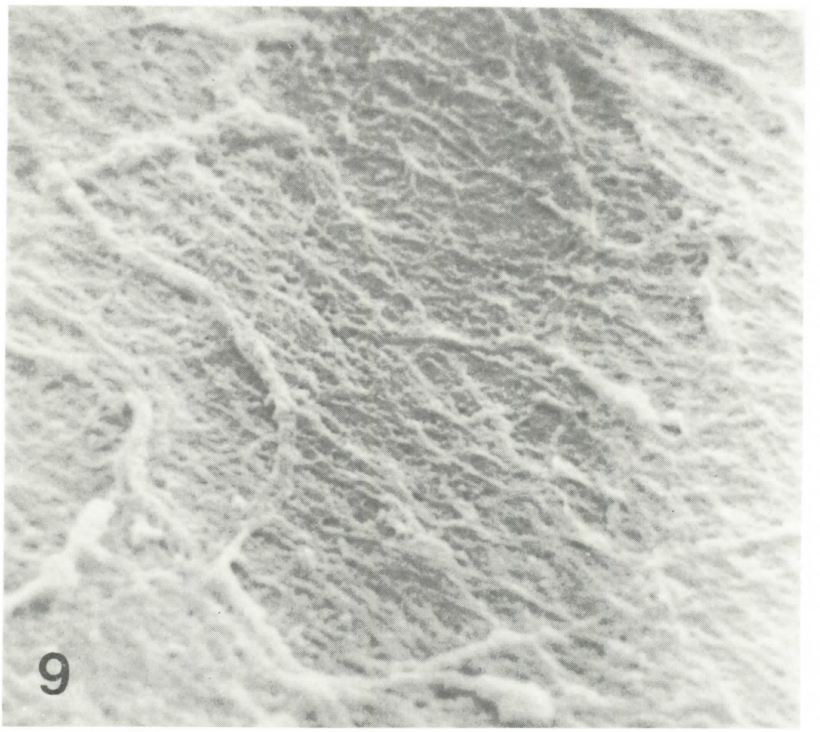
Fig. 9. The outer surface of the vitelline membrane demonstrated by scanning electron microscopy. A network was formed by fine fibrils. x3,000.

Fig. 10. A high-power magnification of Fig. 9. x9,000.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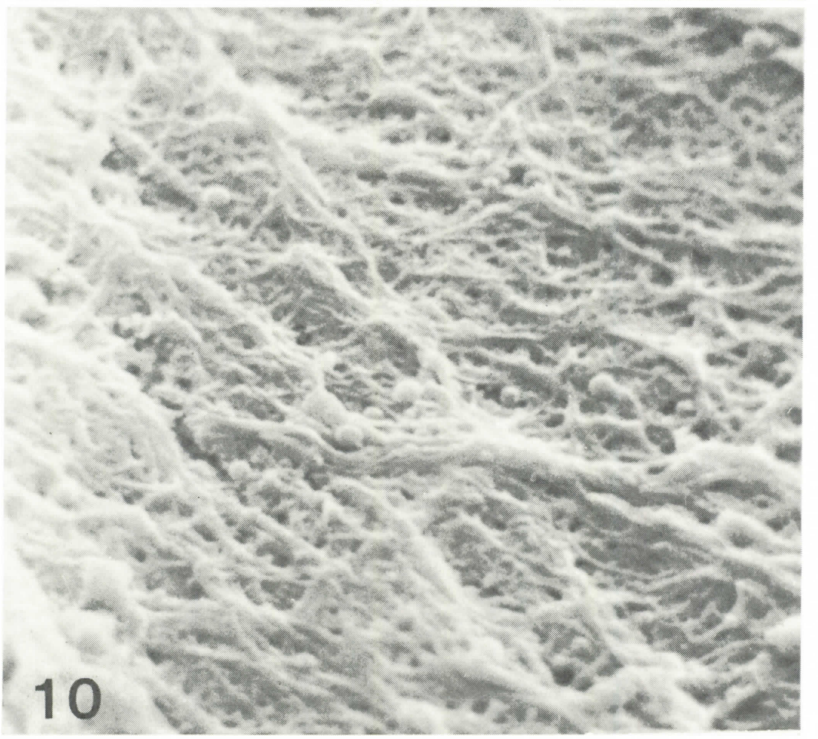
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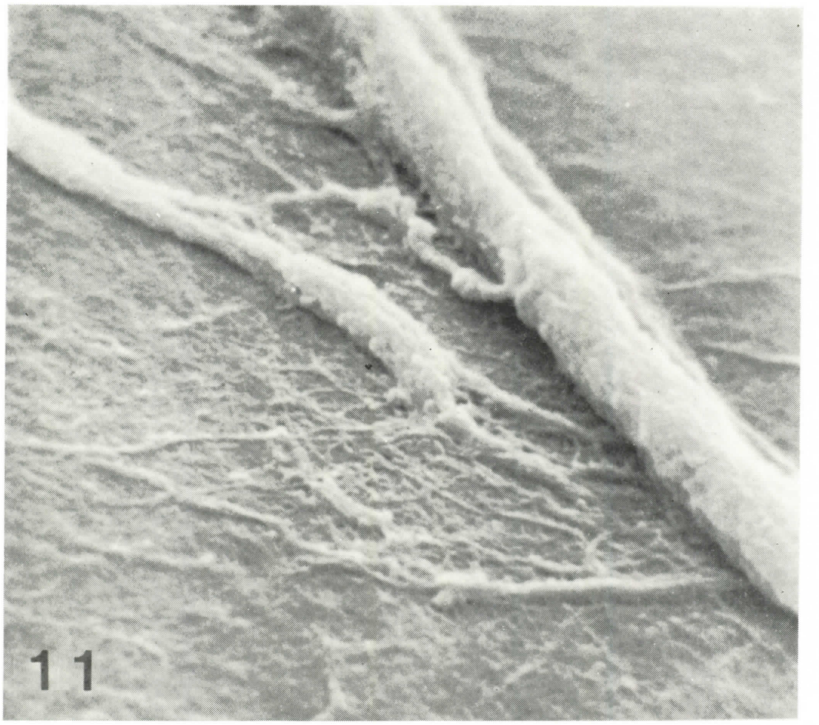
Fig. 11. Bundles of fibrils presented at the beginning of the chalaziferous layer near the equatorial line of the yolk. They were formed by fusion of fine fibrils. x3,000.

Fig. 12. The chalaziferous layer demonstrated by scanning electron microscopy. It was formed by condensation of fibrils and gel-like substance. x3,000.

Fig. 13. The chalaza demonstrated by scanning electron microscopy. It was formed by condensation of gel-like substances. x3,000.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