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Studies on Yolk Formation in Hen's Eggs

I. Light and Scanning Electron Microscopy of the Structure of Yolk Spheres

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(Figs. 1-16)

The yolk of the hen egg is a huge oocyte containing a large quantity of yolk materials in its cytoplasm. These yolk materials are in a form of viscous emulsion in the physico-chemical nature. They are constructed in concentric arrangement of yellow and white yolk layers, except those in the region of the blastodisk, the latebra, and its column. A number of investigators have examined in detail the chemical composition of the yolk.[1)2)] Many workers too have observed the morphological strucutre of the yolk by light, phase contrast, and electron microscopy.[3)4)5)] From his studies by electron microscopy, BELLAIRS (1961)[6)] reported that the yolk contained as gross components; free floating oil drops, yellow and white yolk spheres, and aqueous protein fluids. Of these components, the yolk spheres are the most conspicous. Until now, however, little is yet known of the mechanism of the formation of these yolk spheres, as well as of the relationship between the chemical constituents and their locations within the yolk.

The present studies were carrid out by light and scanning electron microscopy to clarify the structure of the yolk spheres of the hen egg.

MATERIALS AND METHODS

The conventional techniques for the preparation of paraffin or celloidin sections are not always dequate in the observation of the morphology of yolk spheres, since in these techniques lipids of yolk spheres dissolve when passing through alcohol or xylol. Moreover, the yolk of the hen egg has a size too large to be sectioned. For these reasons, a suspension of raw yolk was mainly employed in the present observation.

Preparation of suspension materials

Eggs laid by White Leghorn hens were used. The yolk was collected from them and treated by either of the following two methods.

In one method, the yolk was fixed in a 10% formalin solution as a whole for about one week. The fixed yolk was dispersed in a 0.85% NaCl solution for observation.

In the other method, the yolk was suspended immediately when it was still in state of raw material. For this purpose, eggs were broken and the white adhering to the yolk was separated. The yolk was placed on an 80-mesh filter made of wire gauze, set in a large-sized funnel with its bottom plugged. The funnel had previously been filled with a 0.85% NaCl solution containing saturated lithium carbonate at a rate of 1%. Thereby, the yolk was dissolved gradually and the yolk spheres fell through the filter to the bottom of the funnel. The supernatant was discarded. The precipitate was resuspended in the same solution mentioned above. Again the supernatant was discarded. The precipitated yolk spheres were washed with a NaCl solution containing no lithium carbonate. By this method, large quantities of suspension of yolk spheres were collected in a pure state. The volume of the NaCl solution to be added should best be 20—30 times the volume of yolk. This operation should be done as gently as possible. The lithium carbonate added seems to act as an anticoagulant on the aqueous protein of the yolk and make the yolk spheres more filtrable. When small quantities of yolk spheres are necessary, they are collected in a watch glass, with gentle rotating, by using the same solution as mentioned above.

Observation of yolk spheres

A drop of suspension of yolk spheres was placed on a glass slide and examined directly under a light microscope. For scanning electron microscopy, the suspension of yolk spheres was fixed in a 10% formalin solution or in bichromate-formalin dissolved in a 0.85% NaCl solution. After washing, a drop of suspension was put on a coverslip and dried slowly at room temperature. It was coated with gold and examined by a scanning electron microscope, type JSM U (Japan Electron Optic Laboratory, Ltd.), at an accelerating voltage of 15KV.

RESULTS AND DISCUSSION

It has been known since a long time ago that the yolk spheres are divided into two types, a yellow and a white yolk sphere.[1)3)4)] The classification of these two types is based mainly on the difference in diameter and on the presence or abscence of fine granules contained in yolk spheres.

Light microscopy

The first observation was done on yolk spheres fixed in formalin. Fig. 1 shows the picture of yolk spheres taken from the yellow yolk layer, and Fig. 2 that of yolk spheres taken from the center of the yolk (the latebra). As is seen in these figures, yolk spheres uniformly appeared in a curious form of polyhedral crystal. The yolk spheres of Fig. 1, though they vary in size, are yellow yolk spheres, because of their homogeneous, structureless appearance. Whereas, those of Fig. 2

are generally smaller in size and more distinctly flat in form than the yellow yolk spheres. The most striking feature of the yolk spheres is that they contain comparatively large droplets in the body. The droplets vary in size and number. They are located in the periphery and show a crescent-form gathering on one side of the pole. This type of spheres is the white yolk sphere. The polyhedral appearance of yolk fixed in formalin suggests that yolk spheres may be laid in a tightly-packed form in the yolk under natural conditions, although they are originally spherical in form.

An other observation was performed on the suspension of raw yolk. Figs. 3, 4, and 5 show pictures of yolk spheres collected from the whole yolk, from the yellow yolk layer, and from the latebra region, respectively. Collection of yolk spheres from the latebra was done by plunging the tip of a pipette into the center of the yolk. In those figures, yolk spheres are revealed uniformly in spherical form. In Fig. 3, there are various types of yolk spheres differing in size and appearance. Those which are large in size and homogeneous in appearance are yellow yolk spheres. Those which are comparatively small in size and finely granular in appearance are white yolk spheres. Those which are smaller in size and clear in appearance are free floating drops named as such by BELLAIRS (1961).[6] The yellow yolk sphere (Fig. 4) is characterized by its size and the presence of densely arranged, fine globules whthin it. It ranges from 20 to 150 μ in diameter. Whereas, the white yolk sphere (Fig. 5) is characterized by its relatively smaller size and the presence of more coarsely arranged, rough droplets. It ranges from 5 to 80 μ in diameter.

The yolk sphere is very sensitive to mechanical actions and osmotic changes of the surrounding medium. Morphological changes of yolk spheres induced by osmotic effects have been examined in detail by GRODZINSKI (1951).[7] In the present study, when yolk spheres were nipped with the tip of a needle, they bursted and their contents flowed out into the medium. Fig. 6 presents the bursting of a yellow yolk sphere on the left side and that of a white yellow sphere on the right side. Immediately after bursting, globules contained in yellow yolk spheres started to show the Brownian movement and dispersed in the medium. On the contrary, globules from white yolk spheres became larger and larger in size and turned finally into aqueous fluids. No shadows of yolk spheres remained. When yolk spheres were pressed slightly with a coverslip, the globules contained in them were expelled through the entire surface (Fig. 7). In addition, when a drop of fat solvent was put on yolk spheres and examined by a light microscope, clear fat droplets were seen escaping from the bodies of the spheres (Fig. 8). The above-mentioned strange phenomena of yolk spheres seem to point to the presence of a certain membrane-like structure around the bodies of these spheres.

Scanning electron microscopy

When examined by a scanning electron microscope, yolk spheres fixed in formalin showed such a polyhedral appearance as observed by light microscopy (Fig. 9). In

Fig. 9, the appearance of comparatively large protrusions on the outer surface may be an artifact. When scrutinized at a high-power magnification, the spheres had not always a smooth surface, but occasionally some small granular protrusions formed on the entire surface (Fig. 10). These protrusions were considered to derive from the external exposure of globules contained in the yolk spheres. On the other hand, yolk spheres in untreated suspension are shown in Figs. 11—13. In these figures, they show a spherical appearance like that of a pearl. Yolk spheres taken from the whole yolk consist of both yellow and white ones (Fig. 11). Of them, the large-sized ones are apparently yellow yolk spheres, and the small-sized ones probably white yolk spheres. Yellow yolk spheres taken from the yellow yolk layer (Fig. 12) are occupied by yellow yolk spheres only.

At a high-power magnification, the surface of yellow yolk spheres showed generally a smooth appearance, though somewaht wrinkled (Fig. 13). The wrinkles may have been caused by dryness during the preparation of the samples. In Fig. 13, yolk spheres fused with one another. Although the fusion among the yolk spheres was also brought about by the dryness of the samples, it seems possible that the surface of yolk spheres might be enclosed by certain viscous structures. Yolk spheres were further examined in a transverse section. For this purpose, a mass of yolk spheres was hardened by kneading and then cut with a sharp knife after drying. In Fig. 14, a yellow yolk sphere cut transversely shows numerous globules, which were observed by light microscopy. The globules are distributed over the whole area, embedded in a somewhat solid substance. The margin of the sphere, where a membrane, if present, lies, seems to be framed by certain homogeneous structures, although it is naked in some places. Few globules are distributed in the surrounding area.

At present, the opinions about the presence of membranous structures around yolk spheres are divided. Grodzinski (1951)[7] observed morphological changes of yolk spheres in various osmotic conditions and presumed that there might be a wall built up with a semipermeable membrane. From his electron microscopic studies on yolk spheres, Bellairs (1961)[6] reported that the surface of yolk sphere may be distinguished in three types: a lamellated capsule, a unit membrane, and a naked surface. In the present observation, light microscopy of raw yolk materials revealed that there might be a membrane-like structure around each yolk sphere, as mentioned above. Scanning electron microscopy of yolk spheres also showed that a wall made of viscous substances was present around each sphere, although it was vague. From these results, the probability arises that yolk spheres are surrounded by something like a membranous structure.

Further morphological examination was made on aging yolk spheres. For this purpose, unfertilized eggs were put in an incubator at 38°C in saturated humidity. Figs. 15 and 16 show the pictures of a suspension of yolk spheres of an egg at 16 days of incubation observed by light and scanning electron microscopy, respectively. In these figures, the structure of most of the yolk spheres has been deformed distinctly. The most striking change is the loss of the spherical appearance of the yolk spheres. These spheres have become enlarged, flat, and polyhedral in form. The morphological changes were more prominent in white spheres than in yellow yolk ones. They began to appear around the 10th day of incubation. The decomposition of yolk spheres in incubated eggs is considered to be caused by the emission of the contents of spheres as a result of the change in viscocity of the yolk. SMITH (1935)[8] reported that the viscosity of yolk decreased as a result of the entrance of water from the albumen to the yolk during storage. This effect was associated with the surrounding temperature. The entrance of water from the albumen broke the osmotic equivalence between the inside and outside of the yolk spheres. As a result of this, the yolk spheres swelled and extruded their contents. These changes will be described in detail in a future separate report.

SUMMARY

The structure of yolk spheres of hen yolk was observed in state of suspension of raw and fixed materials under the light and the scanning electron microscope. The yolk spheres were composed of yellow and white yolk spheres. Their structural features were discussed on the basis of figures obtained by both types of microscopy. The surface of yolk spheres seemed to be enclosed in a wall made of certain homogeneous structures. In stored eggs, the appearance of yolk spheres changed distinctly. These morphological changes were considered to have been induced by osmotic changes of the yolk.

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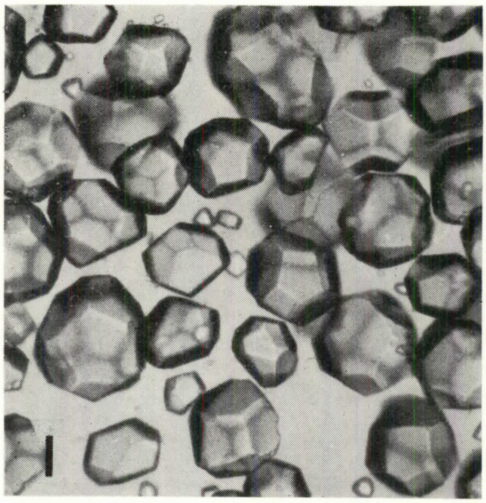
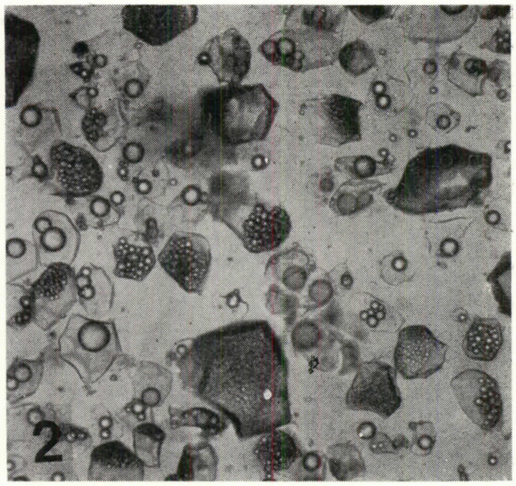
ニワトリの卵黄形成に関する研究

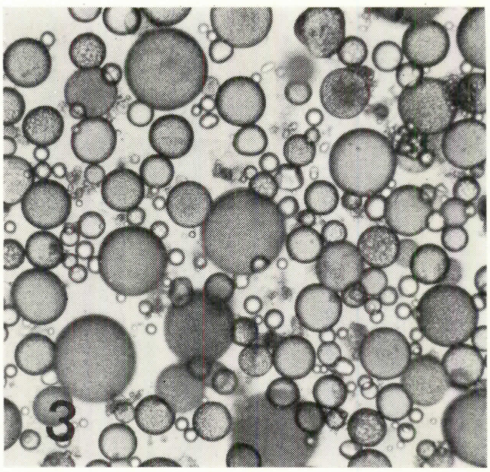
I. 卵黄球の光顕と走査電子鏡検

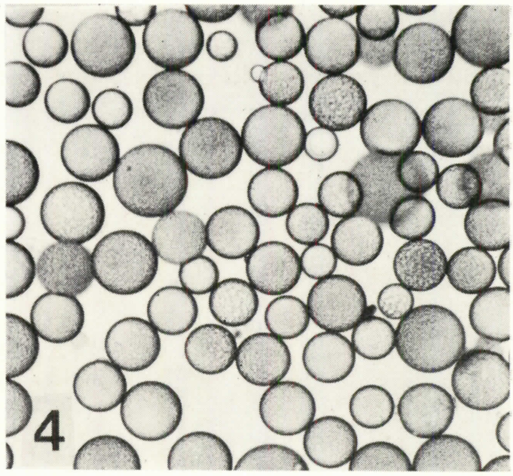
藤井　俊策・田村　達堂・岡本　敏一

卵黄を構成する卵黄球（yolk spheres）の構造を，光学顕微鏡と走査電子顕鏡下で観察した．とくに卵黄球内の粒子と卵黄球表面の膜様構造物について論議した．貯蔵卵の卵黄球の形態的変化についても一部触れた．









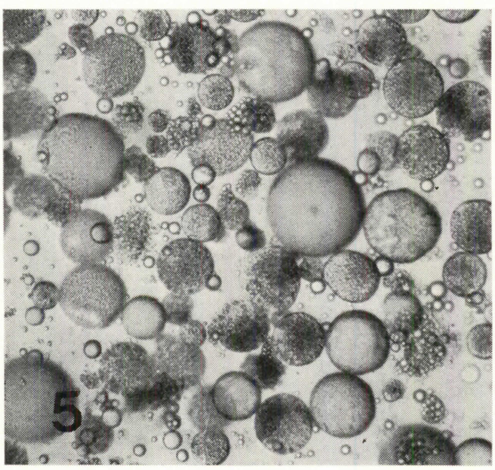


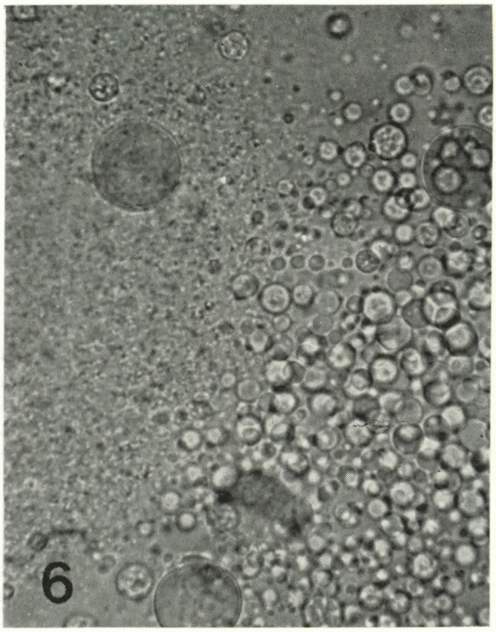
Fig. 1. Yellow yolk spheres collected from the yellow yolk layer. The yolk was fixed in a 10% formalin solution as a whole. ×150.

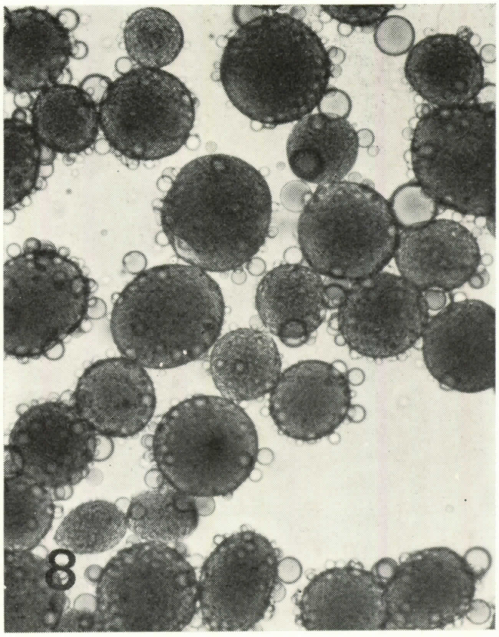
Fig. 2. White yolk spheres taken from the latebra region. The yolk fixed in a 10% formalin as a whole. ×150.

Fig. 3. Various types of yolk spheres collected from suspension materials of whole yolk. ×100.

Fig. 4. Yellow yolk spheres collected from suspension materials of the yellow yolk layer. ×100.

Fig. 5. White yolk spheres collected from suspension materials of the latebra region. ×100.





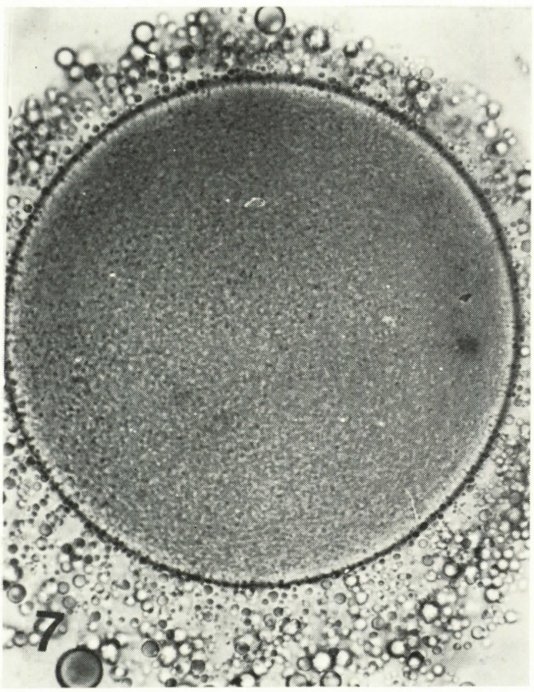


Fig. 6. Bursted yolk spheres. Globules extruded from a yellow yolk sphere are shown on the left side and droplets from a white yolk sphere on the right side. ×400.

Fig. 7. Expulsion of contents of a yellow yolk sphere. The yolk sphere has been pressed slightly. ×800.

Fig. 8. Extrusion of oil drops from yellow yolk spheres. This phenomenon was revealed by adding a drop of fat solvent. ×150.

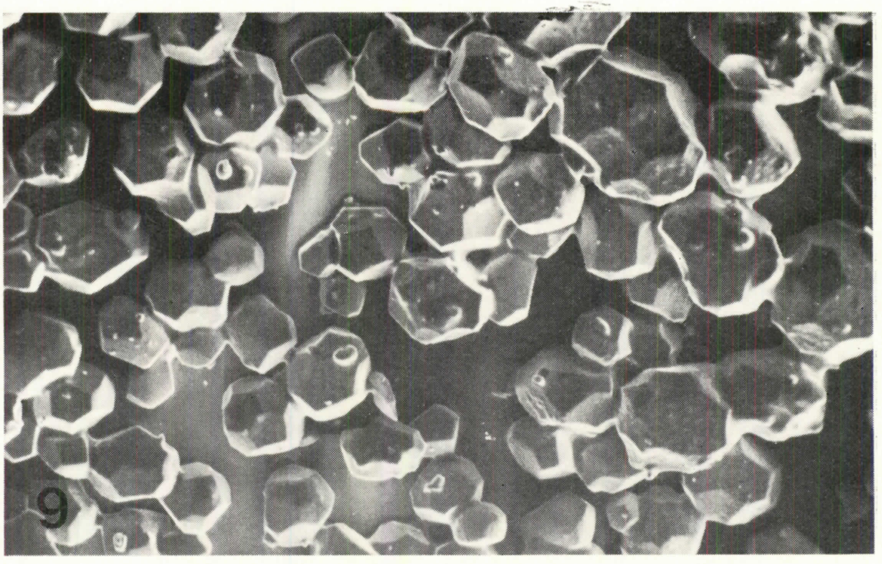


Fig. 9. Yolk spheres observed by scanning electron microscopy. They were obtained from yolk fixed in formalin. ×100.

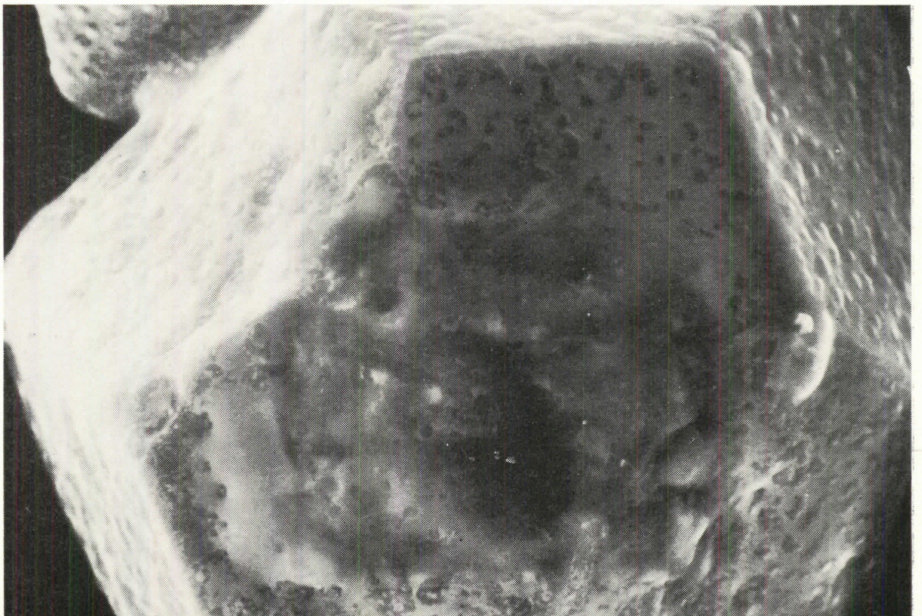


Fig. 10. A high-power magnification of Fig. 9. ×3,000.

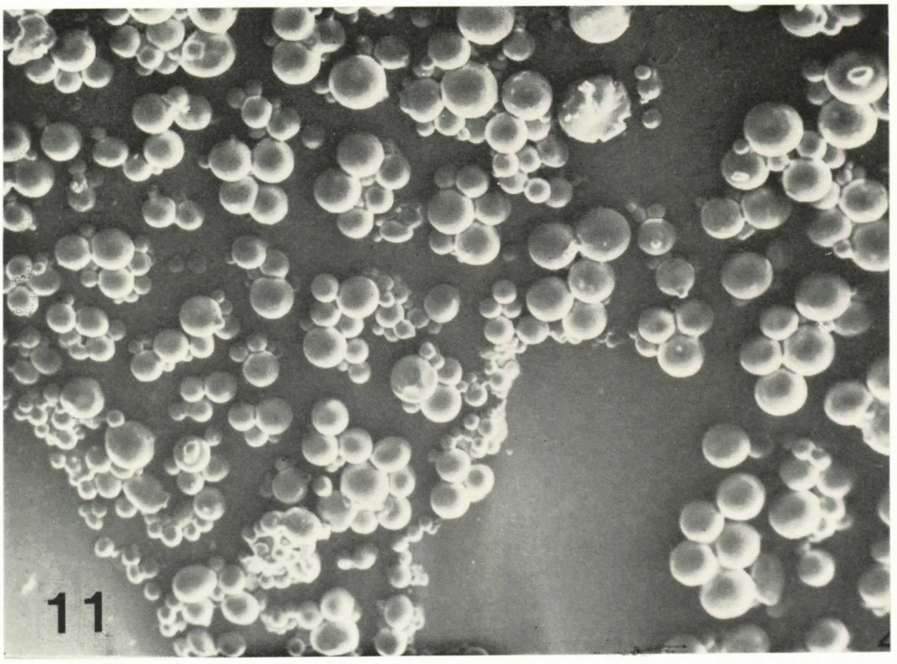


Fig. 11. Yolk spheres observed by scanning electron microscopy. They were collected from suspension of raw whole yolk. Yellow and white yolk spheres are mixed in the figure. ×100.

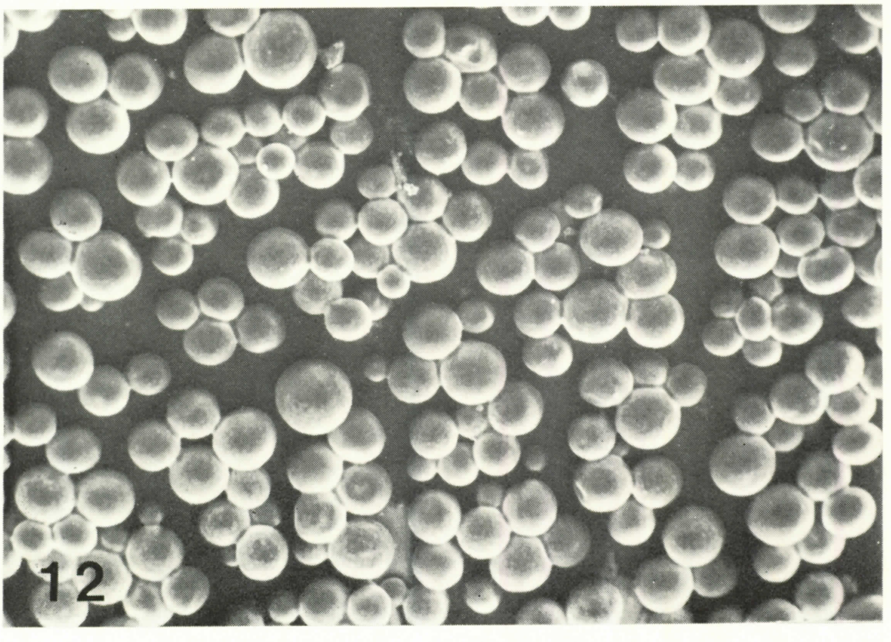


Fig. 12. Yellow yolk spheres observed by scanning electron microscopy. They were collected from suspension of the raw yellow yolk layer. ×100.

Fig. 13. A high-power magnification of Fig. 12. ×3,000.

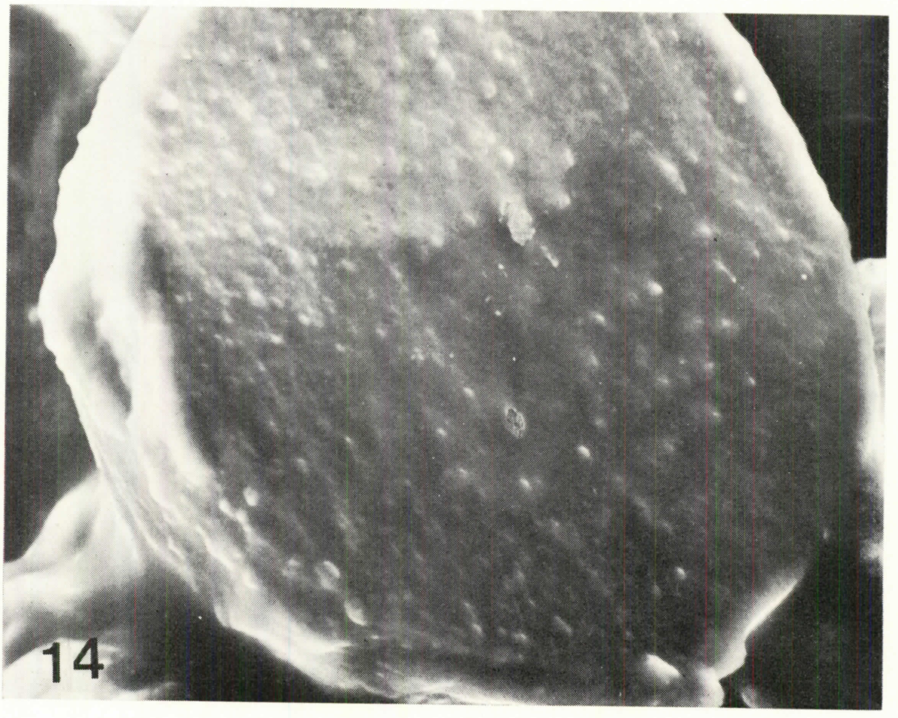


Fig. 14. A transverse section of a yellow yolk sphere. Scanning electron microscopy. ×3,000.

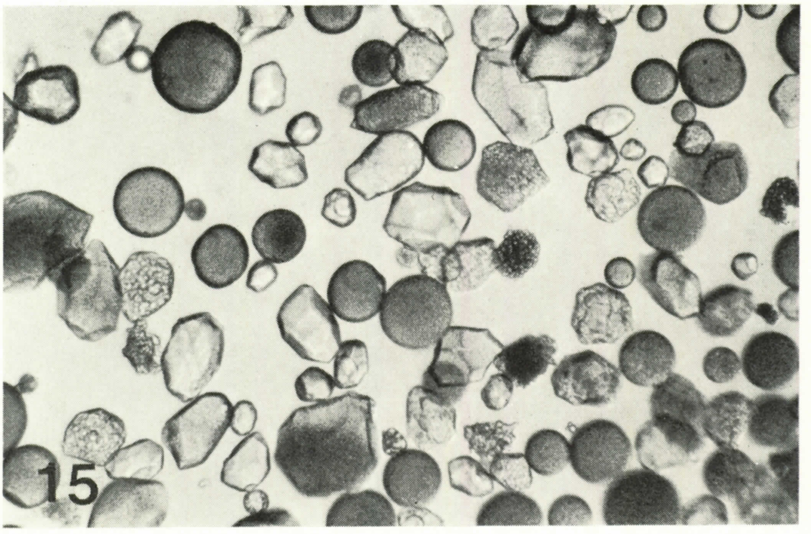


Fig. 15. Yolk spheres from an unfertiliezd egg incubated in an incubator at 38°C for 16 days. ×100.

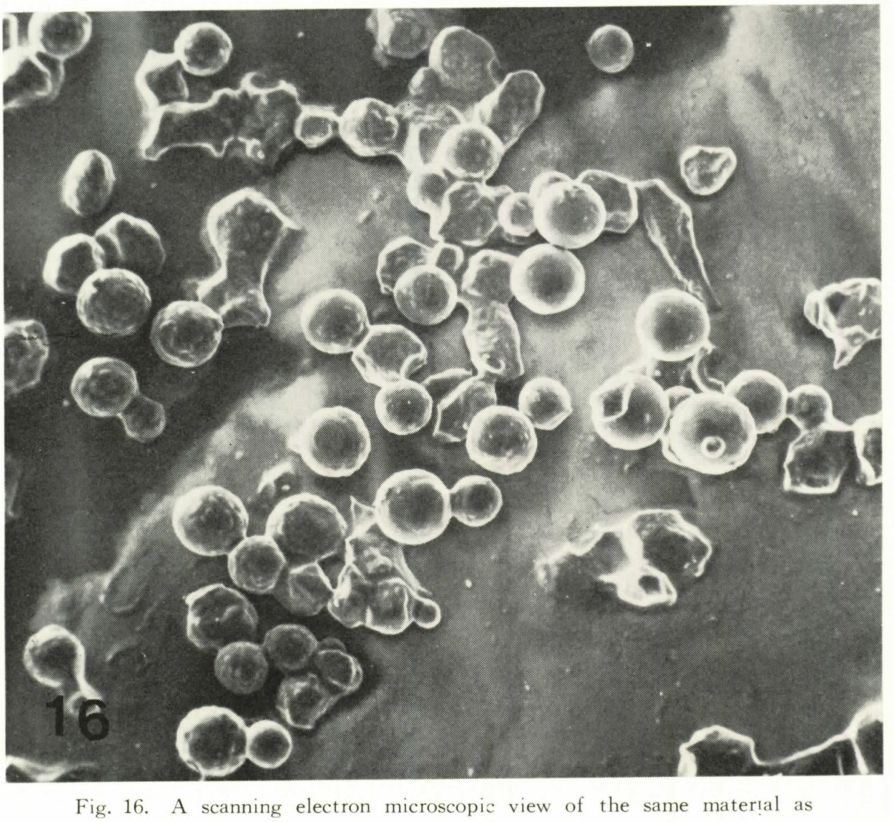


Fig. 16. A scanning electron microscopic view of the same material as Fig. 15. ×150.