THE EGG SHELL OF THE HOUSE CRICKET
(ACHETA DOMESTICUS): AN ELECTRON-MICROSCOPE STUDY

P. J. S. FURNEAUX, C. R. JAMES AND S. A. POTTER
Shell Research Limited, Milstead Laboratory of Chemical Enzymology, Broad Oak Road, Sittingbourne, Kent, England

SUMMARY

Consecutive changes in two discrete layers of the egg shell of the house cricket, Acheta domesticus, have been claimed to control the uptake of water by the eggs. The development of the shell has been re-investigated with the electron microscope by examination of eggs at different stages of embryogenesis and of ovarioles containing oocytes at various stages of maturity.

It is confirmed that fragmentation of the maternal epicuticle and deposition and resorption of the serosal cuticle are the only apparent changes in the shell during development. The existence of a serosal epicuticle is confirmed and a distinction is made between the serosal epicuticle and the vitelline membrane.

Previously unreported features of the shell are (i) an outer zone of the maternal endocuticle which seems to be the most stable part of the maternal cuticle, (ii) a microlaminar organization within the scales of the maternal epicuticle, (iii) a vitelline membrane containing specialized regions, which remains distinct from the serosal epicuticle throughout development, and (iv) the osmiophilic character of the serosal epicuticle, its complex fine structure and its origin.

Observations on eggs which had just begun to absorb water allow us to suggest that fragmentation involves a shrinkage of the material of which the scales are composed.

INTRODUCTION

Water uptake is a necessary event in the development of house-cricket eggs and the time at which water is absorbed is directly related to embryogenesis (McFarlane & Kennard, 1960). The control of water uptake by orthopteran eggs has been studied in detail only in crickets (McFarlane, 1960a, 1961, 1963, 1965; Furneaux & McFarlane, 1965; Browning, 1965, 1967; Browning & Forrest, 1960) and the grasshopper Melanoplus differentialis (Slifer, 1937, 1938, 1946, 1949; Slifer & Sekhon, 1963). The cricket egg shell is a multilaminate structure whose component layers differ in appearance, histochemistry and composition. It was proposed by McFarlane (1960a) that water uptake is controlled by the successive tanning of two thin layers of the shell. The first may alter the permeability of the shell and allow water to enter, while the second may reduce the elasticity of the shell and bring water uptake to an end.

At oviposition the egg is enclosed by a chorion or maternal cuticle (McFarlane, 1962a) laid down by the follicular epithelium. The maternal epicuticle is composed of overlapping scales and is slightly sudanophilic and a site of tyrosinase activity (McFarlane, 1960a, 1961). Simultaneously with the onset of water uptake, the maternal epicuticle develops a pattern visible with the light microscope, consisting of
variably sized, more or less rectangular blocks. The appearance of the pattern is probably the result of the activity of the epicuticular tyrosinase (McFarlane, 1960a). It does not appear to be induced before its normal appearance by pressure applied to the egg, indicating that it is not a consequence of fracture when the chorion is stretched by the uptake of water. The occurrence of the pattern at the onset of water uptake indicates a change in the permeability of the shell which permits water to enter (McFarlane, 1960a).

As water uptake proceeds, the thickness of the shell is increased due to the deposition by the serosa of a serosal cuticle. McFarlane (1960a) recognized a sudano-philic epicuticle which is also a site of tyrosinase activity. Subsequently a thick chitinous endocuticle is secreted. It was suggested that the outer part of the serosal cuticle, either the epicuticle or the outer part of the endocuticle, may be stabilized by quinone tanning and that this event could bring water uptake to an end by limiting the elasticity of the shell (McFarlane, 1960a). No evidence for this proposed change could be discerned with the light microscope. However, after water uptake has finished, most of the endocuticle is digested and only a thin outer portion remains in the vacated shell at eclosion. This suggests that the outer portion is different from the remainder, but does not indicate when the change occurs. Evidence to indicate that a quinone tanning may occur at the end of water absorption was obtained by estimating the concentration of phenols in whole eggs at various stages of development. House-cricket eggs contain several aminophenols and one of these, N-acetyl dopamine, is most abundant at the end of water absorption. A histochemical test for aminophenols showed these to be located mainly in the serosal cells before the end of water absorption (Furneaux & McFarlane, 1965).

The studies reported here were carried out to verify the results obtained with the light microscope, and to see whether knowledge of the fine structure of the shell threw any further light on the mechanism of water uptake.

MATERIALS AND METHODS

Eggs of the house cricket, *Acheta domestica* (L.), were collected by introducing dishes of moist sand into a culture of the insect for up to 12 h. The eggs were separated from the sand and incubated at 34 °C on moist filter paper. At this temperature water uptake begins at 46 h and ends at 65 h incubation. Eggs were removed at 2-h intervals between 30 and 48 h in order to locate the period of deposition of the serosal epicuticle. Ovarioles containing eggs at various stages of maturity were dissected from female crickets at daily intervals in order to locate the period of deposition of the maternal cuticle.

Eggs were punctured with a fine needle in ice-cold 2.5 % glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, adjusted to 0.15 M with sucrose. With the aid of iridectomy scissors the ends of the egg were cut away to leave a cylinder (the middle portion of the egg) about 1.5 mm long. It was found that puncturing the egg under glutaraldehyde facilitated the subsequent trimming with scissors and allowed the operation to be carried out with minimum damage to the egg contents. Fixation was continued for 2 h at 2 °C and the material was then washed in 0.15 M sucrose in 0.1 M cacodylate buffer, pH 7.2, for about 16 h at 2 °C. Post-fixation was carried out for up to 1 h at 40 °C in 1 % OsO₄ in 286 mM veronal acetate-HCl buffer, pH 7.4, adjusted to 0.15 M with sucrose (Caulfield, 1957). Virtually no penetration of the tissue by OsO₄ occurred if post-fixation was performed at 2 °C. The material was dehydrated with a graded series of alcohols and taken through propylene oxide into Araldite as described by Luft (1961). Thin
sections were cut with glass knives on a Huxley Microtome and examined with a Zeiss EM 9 A electron microscope, either unstained or after staining with uranyl acetate followed by lead citrate (Reynolds, 1963) or after staining with potassium permanganate (Lawn, 1960).

RESULTS

General structure

Before water uptake occurs the shape of the house-cricket egg approximates to a cylinder with rounded ends, about 2.5 mm long and 0.3 mm in diameter. During water uptake the shape of the egg changes slightly, the ends of the egg becoming more ellipsoid. The length of the egg hardly changes at all, but the diameter increases to about 0.5 mm.

![Diagram of egg shell layers](image)

Fig. 1. A, diagrammatic representation of the house-cricket egg shell at a time when the serosal endocuticle is at its greatest thickness; B, the serosal epicuticle; C, the maternal epicuticle, in which the pitch of the scales has been exaggerated.

The main regions of the cricket egg shell are shown in Figs. 1 and 2. The nomenclature of the layers is based on previous observations with the light microscope or from the results of experiments performed on the intact egg or isolated shell (McFarlane, 1960a, b, 1961). Figure 1 is a diagram showing the dimensions of the layers of the
shell as measured on electron micrographs. They differ in some cases from measurements made with the light microscope, and this is almost certainly due to the difference in preparation of the material for the different methods of microscopic examination. The components of the shell during water uptake are demonstrated by the survey photographs shown in Fig. 2.

The maternal cuticle

The outside of a newly laid egg is slightly uneven and particles can sometimes be seen adhering to the surface. The particles are not seen in sections of ovarian eggs and could be the product of the accessory ('annex') glands which have been claimed to produce a secretion lubricating the ovipositor (Braesch, 1950). The spongy extrachorion of acridid egg shells (Hartley, 1961) is considered to be a secretion of the accessory glands.

The maternal endocuticle is about 6 μm thick. Beneath the outer surface is a zone 0.5-1.0 μm thick containing inclusions which have circular or elliptical profiles and range in size up to 1 μm diameter (Fig. 3). The inclusions are demarcated from the matrix by an electron-transparent space; they are not noticeably different from the matrix in texture. The matrix of this outer zone appears to be composed of a material which is more stable than the remainder of the endocuticle. When unstained sections were immersed in dilute NaOCl for 20 min, most of the maternal cuticle dissolved, but the matrix of the outer zone was still recognizable (Fig. 4). This zone is found in sections of ovarian eggs and cannot therefore be a product of the accessory glands.

The remainder of the endocuticle appears to be homogenous, even at high magnification. There is no evidence of any pores or other radially oriented structure, nor is there any lamellar appearance. The endocuticle sometimes appears to have folded, suggesting that it expands during fixation and sectioning relative to the remainder of the shell (Fig. 22).

The maternal epicuticle differs strikingly in appearance from the endocuticle. It is 0.6-1.0 μm deep and is composed of overlapping scales arranged like slates on a roof (Fig. 1c). The scales are about 30-60 μm across their surface and vary in thickness from 0.05 μm at their origin next to the vitelline membrane to 0.25 μm at the interface between epi- and endocuticle. The scales are set at an angle of between 0° 30' and 1° 30' to the surface of the vitelline membrane. Three to six scales may overlap in any particular radial section. The scales are separated from one another by a narrow gap of electron-transparent material about 100 Å wide, in the centre of which is a 25-Å wide, electron-dense lamina (Fig. 9).

In transverse or longitudinal sections the scales possess a microlaminar pattern composed of alternating light and dark zones (Figs. 8-10). The repeat from the centre of one dark zone to the next is about 50 Å. This microlaminar pattern is found in shells of any age, including ovarian shells, but is visible only after staining.

Beneath the maternal epicuticle there is a distinct vitelline membrane 0.15 μm thick. Sections of the shell occasionally pass through elaborate regions of the vitelline membrane which probably correspond to the sparsely distributed spots described by McFarlane (1960b, 1961). In these regions the vitelline membrane is thickened
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towards the inside of the shell (Fig. 17). The thickening contains material of a spongy appearance at its edges, and fibrous or tubular material centrally. The fibres or tubes appear to converge on a darkly staining, but otherwise homogeneous, portion of the vitelline membrane. Before the vitelline membrane has been formed, the oocyte is surrounded by a thin ‘oocyte membrane’ (Favard-Séréno, 1964). We have noticed an oocyte membrane distinct from the vitelline membrane in newly laid eggs.

Simultaneously with the beginning of water uptake, a change is noticed in the maternal epicuticle. Clefts develop across this layer corresponding to the fragmentation pattern observed in surface view. The result is the formation of blocks which are often rectangular or triangular in section, containing portions of several scales (Figs. 2, 5–7, 10 and 22). In some cases the clefts do not penetrate the epicuticle completely but leave a few inner scales intact, suggesting that fragmentation may begin at the outside of the epicuticle and spread towards the vitelline membrane. An incomplete cleft is shown in Fig. 10. The edges of the fragmented scales are normally very smooth (Figs. 5, 6), but in a few cases strands of material appear to connect opposite sides of the cleft. The area shown in Fig. 5 also indicates that the material of the scales shrinks at fragmentation; the outer end of the scale has clearly been withdrawn, leaving the imprint of its original position against the inner face of the maternal endocuticle. The microlaminae persist during and after fragmentation (Fig. 10).

Early stages in the formation of the maternal cuticle of another cricket, *Acheta bimaculatus*, have been described by Favard-Séréno (1966). The scales of the maternal epicuticle appear to be constructed from material forming the contents of certain vesicles which originate in the Golgi region of the follicle cells. The contents of these vesicles are liberated as electron-dense granules into the space between the plasma membrane of the follicle cell and the vitelline membrane. In *A. domesticus* these granules are still present between the loosely packed scales of the epicuticle after the formation of the maternal endocuticle has begun (Fig. 11.) The maternal endocuticle at this stage is only 0.5–1.0 μm thick and is composed of blocks between which are projections of the plasma membrane. At a later stage (as judged by the thickness of the maternal endocuticle) the blocks have fused (Fig. 12). The plasma membrane is closely apposed to the endocuticle, but does not penetrate it. Small vesicles appear to be discharging their contents at the interface (Fig. 13).

The serosal cuticle

The serosa begins to secrete the serosal cuticle against the inner face of the vitelline membrane at about the time that the egg begins to swell. When fully formed, the serosal cuticle is about 20–25 μm thick. The serosal epicuticle has been described as a thin, refractile, strongly sudanophilic membrane on the inner side of the vitelline membrane (McFarlane, 1960a). It is assumed to confer resistance to the action of hypochlorite, which removes the chorion and vitelline membrane from eggs immersed in it. In many thin sections, this layer is intensely osmiophilic (Figs. 8, 10, 18, 22). The dark region is about 600 Å thick, and beneath is a less-dense zone about 250 Å
thick (Fig. 1B). Between the denser zone of the epicuticle and the vitelline membrane there is a space 150 Å wide containing a 90-Å wide dense line.

By sampling eggs at 2 h intervals we have found that the formation of the serosal epicuticle occurs after 34-40 h incubation at 34 °C. This precedes the onset of swelling by about 10 h. The earliest stages of secretion can be found in different serosal cells of eggs incubated for 34-36 h. Within one sample of eggs there was some variability of stage of serosal epicuticle formation. Accordingly, we have based our interpretation of serosal epicuticle formation on the complexity of potential epicuticle that we observed, rather than on a strict time schedule. Cells that are just beginning to secrete the serosal epicuticle are almost entirely filled by the nucleus. Near the plasma membrane bordering the vitelline membrane are numerous mitochondria and clusters of ribosomes (Fig. 14). The plasma membrane of other serosal cells is slightly folded (Fig. 15). Above the folds are patches of membrane, the profiles of which are about 250 Å thick.

In the space between the plasma membrane and the vitelline membrane there is also an irregular layer of electron-dense material. In other eggs (Fig. 16) a more or less continuous membrane 250 Å thick is present between the plasma membrane of the serosal cells and the vitelline membrane. At a later stage the membrane has become more complex, and is now about 500 Å thick (Fig. 17). The irregular layer of electron-dense material has now disappeared. The increase in thickness of the original membrane seems to take place by addition to the inner side. The outer electron-dense line (about 90 Å thick) persists throughout the remainder of development and is separated from the vitelline membrane on the outside and the rest of the serosal epicuticle on the inside by electron-transparent spaces about 30 Å wide (Figs. 8, 18).

The serosal endocuticle is secreted during water uptake and for up to 24 h afterwards. It is known to contain chitin and protein (McFarlane, 1962a) and its structure is very similar to the endocuticles of post-embryonic insects. When fully formed it is composed of 30–40 lamellae, of which the first 5–10 to be formed are markedly different from the remainder (Figs. 2, 19). The relative disorder of these first-formed lamellae may be a consequence of the secretion of endocuticle while the volume of the egg is increasing. The disordered outer lamellae can be observed in sections of eggs fixed while water uptake is in progress. During the secretion of the disordered lamellae several long microvilli reaching almost to the serosal epicuticle are interspersed with numerous short microvilli (Figs. 19, 20). During the secretion of the remaining lamellae only short microvilli are present (Fig. 2).

After about 108 h incubation at 34 °C the microvilli at the serosal cell border become fewer. A space separates them from the innermost layer of the serosal endocuticle (Fig. 21). The inner layers of the endocuticle now progressively lose their lamellar structure. At about this time the pleuropodia are showing signs of secretory activity (McFarlane & Furneaux, 1964). It is likely that the pleuropodia secrete digestive enzymes which break down the serosal endocuticle in a similar fashion to that demonstrated for the egg of *Melanoplus* (Slifer, 1937). Sections of the vacated shell reveal only one or two lamellae remaining (Fig. 22). McFarlane (1962a) has suggested that these may resist digestion by the hatching enzymes by virtue of being stabilized by quinone tanning.
DISCUSSION

Our observations on the fine structure of the house-cricket egg shell agree to a large extent with previous descriptions of the shell (McFarlane, 1960a, 1961). They confirm that fragmentation of the maternal epicuticle is the only apparent change in shell structure which seems to be related to water uptake, and that the maternal and serosal epicuticles differ in structure. They also reveal a degree of organization of the shell layers which was previously unsuspected.

The maternal endocuticle was previously thought to be homogeneous. However, the outermost part in which we have noticed spherical inclusions of various sizes shows a considerable resistance to dilute NaOCl and appears to be the most highly stabilized region of the chorion.

The inner layer of the chorion, the maternal epicuticle, is composed of overlapping scales which can be separated one from another (McFarlane, 1961, 1962a). This is the only part of the shell to be affected by fragmentation. Little is known about the composition of the scales, except that they are largely protein and perhaps contain lipid (McFarlane, 1960a, 1962a). We have shown that they have a microlaminar appearance in section and interpret the microlaminae as local accumulations of stain on the surface of the section corresponding to some highly organized component of the scales. The scales appear to be constructed from electron-dense granules liberated into the space between the follicle cell plasma membrane and the vitelline membrane (Favard-Séréno, 1966). It is not clear how the material is organized into overlapping scales and not into, for instance, concentric lamellae.

The clefts which separate blocks of scales after fragmentation generally penetrate completely to the vitelline membrane, but examination of shells shortly after the onset of water uptake (60 h incubation) revealed a proportion of clefts which penetrated the maternal epicuticle incompletely. These clefts were almost always open at the interface between the epicuticle and endocuticle. This suggests that fragmentation is progressive and usually develops across the epicuticle from the outside inwards. Fine threads were sometimes observed to be stretched between opposite sides of small incomplete clefts and, in some areas of the fragmented epicuticle, the relationship of the blocks of scales to the clefts between them indicated that fragmentation had occurred as a result of shrinkage of the material of the scales. This interpretation of the fragmentation must, however, be tentative in the absence of a more detailed analysis of the composition of the maternal epicuticle.

The chorion of the house-cricket egg shell appears to be quite unlike the chorion of other orthopteran eggs. In acridids (Hartley, 1961; Slifer & Sekhon, 1963) and tettigoniids (Hartley, 1962, 1964) part of the chorion is composed of an open, three-dimensional meshwork of fibres or of discrete struts. Such an open system is lacking in the house cricket. However, the possession of an endochorion (maternal epicuticle) composed of overlapping scales may be a general and unique feature of crickets (McFarlane, 1966).

Evidence for the presence of a vitelline membrane in the house-cricket egg has previously been indirect. It stains in a similar way to the maternal epicuticle but, when
whole eggs were immersed in saturated basic fuchsin before water uptake had begun and examined after water uptake, randomly and sparsely distributed accumulations of stain were observed in sections of the shell beneath the maternal cuticle. They were removed if the egg was treated with hypochlorite after staining, thus indicating that the spots were located outside the serosal epicuticle. The thin layer in which the spots were located was not subject to fragmentation at the beginning of water uptake. Similar results were obtained when eggs were immersed in a solution of tyrosine (McFarlane, 1960, 1961). It was concluded that a vitelline membrane is present between maternal and serosal epicuticles and that this membrane probably contains radially oriented pores. Our observations confirm the presence of a vitelline membrane in the mature oocyte and throughout the remainder of development of the egg. The elaborate areas occasionally seen in thin sections probably correspond to the accumulations of stain seen by McFarlane. Further work will be necessary to establish the significance of these structures. At present, there is no evidence that they are involved in water uptake. No similar structures have been reported in previous studies on the fine structure of the vitelline membrane of insect eggs (Gerrity, Rempel, Sweeny & Church, 1967; Hopkins & King, 1966; King & Koch, 1963; Slifer & Sekhon, 1963).

A serosal epicuticle was recognized in acridid eggs because of chemical similarity to the epicuticle of post-embryonic insects (Jahn, 1935) and on electron-microscopical evidence (Slifer & Sekhon, 1963). A thin, sudanophilic outer part of the serosal cuticle of the house-cricket egg was interpreted as an epicuticle by McFarlane (1960a). Browning (1967) has confused the serosal epicuticle and the vitelline membrane in his interpretation of the illustrations in his review (see his Plate 33b, c). These 2 thin layers of the cricket egg shell can be distinguished readily. The vitelline membrane is present in the mature ovarian egg and throughout the remainder of development. It remains intact after fragmentation of the maternal epicuticle. The serosal epicuticle is not formed until shortly before water uptake begins. In our experience it is usually intensely osmiophilic. It remains distinct from the vitelline membrane throughout development. In contrast, the vitelline membrane of the egg of *Melanoplus* becomes incorporated into the serosal epicuticle (Slifer & Sekhon, 1963).

The early stages of the formation of the serosal epicuticle of the house-cricket egg are reminiscent of the description of the formation of the cuticulin layer in the larval epicuticle of the hesperid caterpillar *Calpodes ethlius* (Locke, 1966). In both instances membrane profiles appear as patches a short distance above projections of the plasma membrane of the secretory cell. These projections are long microvilli in *Calpodes* larvae and low hummocks in the cricket egg. The patches of membrane profile are resolved as 3 dense laminae in the formation of *Calpodes* cuticulin; we can resolve with certainty only 2 dense laminae in the membrane profile of the early serosal epicuticle of the cricket egg. The thickness of the membrane is rather similar in both instances: 160–190 Å in *Calpodes* and about 250 Å in the cricket egg. We have been unable to resolve any finer structure of this membrane which might indicate further resemblance to the precursor of cuticulin. The outer lamina persists and can be recognized at later stages as a thin, electron-dense line marking the outer edge of the serosal epicuticle. The central and inner laminae in *Calpodes* larvae and the inner lamina in the cricket
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egg are incorporated into the homogenous layer of the epicuticle. The stages which we
have been able to observe indicate that the serosal epicuticle of the house-cricket egg
shell is laid down in a similar way to the cuticulin layer of the epicuticle of Calpodes
larvae, and that the origin of the serosal epicuticle, as well as its structure and composi-
tion, reveals a considerable similarity to the epicuticle of post-embryonic insects.

It is a pleasure to thank Professor J. E. McFarlane for his encouragement during the course
of this work, and Professor G. Popjak, Professor J. W. Cornforth and Dr A. N. Clements for
their helpful suggestions regarding the preparation of the manuscript.

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Fig. 2. Low-power electron micrograph of the shell and parts of several serosal cells. Sixty hours incubation. Stained with uranyl acetate and lead citrate. All of the following photographs, except Fig. 17, are of sections stained in a similar way. (mm, maternal endocuticle; mp, maternal epicuticle; oz, outer zone of maternal endocuticle; s, part of serosal cell; sn 1, outer region of serosal endocuticle; sn 2, inner region of serosal endocuticle.)
Fig. 3. The outer part of the maternal endocuticle of a newly laid egg. The outer zone contains inclusions (i) of various sizes which often have elliptical profiles. Fluffy material (m) outside the shell may be the product of the accessory glands.

Fig. 4. As Fig. 3, but section immersed in dilute NaOCl for 20 min before staining. Only the matrix of the outer zone (oz) survives this treatment. Newly laid egg.

Fig. 5. The fragmented maternal epicuticle. The clefts that are formed sometimes cut radially (c 1) or diagonally (c 2) across several scales or appear to be the space left after the tip of a scale has receded (c 3). Sixty hours incubation.

Fig. 6. A diagonal cleft in the maternal epicuticle which appears to be developing from the maternal endocuticle (mri) towards the vitelline membrane (vm). The edges of the cleft in the outside scale are smooth, but as the cleft passes into the next scale, its edges are less regular and are joined by fine threads. Sixty hours incubation.

Fig. 7. The formation of a block whose profile will be triangular after fragmentation of the maternal epicuticle. The threads joining the edges of the cleft are more or less in the plane of the scale undergoing fragmentation, suggesting that they may be related to the microlaminae illustrated in Figs. 8–10. Sixty hours incubation.
The scales of the maternal epicuticle are broadest towards the outside of the shell (right of photograph) and possess a microlaminar appearance. The serosal epicuticle has three components: a line of medium electron density (1) separated by electron-transparent spaces from the vitelline membrane (vm) and the main region (2) of extreme electron density; the main region merges into a region of medium electron density (3). Forty hours incubation.

Fig. 9. Parts of 3 scales from the maternal epicuticle. The spaces between the scales are often electron-transparent except for a thin central lamina. Thirty-four hours incubation.

Fig. 10. An incomplete cleft apparently developing across the maternal epicuticle (mp) from the outside (right of photograph) towards the vitelline membrane (vm). Sixty hours incubation.
Fig. 11. Maternal cuticle and part of a follicular epithelial cell (f). The follicular epithelial cell extends into the maternal endocuticle (mn) at the place marked with an arrow. At this stage of deposition, the maternal endocuticle is composed of irregular blocks. The scales of the maternal epicuticle (mp) are separated by spaces packed with granules. Part of the ovariole containing an almost fully developed oocyte from a cricket 7 days after moult to adult.

Fig. 12. Follicular epithelial cell (f) and maternal endocuticle (mn). At this stage of deposition the blocks of endocuticle have fused. Same age as Fig. 11.

Fig. 13. The secretory edge of a follicular epithelial cell. Small vesicles (arrows) are discharging material against the outer face of the maternal endocuticle. Same age as Fig. 11.
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Fig. 14. Edge of a serosal cell (m, mitochondrion; n, nucleus; r, clusters of ribosomes) from an egg incubated for 34 h. There is only a narrow space between the plasma membrane and the vitelline membrane (vm).

Fig. 15. Edge of a serosal cell of an egg incubated for 36 h. The plasma membrane (solid arrow) of the serosal cell is folded into low hummocks above which the profiles of patches of membrane can be seen (open arrow). Beneath the vitelline membrane (vm) there is an irregular layer of electron-dense material.
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Fig. 16. The plasma membrane (solid arrow) of a serosal cell from an egg incubated for 34 h. A more or less continuous membrane (open arrow) several times thicker than the plasma membrane is present between the latter and the vitelline membrane (vm).

Fig. 17. The plasma membrane (solid arrow) is becoming microvillate in the serosal cells of this egg, incubated for 36 h. The section is stained with Lawn’s permanganate. The serosal epicuticle is now thicker than at the stage shown in Fig. 16. The outer lamina of the original membrane is still visible (open arrow, 1) while the inner lamina forms the edge of what will become the main osmiophilic region of the epicuticle (open arrow, 2). The section passes through an elaborate portion of the vitelline membrane (vm).

Fig. 18. The serosal epicuticle is now complete and the main region has become intensely osmiophilic. The first lamella of the serosal endocuticle (sn 1) is in process of secretion. Forty hours incubation.
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Fig. 19. Part of a serosal cell (s) during secretion of the outer part of the serosal endocuticle (sn 1). Long microvilli (mv) penetrate between the lamellae. Fifty-six hours incubation.

Fig. 20. A single long microvillus. Forty-eight hours incubation.

Fig. 21. Part of a serosal cell (s) with prominent nucleus (n) and irregular short microvilli protruding into the space between the serosa and the serosal endocuticle which appears during the digestion of the latter. Ninety-six hours incubation.

Fig. 22. Digestion of the serosal endocuticle (sn) removes all but one or two lamellae. Vacated shell.