THE STRUCTURE OF THE YOLK OF THE HEN'S EGG AS STUDIED BY ELECTRON MICROSCOPY

I. The Yolk of the Unincubated Egg

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ABSTRACT

A description of the fine structure of the yolk of the unincubated hen's egg has been provided, which will serve as a basis for further studies on yolk digestion. The gross components of the yolk (that is, free-floating lipid drops, yellow and white yolk spheres together with their enclosed lipid subdroplets, and aqueous protein fluid) could be recognized by phase contrast and low power electron microscopy. The majority of the lipid drops, whether free floating or enclosed within yolk spheres, were composed of particles about 30 to 60 A in diameter. The protein component of the yolk was found to consist of round profiles about 250 A in diameter. The surfaces of the yolk spheres were of three types, and it is difficult to decide which represents the true structure although reasons are given for believing that yolk spheres are not normally enclosed by membranes identical with cell membranes.

INTRODUCTION

The yolk of the hen's egg not only contains most of the raw materials for the developing embryo, but, as a number of light microscope studies have shown, possesses a definite configuration of its own. The major aim of the present work has therefore been to provide a description of the fine structure of the unincubated yolk which may then serve as a basis for further studies on yolk digestion. A further aim has been to study the structure of certain membranes present in the yolk. These membranes have been reported to resemble cell membranes in having semipermeable properties (Grodzinski, 1951); it is therefore of wide biological interest to know whether they also resemble cell membranes in their fine structure.

MATERIAL AND METHODS

Yolk from 25 unincubated eggs has been examined. Of these, 3 were fixed immediately after removal from the oviduct of a hen. Others were kept for several days after laying, stored at room temperature, which was about 18°C. In addition, yolk from the yolk sac of a chick which had been incubated for 20 days was examined.

Generally the yolk was spread as a smear on a clean glass slide and examined rapidly with a phase contrast microscope to determine whether white yolk or yellow yolk (see below) was being fixed; the glass slide was then immersed in the fixative. In 8 instances the fixative was added drop by drop to the smear as the slide lay on the stage of the microscope; this enabled individual yolk spheres to be kept under observation during fixation. The white yolk was obtained by plunging the tip of a pipette into either the nucleus of Pander or the latebra (Fig. 1). The yellow yolk was obtained by inserting the pipette into the side of the bag of yolk (position as shown in Fig. 1). The entire process of withdrawing the sample of yolk, examining it, and putting it into the fixative took about 20 seconds.

Five specimens were fixed in a different way. Fixative was injected beneath the blastoderm with a

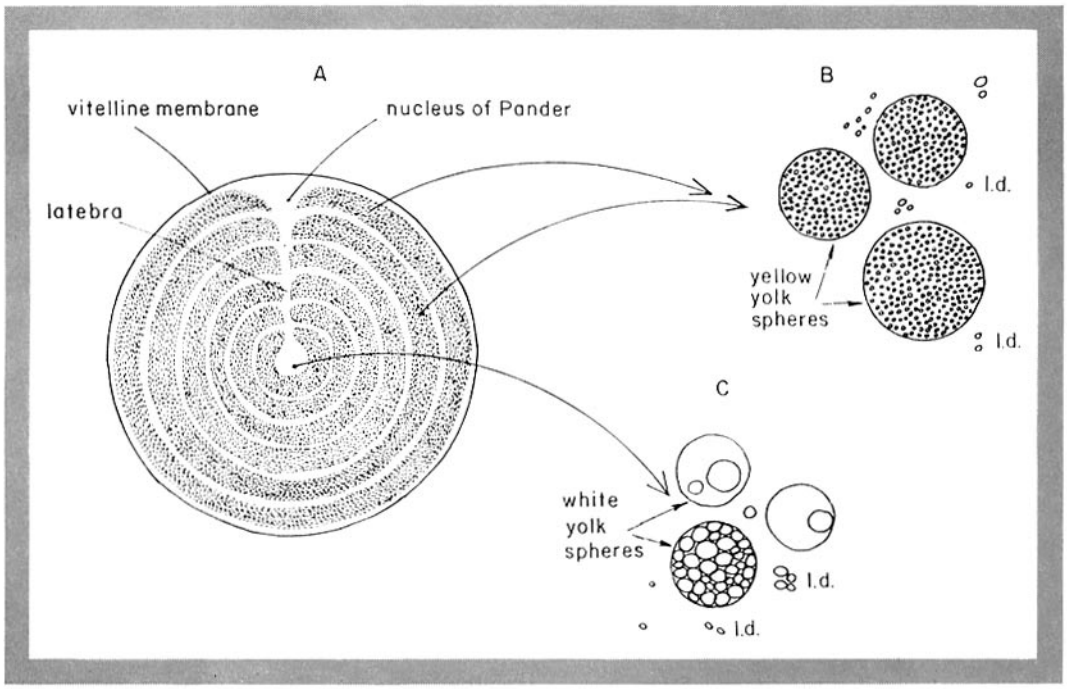


FIGURE 1

Diagram showing the yellow and white yolk in an unincubated egg. *A*, longitudinal section showing alternate banding of white and yellow yolk. *B*, yellow yolk taken from the position indicated in *a*; note the large yellow yolk spheres containing small subdroplets, and the free-floating lipid drops (*l.d.*); *C*, white yolk taken from the centre of the egg (latebra) as indicated; note the white yolk spheres containing one, two, or many large subdroplets, and the free-floating lipid drops.

hypodermic syringe, and the blastoderm, together with its adherent yolk, was then cut off and removed to a fresh pot of fixative. With these 5 specimens it was possible to compare the membranes of the embryonic cells with those of the yolk spheres in the same preparation.

The fixatives were: osmium tetroxide buffered to a pH of 7.4 (Palade, 1952), potassium permanganate also buffered to a pH of 7.4 (Luft, 1956), and unbuffered 10 per cent formalin (followed immediately by buffered osmium tetroxide), the permanganate being especially favoured because of the clarity it imparts to membranes. Fixation was carried out either at about 4°C or at room temperature, and lasted for between 1 and 2 hours. After dehydration in graded alcohols or acetone, specimens were embedded in Araldite (see Glauert and Glauert, 1958). Sections were examined with a Siemens Elmiskop Ib electron microscope. The sections either were unstained or were stained with phosphotungstic acid (after osmium tetroxide fixation), with uranyl acetate (see Watson, 1958*a*), with potassium permanganate (see Lawn, 1960), or with lead hydroxide (see Watson, 1958*b*). The purpose of the stains was to increase contrast.

Fresh unfixed smears of yolk have also been examined with a polarising microscope. To prevent dehydration a coverslip was placed on the top of each of these smears during examination.

GROSS MORPHOLOGY OF THE UNINCUBATED YOLK

The yolk to be described in this paper is entirely extraembryonic and lies beneath the blastoderm. In a previous paper (Bellairs, 1958) I have called it *primary yolk* to distinguish it from intracellular or *secondary* yolk.

The large mass of yolk of the hen's egg is not uniform throughout but consists of concentrically arranged bands of yellow and white yolk (see Fig. 1). The banding is thought to correspond to a diurnal rhythm in the formation of the yolk, the wide yellow bands being laid down during the

day, the narrower white bands during the night (Riddle, 1911). Yellow pigments are present in the yellow yolk but not in the white (see Romanoff and Romanoff, 1949). The yolk, both yellow and white, is an emulsion (see Grodzinski, 1946, for a full description). The continuous phase of the emulsion is a fluid in which yolk spheres and lipid drops float, these forming the dispersed phase (see Figs. 2 and 2 *a*).

There are two main types of yolk sphere, the yellow yolk sphere (found in yellow yolk) and the white yolk sphere (found in white yolk) (Fig. 1). These types were described by Schwann (1847). Both contain structures that I shall call subdroplets, but those of the white yolk spheres are highly refractile whereas those of the yellow are not. The subdroplets of the white yolk spheres are larger and less numerous than those of the yellow, although there is no uniformity in the size or the number of subdroplets even in the same type of yolk sphere. For instance, some white yolk spheres possess only a single large subdroplet whereas others possess perhaps 50 smaller ones.

According to Grodzinski (1946) the subdroplets of both white and yellow yolk spheres consist at least in part of fat. The white yolk spheres with their aggregated subdroplets vary in diameter from about 4 μ to about 75 μ, the yellow yolk spheres from about 25 μ to about 150 μ (Romanoff and Romanoff, 1949).

The free-floating lipid drops in the dispersed phase are smaller than the yolk spheres, more numerous, and highly refractile. They are relatively uniform throughout and do not contain subdroplets.

The gross chemical composition of the yolk has been studied by many workers (see Needham, 1931). According to Romanoff and Romanoff (1949), the proportions of the major chemical compounds in the yolk of the new-laid egg are: water, 48.7 per cent; lipids, 32.6 per cent; proteins, 16.6 per cent; carbohydrates, 1 per cent. When smears of fresh yolk are stained with Sudan III, the fat component can be seen to be present either as subdroplets within the yolk spheres or as droplets floating freely in the continuous phase of the yolk (the subdroplets actually become stained only if the yolk sphere bursts, so that they come to lie freely in the continuous phase). The remainder of the yolk (that is, the continuous phase as well as the fluid part of the yolk spheres, both of which remain unstained), can therefore be assumed to consist largely of proteins (or possibly lipoproteins) in aqueous solution.

RESULTS

A. *Identification of the Various Elements of the Yolk*

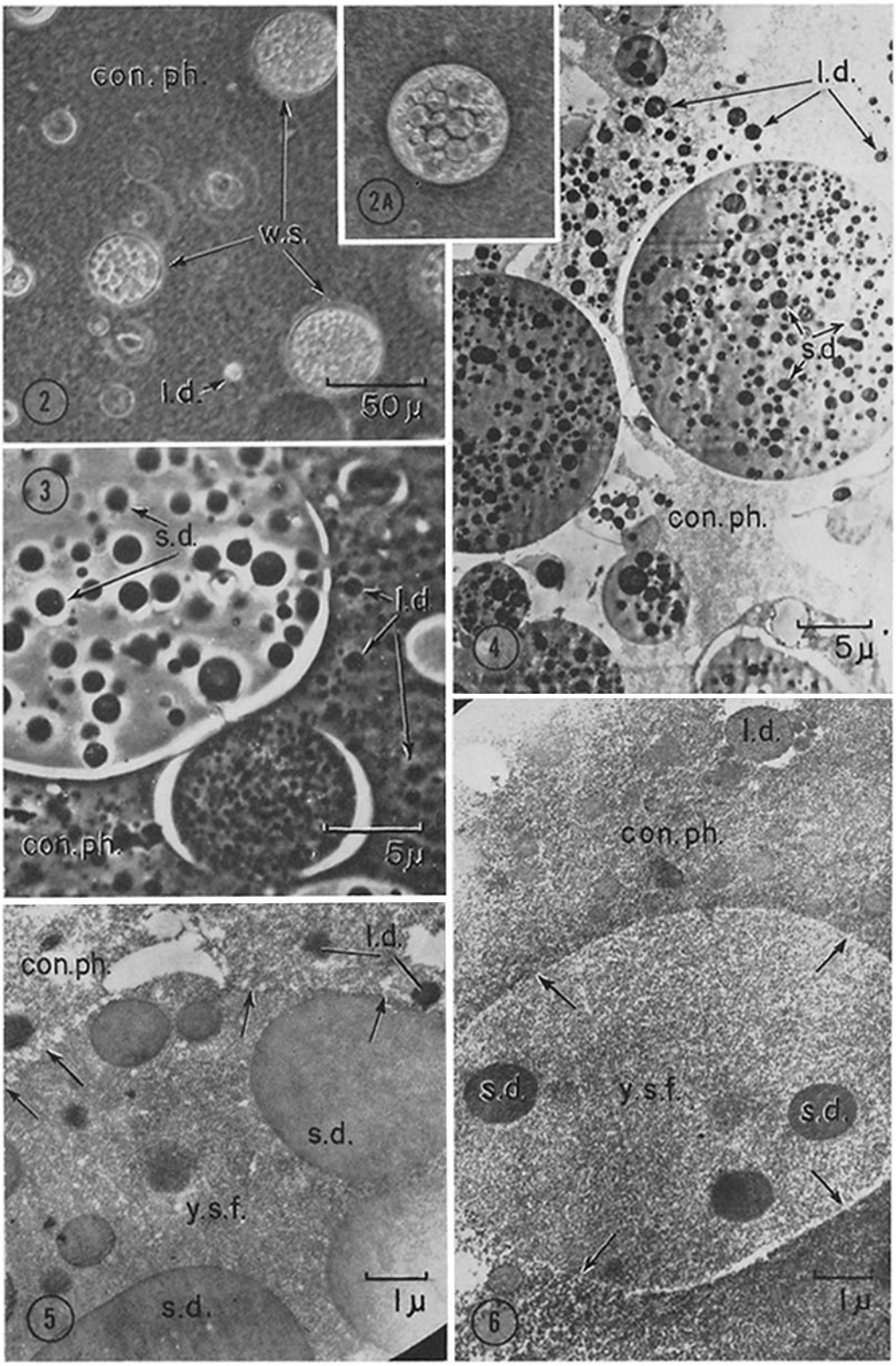
Figs. 2 and 2 *a* show phase contrast photomicrographs of a smear of fresh yolk taken from the nucleus of Pander (see Fig. 1). Fig. 3 shows a thick section cut from a similar smear after it had been fixed and embedded, photographed by phase contrast microscopy. Fig. 4 is a low magnification electron micrograph of a white yolk sphere from the same block. Figs. 5 and 6 show white yolk spheres examined by electron microscopy at higher magnifications.

Figs. 2 to 6 indicate that it is possible to recognise the subdroplets in the white yolk spheres at various stages of preparation and at varying magnifications. Similarly, the subdroplets can be recognised in the yellow yolk spheres at various stages of preparation. Fig. 7 shows a low power electron micrograph of a yellow yolk sphere.

The lipid droplets which help to make up the dispersed phase of the yolk emulsion differ from the yolk spheres in being considerably smaller. They are very numerous and do not contain subdroplets. They are thus easy to recognise in electron micrograph sections (see Figs. 4, 5, 7, and 24). The continuous phase of the yolk can easily be identified (for example, see Figs. 4 and 6).

One of the interesting facts to emerge from this study is that although a large number of different chemicals are present in the yolk (see Needham, 1931; Romanoff and Romanoff, 1949), only a limited number of fine structures can be seen by present electron microscope techniques. The subdroplets of the yellow and white yolk spheres have the same appearance as the free-floating lipid drops. Similarly, the fluid component (presumed to be protein) of the yellow and the white spheres has the same appearance as the fluid that forms the continuous phase of the yolk.

Despite these similarities in their fine structure, however, the two types of yolk sphere are recognisably distinct when sections are examined by electron microscopy. Within the white yolk spheres the subdroplets tend to be denser than the fluid (*i.e.* yolk sphere fluid) around them; within the yellow yolk spheres, however, the reverse is the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case. To some extent the difference between the two types may be the result of differences in concentration of the individual particles. It is difficult to decide whether the yolk sphere shown in Fig. 8 is a yellow or a white one, however, for its subdroplets appear to be unusually lacking in density. Yolk spheres possessing contrast of this type are rare.

B. *The Lipid Component of the Yolk*

Fig. 9 is an electron micrograph of the most common type of lipid drop. Its diameter is about 2 μ and it contains no subdroplets. Lipid drops are more optically dense than the continuous phase yolk, but of about the same density as the subdroplets in the adjacent yolk spheres.

With electron microscopy at high magnifications it becomes apparent that lipid drops of this type are not uniform throughout, for patches of greater and less density can be seen (see Fig. 10). The darker regions are composed of small particles, each about 30 to 60 A in diameter, some of which are less dense than others. The possibility that these particles might not be genuine structures but might be the result of out-of-focus effects (see Robertson, 1958) was tested by taking a through-focus series of electron micrographs. It was found that the individual particles could be recognised at every level of focus. The particles are therefore considered to be real structures.

It is possible that a second type of lipid drop may also be present in the yolk, although the evidence is by no means conclusive. Occasionally, in sections of yolk smears fixed in potassium permanganate a

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FIGURE 2

Unfixed fresh smear of white yolk photographed by phase contrast microscopy. Note: continuous phase fluid(*con.ph.*); free-floating lipid drops (*l.d.*); white yolk spheres (*w.s.*). × 300.

FIGURE 2 *a*

A similar white yolk sphere photographed by phase contrast microscopy. Note: subdroplets within the yolk sphere. × 500.

FIGURE 3

Thick section (about 1 μ) of a white yolk smear photographed by phase contrast microscopy. The edge of a large yellow yolk sphere occupies the top left corner of the figure. A smaller yolk sphere lies towards the bottom of the figure. There is a great variation in the size of the subdroplets. Note: continuous phase fluid (*con.ph.*); free-floating lipid drops (*l.d.*); subdroplets (*s.d.*). *Fixed in potassium permanganate.* × 3000.

FIGURE 4

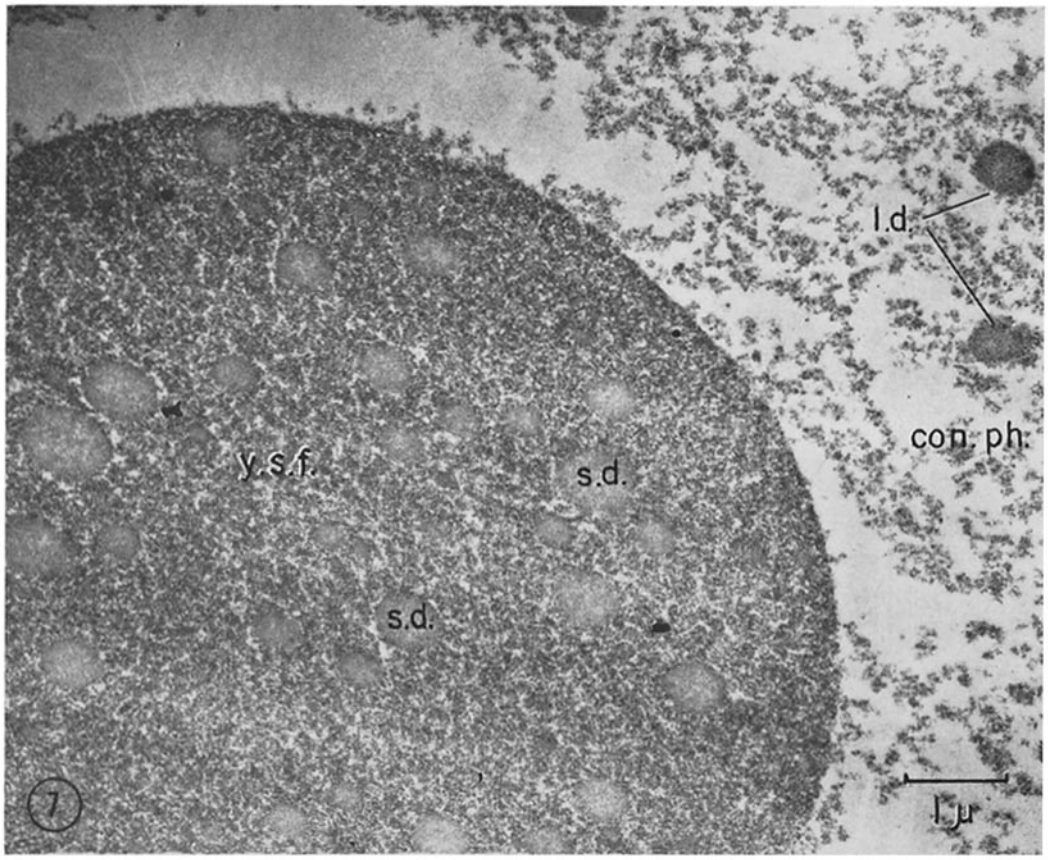
Electron micrograph of a section of white yolk to show the variability in size of the yolk spheres. Note: continuous phase fluid (*con.ph.*); free-floating lipid drops (*l.d.*); subdroplets within the yolk spheres (*s.d.*). Fixed in osmium tetroxide. × 2000.

FIGURE 5

Electron micrograph of part of a white yolk sphere. Arrows indicate the edge of the yolk sphere, which is surrounded by a "membrane" (see text). Note: continuous phase fluid (*con.ph.*); fluid within yolk sphere (*y.s.f.*); free-floating lipid drops (*l.d.*); subdroplets (*s.d.*). Fixed in potassium permanganate. × 9000.

FIGURE 6

Electron micrograph of a white yolk sphere. Arrows indicate the edge of the yolk sphere, where no membrane can be seen. Note: continuous phase fluid (*con.ph.*); fluid within yolk sphere (*y.s.f.*); free-floating lipid drop (*l.d.*); subdroplet (*s.d.*). Fixed in potassium permanganate. × 8000.



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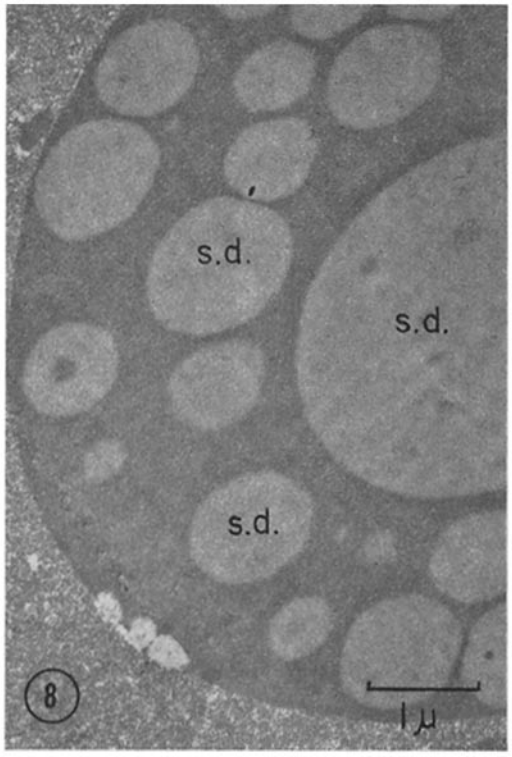

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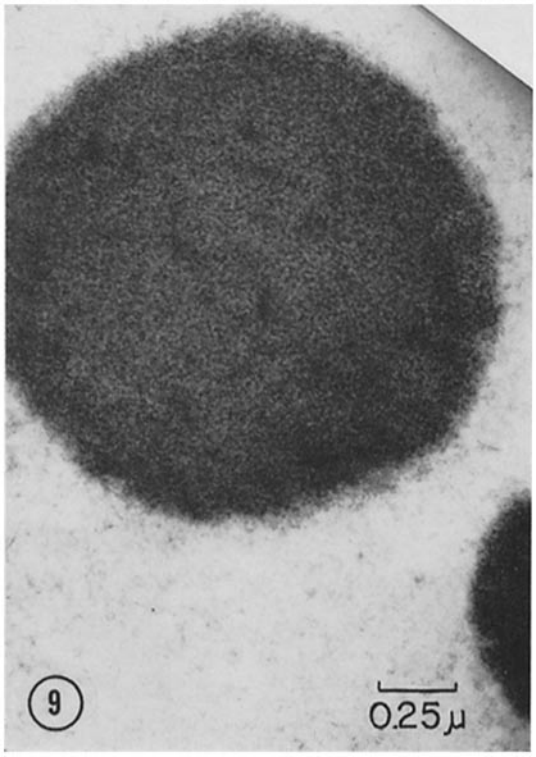

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clear hole can be seen with a dark line around it (*e.g.* Fig. 11); presumably, some material has failed to fix and has subsequently been washed out during dehydration. Perhaps these holes should be regarded as preparation artefacts, though there are several reasons for considering that they may have contained lipid. For instance, if blastoderms of the primitive streak stage are fixed in osmium tetroxide, many intracellular lipid drops can be seen (Bellairs, 1958), but if they are fixed in potassium permanganate, the lipid fails to fix and holes are left in the blastoderm in their place (see Fig. 12). The holes seen in yolk after fixation in potassium permanganate closely resemble the holes in the blastoderm after fixation in potassium permanganate (*cf.* Figs. 11 and 12). By analogy, therefore, it seems possible that these holes in the yolk are the remains of a second type of lipid drop.

Occasionally a small amount of dark material can be seen lining the hole. The dark line surrounding the hole varies in width, perhaps according to the thickness of the section. In Figs. 11 and 13 it is about 70 A wide.

C. *The Protein Component of the Yolk*

When relatively thick (golden yellow) sections are examined by electron microscopy, the continuous phase of the yolk appears to be composed entirely of small round profiles, each about 250 A in diameter (Figs. 14 and 15), which may be sections across small spheres or tubes. Groups of these round profiles are frequently arranged to form a larger ring or a chain (Figs. 14 and 15). It is possible, however, that under normal circumstances in the egg the 250 A profiles maintain a discrete distance from one another and that the arrangement into larger rings and chains is a preparation artefact. The protein fluid of the yellow and white yolk spheres appears to have the same structure. Generally, however, the granules appear to be less densely packed together within the yolk spheres than they are in the continuous phase fluid. Fig. 16 shows a higher magnification electron micrograph of a group of these 250 A profiles.

In one specimen of white yolk which had been taken from the nucleus of Pander (Fig. 1) a mitochondrion was found lying free in the continuous phase fluid (Fig. 17) (see comment in Discussion).

D. *The Surfaces of the Yolk Spheres*

The appearance of the surface of the yolk spheres was not always the same. Three different conditions were found: the "lamellated capsule," the "unit membrane-like structure," and the "naked surface." Each will be described. It is not easy to decide with complete confidence which of these best represents the original state of the yolk spheres in the unfixed egg.

a) The Lamellated Capsule: Both white and yellow yolk spheres may be surrounded by a number of dark lines. For example, Fig. 19 shows a section across a yolk sphere which is apparently surrounded by a lamellated capsule. The number of layers in these capsules varies from one yolk sphere to another, even in the same section (*cf.* Figs. 18 and 19). These lamellated capsules are not, however, specific to the surfaces of the yolk spheres; they have also been seen surrounding lipid drops and even small patches of continuous phase fluid (Fig. 18). They were most common in white yolk taken from eggs which had been stored for several days before fixing, but appeared to be

FIGURE 7

Electron micrograph of part of a yellow yolk sphere. Note: continuous phase fluid (*con.ph.*); fluid within yolk sphere (*y.s.f.*); free-floating lipid drops (*l.d.*); subdroplets (*s.d.*). Fixed in potassium permanganate. × 13,000.

FIGURE 8

Electron micrograph of part of an unusual yolk sphere. The subdroplets are exceptionally lacking in density. Fixed in potassium permanganate. × 14,500.

FIGURE 9

Electron micrograph of the most common type of free-floating lipid droplet. Fixed in potassium permanganate and stained with uranyl acetate. × 36,000.

totally lacking in other eggs. They were especially common in samples of yolk taken from close beneath the blastoderm.

The periodicity of the bands was measured at higher magnifications (see Fig. 20). The average measurement of the full width of one dark line plus one light line was about 40 A (see Fig. 21). The average measurement of the full width of two dark lines plus the one light line which separates them was about 60 to 65 A. Myelin figures having a similar periodicity have been produced experimentally by others (see Discussion) using extracts of egg yolk. These lamellated capsules are therefore interpreted as bearing some relation to myelin figures in their fine structure.

b) The Unit Membrane-like Structure: In a minority of cases a structure consisting of two dark lines separated by a light line was present around at least part of an individual yolk sphere (Figs. 22 and 23). This group of yolk spheres was obtained entirely from the nucleus of Pander (see Fig. 1). A structure of this type could be interpreted in two different ways: it could be regarded as the first step in the formation of a lamellated capsule, or as a naturally occurring "unit membrane" of the type described by Robertson (see Discussion) and illustrated in Fig. 26. Indeed, the width of the membrane-like structure described here (*i.e.* the two dark lines separated by the light line) is about 75 A, which is comparable to the total width of two dark lines separated by one light line in the lamellated capsule (about 60 to 65 A) (see also Fig. 21). This small difference seems to be well within the range of experimental error.

c) The Naked Surface: Around the majority of the yolk spheres it was not possible to resolve even a single unit of the type described above (*cf.* Figs. 5 and 6). The edge of each of these "naked" yolk spheres is nevertheless clearly defined (Figs. 24 and 25), although frequently there appears to have been a shrinkage of the contents away from the edge of the sphere (see Fig. 6).

The findings reported above may be explained in a number of different ways. For example, a unit membrane may normally exist at the surface of each yolk sphere but be destroyed during preparation. Alternatively, no unit membrane may be present normally, and anything resembling one (such as the structure in Fig. 23) may, as already stated, be but the first stage in the formation of a lamellated capsule. The problem will be considered in the Discussion, but additional experimental evidence is as follows:

Firstly, the absence of a unit membrane is unlikely to be due to deficiencies in the preparation techniques *per se*, for the condition of "naked surface" has been seen in sections where other membranes are well fixed. For example, in Fig. 29 the cell membrane of the overlying blastoderm cell is well defined, and in Fig. 28 a 75 A unit membrane

FIGURE 10

Electron micrograph showing the fine structure of the most common type of lipid droplet. Note: patches of greater and less density; small dense particles 30 to 60 A in diameter (arrows). Fixed in potassium permanganate. × 290,000. *Inset:* Similar lipid drop at higher magnification. × 400,000.

FIGURE 11

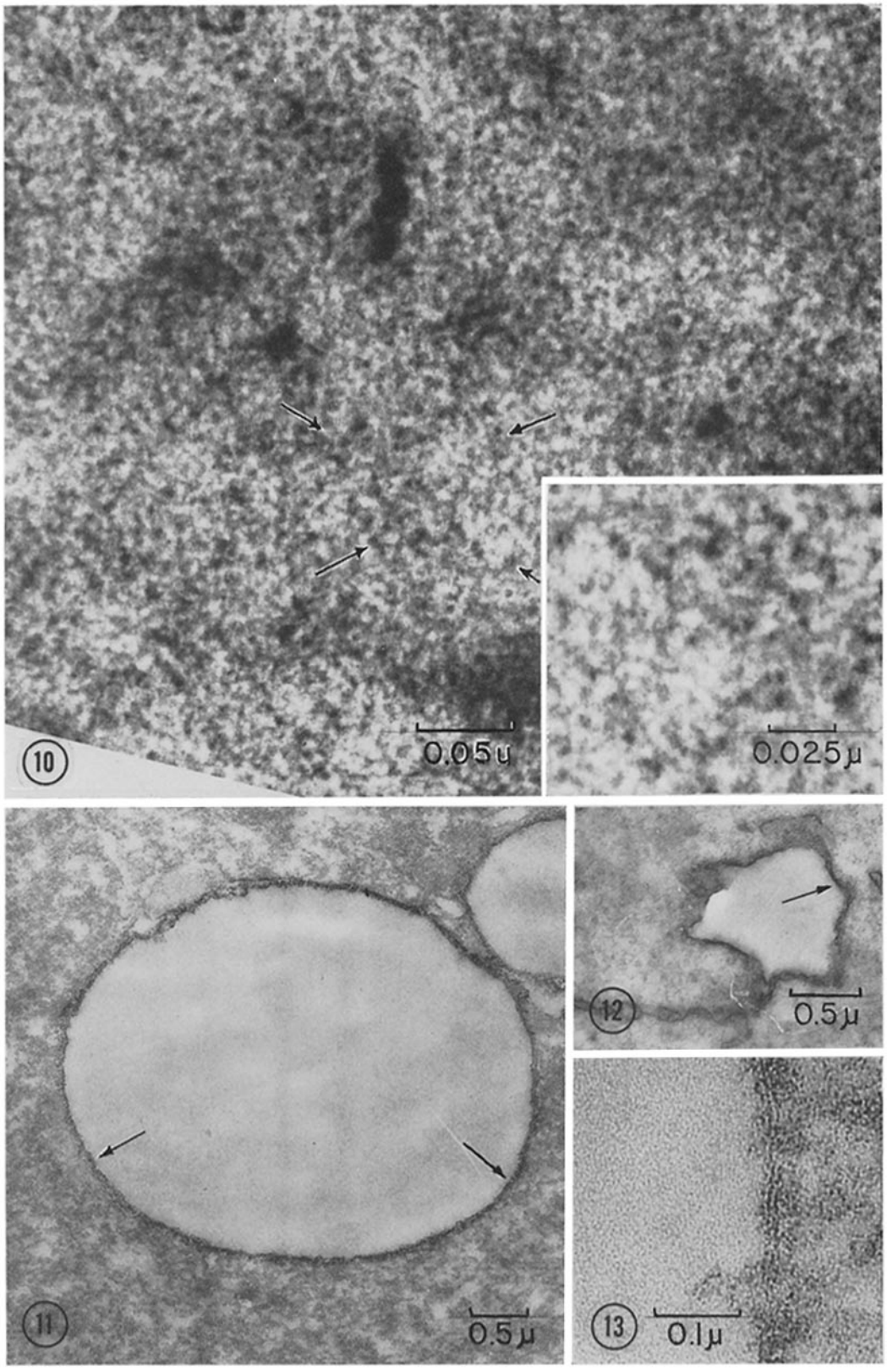
Electron micrograph of the other type of supposed free lipid drop of the yolk. The contents have failed to fix (*cf.* Fig. 12). The edge of the hole is indicated by a dark line (arrows). Fixed in potassium permanganate. × 17,500.

FIGURE 12

Electron micrograph of intracellular fat drop in a tissue fixed in potassium permanganate. The contents of the fat drop have failed to fix and have presumably been washed out during dehydration. The edge of the hole is bounded by a dark line (arrow). × 22,000.

FIGURE 13

Electron micrograph of the edge of a lipid droplet of the type shown in Fig. 11. Note the lamellated border. Fixed in potassium permanganate. × 128,000.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is present around one yolk sphere but not another. Similar results were obtained using several different fixatives (*i.e.* potassium permanganate, osmium tetroxide, 10 per cent formalin). It is moreover unlikely that special properties of yolk itself (*e.g.* a low pH) make fixation of membranes particularly difficult, for almost without exception a clearly defined unit membrane surrounds each intracellular yolk sphere (Fig. 27).

Absence of a unit membrane at the surface of a yolk sphere was not an ageing phenomenon confined to eggs which had been stored for several days, for it was found not only in new-laid eggs but even in those removed from the oviduct of a hen prior to laying (Fig. 25). Certain spheres were kept under observation with the phase contrast microscope during fixation but were not seen to burst.

In an attempt to discover more about the yolk sphere surfaces, a number of fresh yolk smears were examined with a polarising microscope. Only the yellow yolk spheres were suitable for this purpose, for the subdroplets in the white yolk spheres are highly refractile. Fig. 31 is a photomicrograph of a yellow yolk sphere taken by phase contrast microscopy. Fig. 30 is a photomicrograph of the same yellow yolk sphere taken by polarised light; a bright ring of light is visible at the surface. The birefringence was always positive but was in fact visible in only a few cases, and these were all smears of yolk taken from eggs stored for several days. It seems probable, therefore, that when birefringence is present at the surface of the yellow yolk spheres it is indicative of the presence of a lamellated capsule; when no birefringence can be seen, then it is indicative of the presence of a "naked surface."

DISCUSSION

Although little is understood about the differences between yolk spheres, a certain amount is known about the similarities. For instance, Grodzinski (1951) states that "the chemical composition of both kinds of yolk"—*i.e.* yellow and white—"is fundamentally similar and differs only in the quantity of water and neutral fats." The present results are of interest in this connection, for they show that there is also a similarity in the fine structure of the two kinds of yolk. Riddle (1911) even suggested that white yolk could become converted into yellow and *vice versa*, according to the conditions in the egg, but unfortunately the present results do not provide enough evidence to enable a decision to be made upon this point.

One type of (presumed) lipid drop may have a constitution similar to that of the intracellular fat drops, for they both fail to fix with potassium permanganate (Figs. 11 and 12). Supposed lipid drops of this type are also present in the yolk of the adder *Vipera berus* (Bellairs, 1959). The most fre-

FIGURE 14

Electron micrograph of a thick section of the continuous phase fluid showing ring-like granules, each about 250 A in diameter (arrow). Note how some of these rings are arranged in a chain. Fixed in potassium permanganate and stained with lead hydroxide. × 100,000.

FIGURE 15

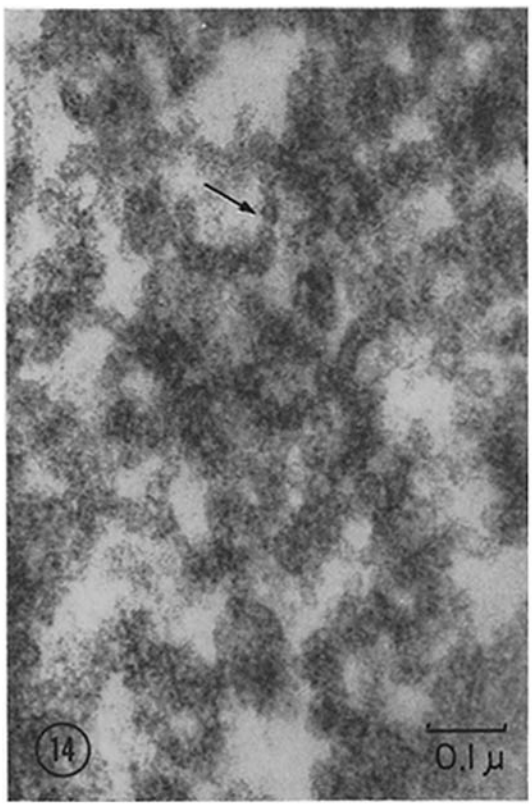
Electron micrograph of continuous phase fluid. Note how the granules are arranged in chains (arrows) which may be an early stage in the formation of lamellated structures. Fixed in potassium permanganate. × 80,000.

FIGURE 16

Electron micrograph of a thin section of the continuous phase fluid. Fixed in potassium permanganate. × 340,000.

FIGURE 17

Electron micrograph of a mitochondrion lying free in the continuous phase fluid. This mitochondrion may have fallen from a damaged cell in the blastoderm. Fixed in potassium permanganate. × 80,000.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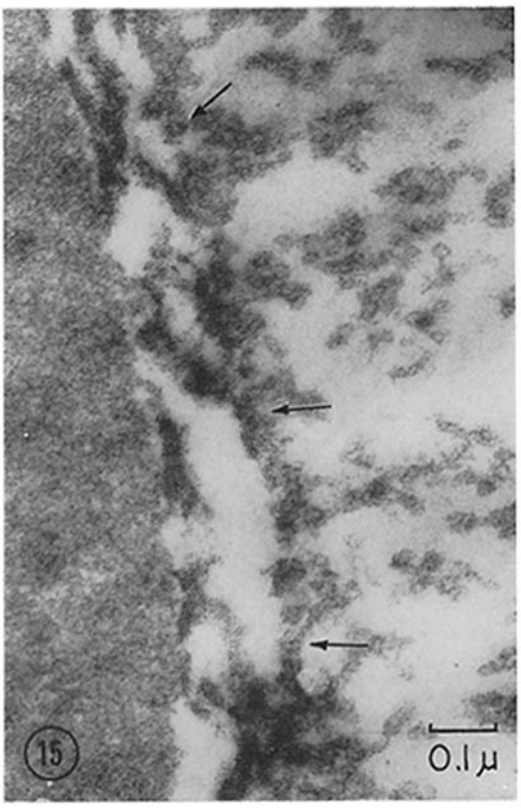
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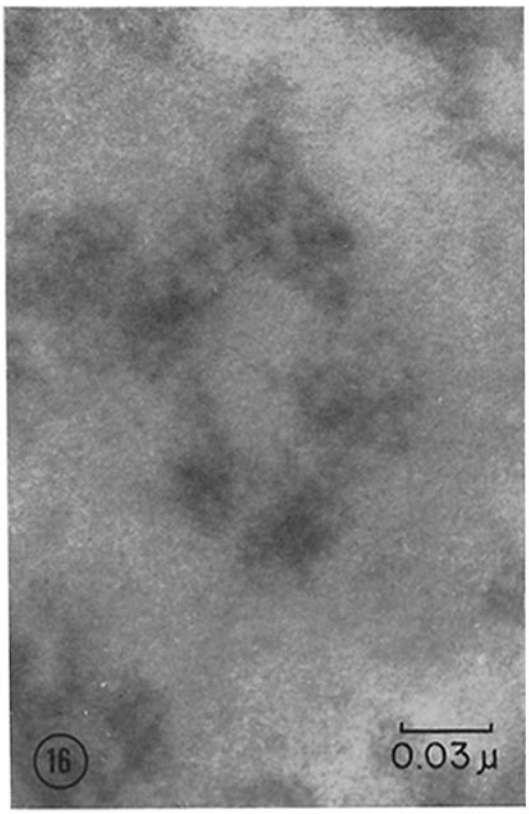
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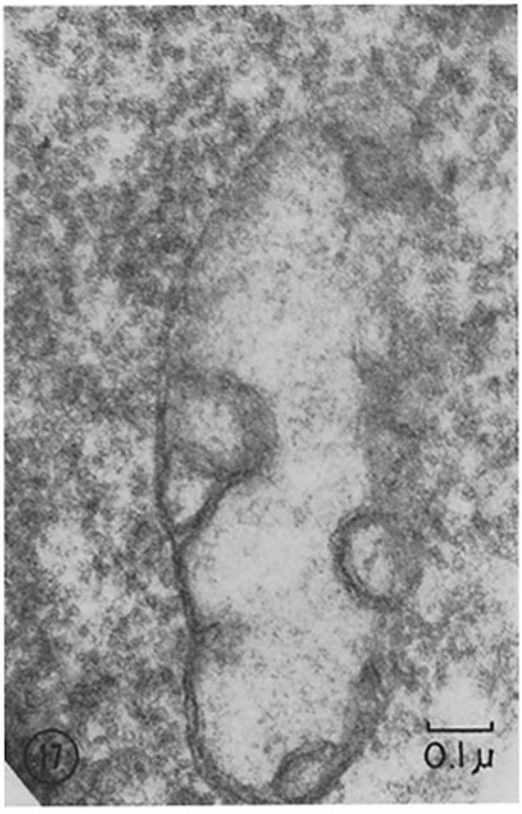
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quent type of yolk lipid drop, however, differs from the intracellular fat drops in staining intensely with potassium permanganate (Fig. 9). Grodzinski (1946) has suggested that glycerides in the yolk become phosphatides during incubation. At present it seems premature to discuss whether or not two types of lipid drop, the dense (Fig. 9) and the unfixed (if indeed this is a lipid drop) (Fig. 12), represent steps in this series. Nevertheless, in a preliminary investigation of yolk taken from the yolk sac of a 20-day-old embryo, it was possible to see a greater proportion of these "holes" than in the earlier stages. For instance, six of the "holes" were seen in the first section of 20 day yolk examined, whereas only about twenty have been seen in thousands of sections of unincubated yolk.

Substantial yolk sphere "membranes" have been illustrated in a light microscope study by Grodzinski (1938) of yolk smears treated with a hypotonic solution (his Fig. 8) or with iodine (his Fig. 14). A "membrane" of this type can also be seen in Fig. 32. Unfortunately, it is not possible when using a light microscope to distinguish between a true (unit) membrane and a lamellated capsule, or between either of these and phase effects at a "naked surface."

The experiments of Grodzinski and his school suggest that a physiologically semipermeable membrane surrounds each yolk sphere. Their main evidence is as follows: (*a*) If the concentration of the continuous phase of the yolk is increased by adding sodium chloride crystals, the diameter of the yolk spheres decreases (Grodzinski, 1946). (*b*) If the concentration of the continuous phase is reduced by addition of water, this often results in the bursting of the yolk spheres (Grodzinski, 1951). Such evidence is perhaps not completely conclusive, for gels also shrink and swell (and disperse) in this way (see Alexander and Johnson, 1949) and it is thus not impossible that each yolk sphere is a gel.

In view of Grodzinski's findings, therefore, it is important when considering the digestion and absorption of yolk by the embryo to know whether this "semipermeable membrane" corresponds to the semipermeable membrane of cells as Grodzinski has suggested. There has for several years been some lack of unanimity among electron microscopists as to what visible structures constitute a true membrane. Robertson (1959), who has recently discussed the matter, has been able to show a remarkable, though not necessarily universal, conformity of structure in the cell membranes of many different types of animal cells. He has shown that a cell membrane normally consists of two dark lines separated by a light line, the total width being about 75 A, and has introduced the term "unit membrane" for the entire structure of the three lines. Many of the internal membranes of cells (for example, those of mitochondria) also have this appearance (for a discussion, see Robertson, 1959). It seems reasonable to conclude, therefore, that in general physiologically semipermeable membranes possess a unit membrane structure.

It is thus of interest that a unit membrane–like structure may also surround each yolk sphere, for a 75 A wide unit of this type was sometimes present (Fig. 24). As was pointed out under Results, however, it is difficult to be certain that a single unit of this type is not the first stage in formation of a lamellated capsule rather than the original structure.

FIGURE 18

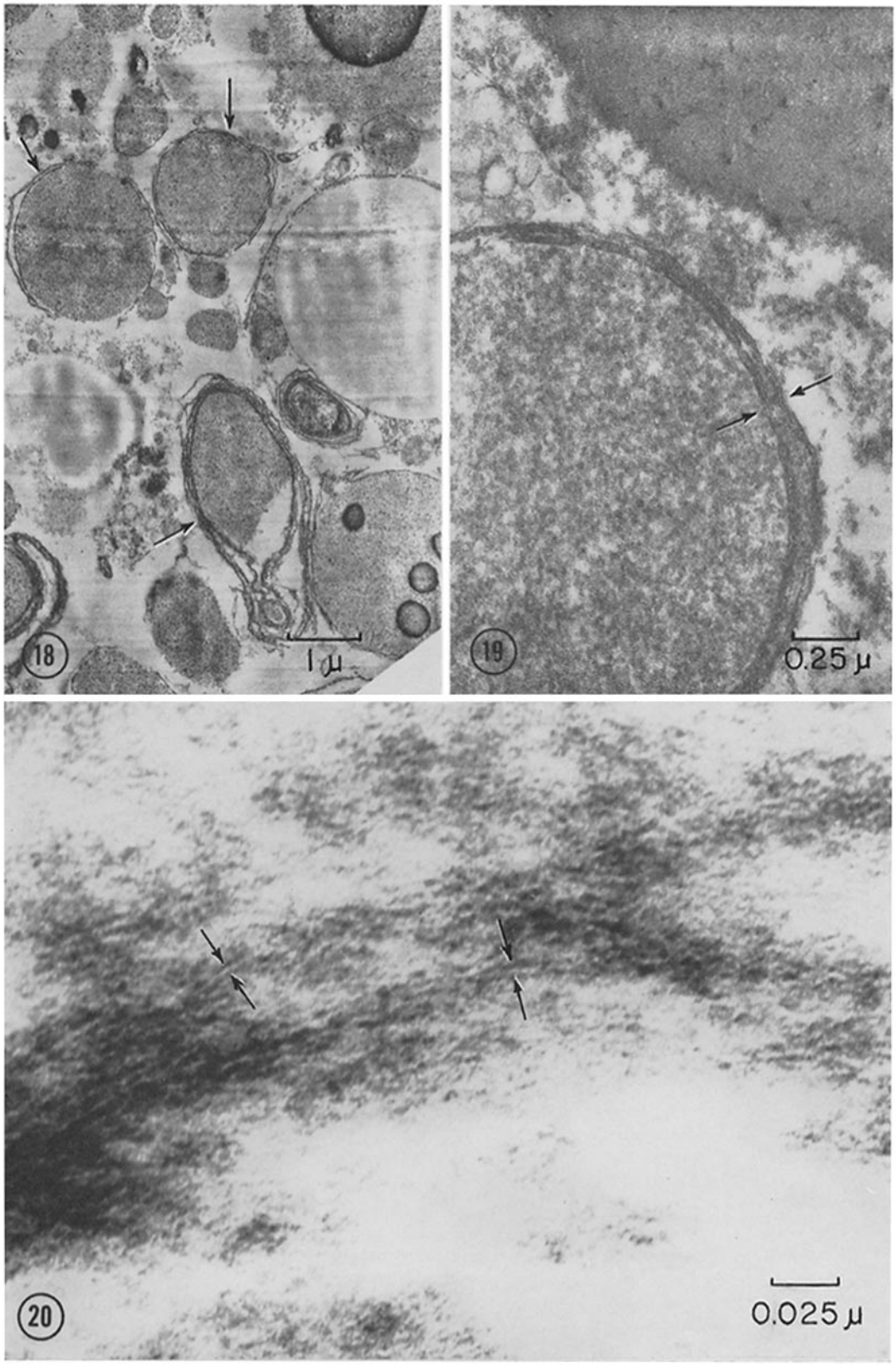
Electron micrograph of white yolk showing lamellated capsules (arrows). Fixed in potassium permanganate. × 11,250.

FIGURE 19

Electron micrograph of part of a lamellated capsule (arrows). Fixed in potassium permanganate. × 40,000.

FIGURE 20

Electron micrograph of part of a wide lamellated capsule. The full width of the region between the arrows, *i.e.* two dark lines separated by a single light line, is about 60 to 65 A. Fixed in potassium permanganate. × 400,000.


18
1 μ
19
0.25 μ
20
0.025 μ

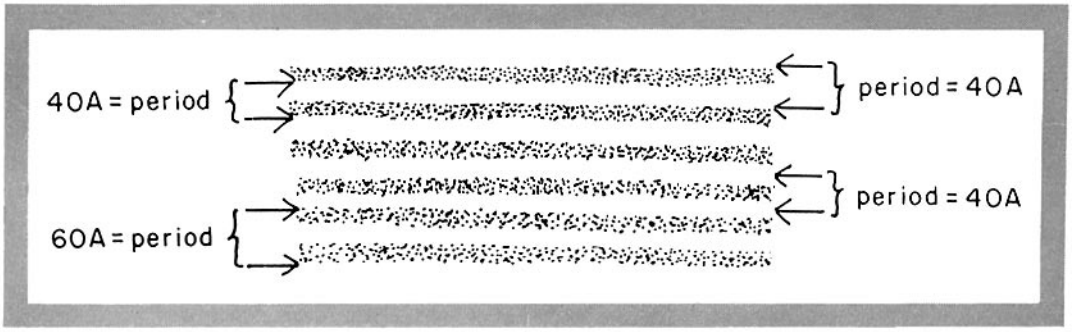


FIGURE 21

Diagram to illustrate the periodicity of the components of the lamellated capsules.

The lamellated capsules bear some resemblance to the myelin figures described by Stoeckenius (1959), (the periodicity is comparable—about 40 A for a repeating unit) and by Revel, Ito, and Fawcett (1958). The latter authors obtained myelin figures by experimental hydration of the lecithins of egg yolk, actually using chick yolk as raw material. It seems reasonable to conclude, therefore,

FIGURE 22

Electron micrograph of the edge of a white yolk sphere; there has been a shrinkage of the contents. Note: continuous phase fluid (*con.ph.*); fluid within yolk sphere (*y.s.f.*). Fixed in potassium permanganate. × 6500. Region enclosed by rectangle shown enlarged in Fig. 23.

FIGURE 23

Electron micrograph of the edge of a white yolk sphere. Note: continuous phase fluid (*con.ph.*); fluid of yolk sphere (*y.s.f.*); the "unit membrane" ("*u.m.*"). Fixed in potassium permanganate. × 120,000.

FIGURE 24

Electron micrograph of the edge of a white yolk sphere. Note: continuous phase fluid (*con.ph.*); fluid of yolk sphere (*y.s.f.*); free-floating lipid drop (*l.d.*); subdroplet (*s.d.*); "naked surface" (arrows). Fixed in potassium permanganate and stained with uranyl acetate. × 21,000.

FIGURE 25

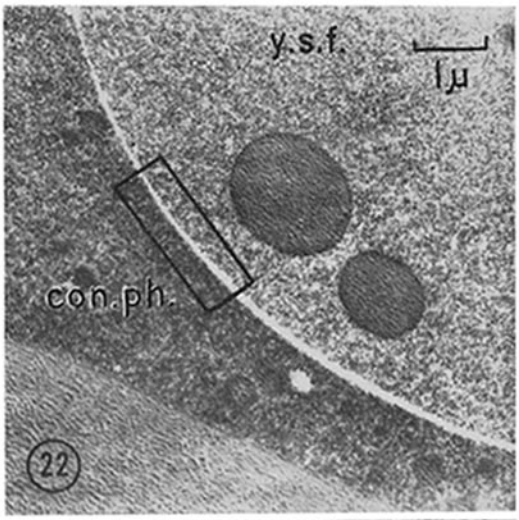
Electron micrograph of the edge of a white yolk sphere from an unlaid egg. Note: continuous phase fluid (*con.ph.*); fluid of yolk sphere (*y.s.f.*); "naked surface" (arrows). Fixed in potassium permanganate. × 64,000.

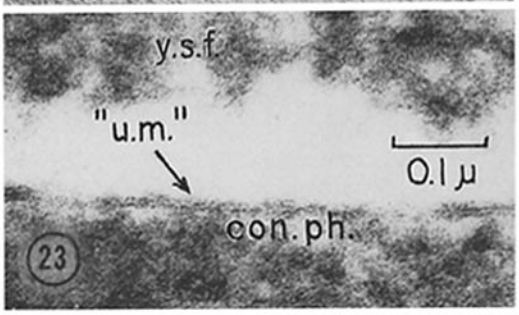
FIGURE 26

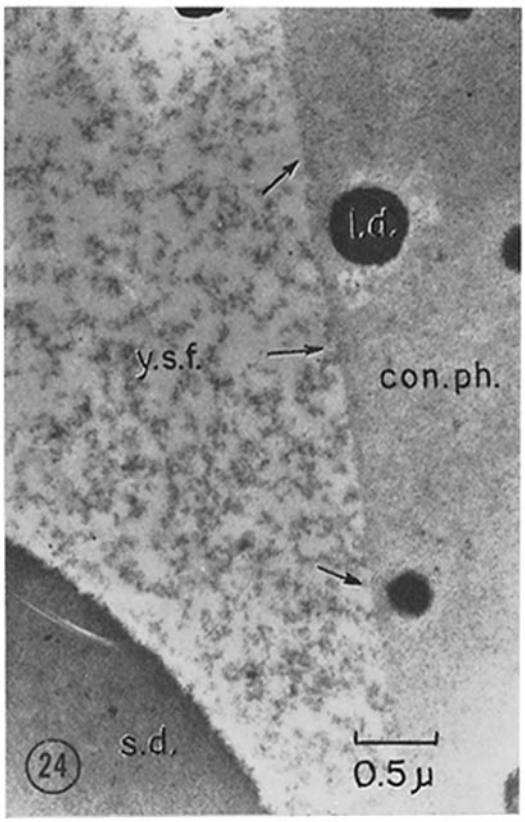
"Unit membrane" lying within the cytoplasm of a chick blastoderm. Fixed in potassium permanganate. × 62,000.

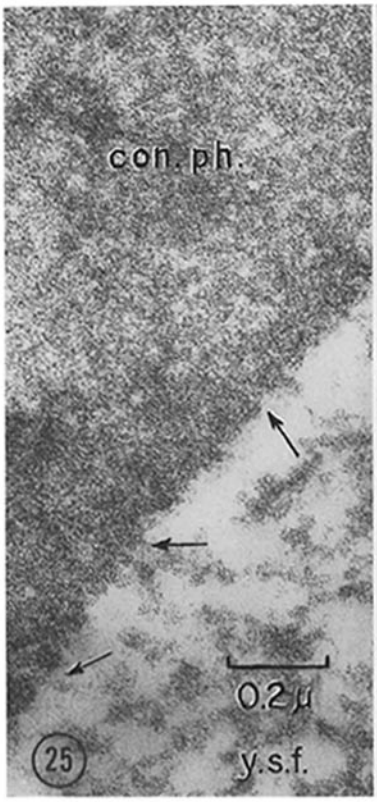
FIGURE 27

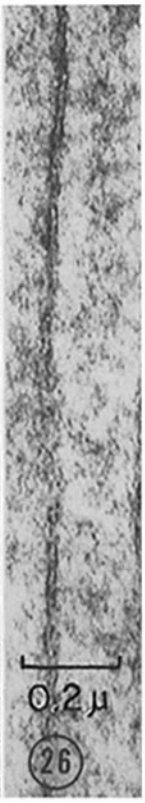
Electron micrograph of part of an intracellular yolk drop which lies on the right of the picture. Note: a well formed unit membrane surrounds the yolk sphere; the yolk granules (*y.g.*) are denser and more conspicuous than the cytoplasmic particles (*c.p.*). Fixed in potassium permanganate. × 40,000.

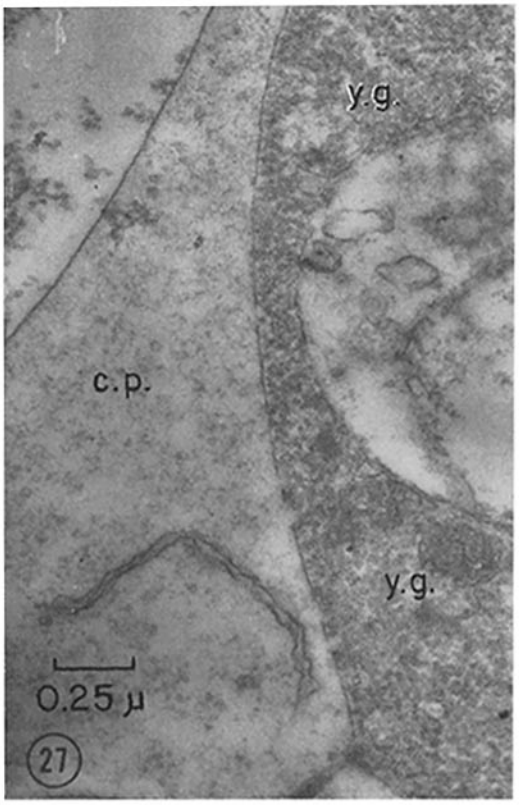

y.s.f.
1μ
con.ph.
22


y.s.f.
"u.m."
0.1μ
con.ph.
23


l.d.
y.s.f.
con.ph.
s.d.
0.5μ
24


con.ph.
0.2μ
y.s.f.
25


0.2μ
26


y.g.
c.p.
y.g.
0.25μ
27

that in specimens exhibiting myelin figure-like structures a similar hydration has occurred, perhaps as an ageing phenomenon in stored eggs. It is of interest in this connection that both the lamellated capsules and the unit membrane-like structures were found only in the vicinity of the blastoderm.

Whether or not a yolk sphere "membrane" and a cell membrane resemble each other in having a unit membrane structure, a problem which cannot be regarded as settled on the present results, they nevertheless must be considered to differ in at least one important characteristic; that is, that although considerable difficulty was experienced in preserving anything resembling a unit membrane around individual yolk spheres, which apparently often resulted in the total absence of the membrane, this difficulty has never been experienced when dealing with membranes in and around cells. Evidence has been presented to suggest that this difficulty is unlikely to be due to poor fixation resulting from peculiar conditions in yolk, or to ageing of the yolk sphere membrane itself, or to unsuitability of one fixative rather than another. It seems probable, therefore, that the yolk sphere "membrane" and the cell membrane differ in certain chemical characteristics. This conclusion is supported by the fact that if the contents are expelled from a red blood corpuscle, a "ghost" membrane remains; but if the contents are expelled from a yolk sphere, the "membrane" disappears within a few minutes (Grodzinski, 1951). It is therefore suggested here that although the surfaces of the yolk spheres exhibit interesting semipermeable characteristics, they do not possess membranes completely comparable with those of cells. Indeed, it is tentatively suggested that since the condition of "naked surface" is found in the majority of the yolk spheres, and since it is not confined to a limited region of the egg, this may be the "true" surface condition.

The presence of a mitochondrion in the extraembryonic yolk is of interest since mitochondria are occasionally found inside intracellular yolk spheres of the area pellucida (Bellairs, 1958). It is not suggested that these mitochondria are formed except under the influence of living cytoplasm, though the mechanism is not understood. It seems possible that the mitochondrion illustrated in Fig. 17 had fallen out of a damaged cell in the overlying blastoderm before fixation. It is included here because it is the only true cytoplasmic structure

FIGURE 28

Electron micrograph of white yolk. Note: some of the yolk spheres appear to be surrounded each by a membrane (arrows 1), whereas others appear to have a naked surface (arrows 2). Fixed in osmium tetroxide and stained with phosphotungstic acid. × 8500.

FIGURE 29

Electron micrograph of a white yolk sphere lying immediately beneath a young chick blastoderm. Note: membranes are present in the blastoderm but not around the yolk sphere. Fixed in osmium tetroxide and stained with phosphotungstic acid. × 6750.

FIGURE 30

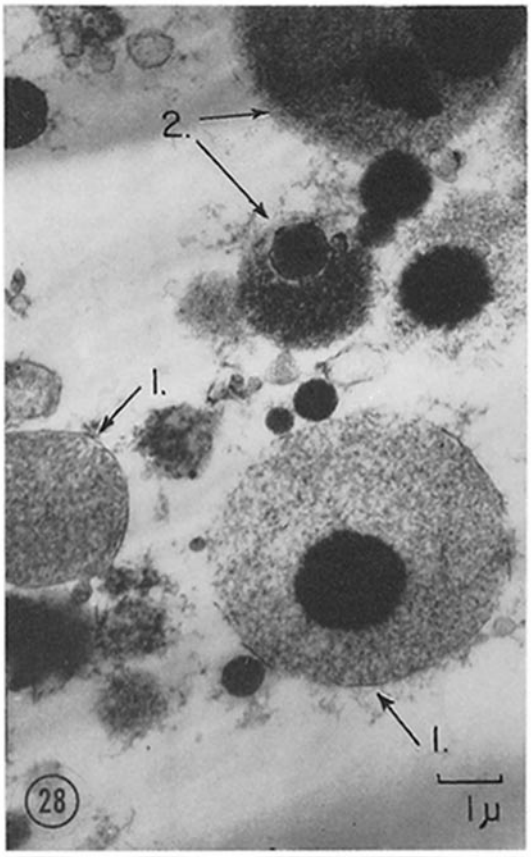
Polarised light micrograph of the yellow yolk sphere shown in Fig. 31. Note the bright ring of light at the surface of the yolk sphere. × 5000.

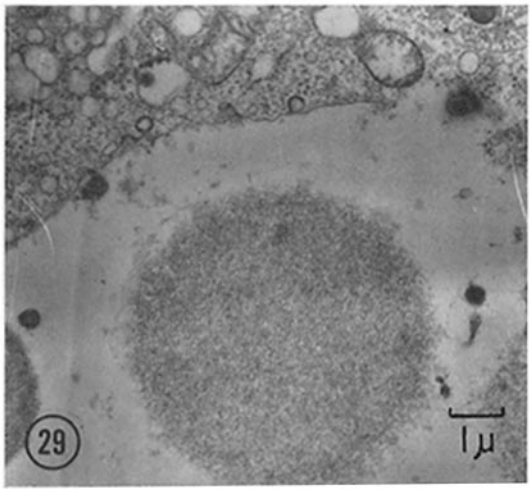
FIGURE 31

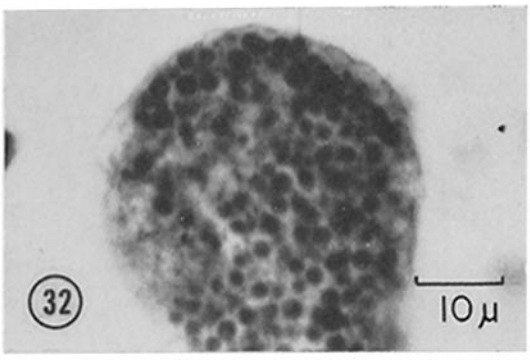
Phase contrast micrograph of an unfixed yellow yolk sphere. Egg white was added to this yolk smear to improve contrast. × 5000.

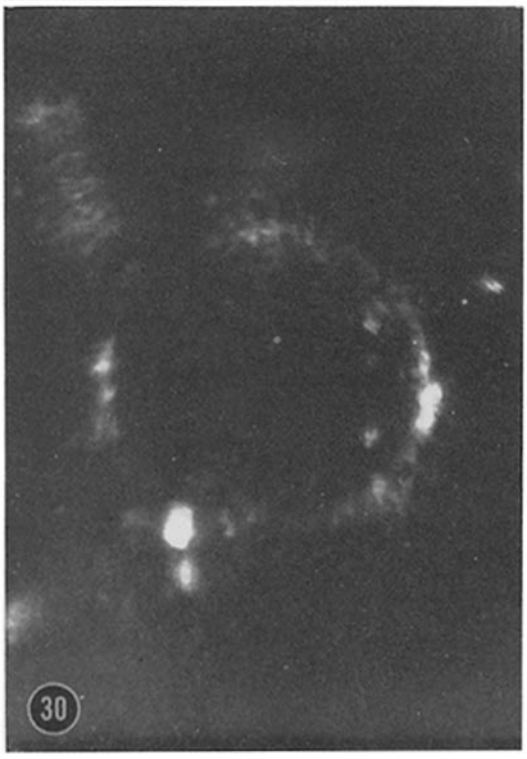
FIGURE 32

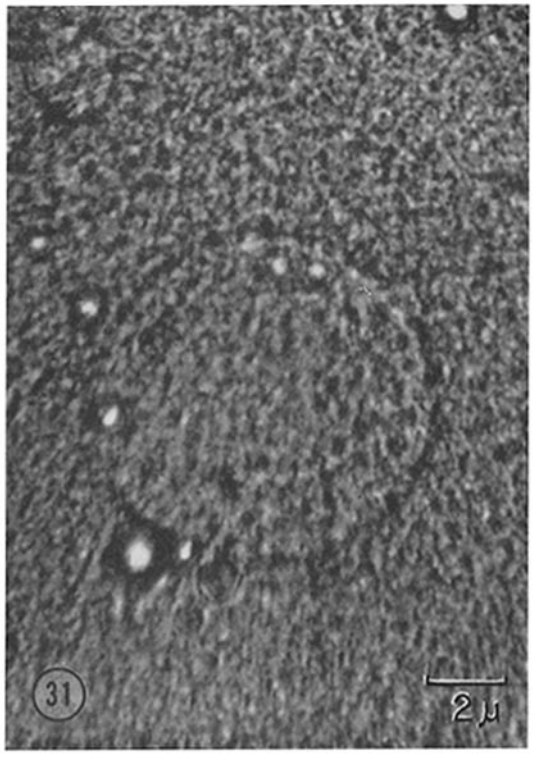
Light micrograph of a 5 μ section of a white yolk sphere. Note the line (a membrane?) which surrounds the yolk sphere. Fixed in Bouin's fluid and stained with haematoxylin and eosin. × 1200.


2.
1.
1.
28
1μ


29
1μ


32
10μ


30


31
2μ

which I have ever seen in extraembryonic (primary) yolk. The relationship between mitochondria and intracellular yolk will be discussed in a later paper.

It has been suggested in the literature at various times that yolk spheres may become transformed directly into cells (Lavodsky, 1901; Golgi, 1923; Pierantoni, 1951; Lepeschinskaya, 1954; Jordanov and Georgiev, 1957). Throughout the present investigation, however, no evidence has been found which supports that idea in any way. In a recent paper Roskin (1955) has discussed the histological and staining techniques employed by some of the above authors and has put forward possible explanations to account for their findings.

CONCLUSIONS

1. Both the yellow and white yolk of the hen's egg have been examined by phase contrast, polarised light, and electron microscopy. Particular attention has been paid to the fine structure of the lipid and protein components and to the surfaces of the yolk spheres.

2. The lipid drops were composed of particles about 30 to 60 A in diameter.

3. The protein (or perhaps lipo-protein) component of the yolk was found to consist of round profiles about 250 A in diameter.

4. The surfaces of the yolk spheres were of three types. The first type was covered with a lamellated capsule; this capsule was considered to be the result of hydration changes occurring during storage of the eggs. The second type, which was extremely uncommon, possessed a 75 A wide structure closely resembling the unit membrane of Robertson (1959), although it is suggested that this may represent the first stage in formation of a lamellated capsule. The third type was apparently naked at the surface, and it is tentatively suggested that this type may represent the true structure of the yolk sphere "membrane." It is noted, however, that even if the second type (which closely resembles a cell membrane in appearance) is the true yolk sphere "membrane," it nevertheless differs from a cell membrane in certain respects.

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