THE FORMATION AND STRUCTURE OF THE MICROPYLER COMPLEX IN THE EGG-SHELL OF RHODNIUS PROLIXUS STAHL. (HETEROPTERA REDUVIIDAE)

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(With Fifteen Text-figures)

INTRODUCTION

The micropyle and associated structures of insect eggs have received little detailed attention, although they are of great importance. For not only do they play a vital part in fertilization but also, since they penetrate the whole of the chorion layers, they form a most likely site of entry for ovicidal materials. They may also be of importance in the water-exchange and respiratory systems of the egg.

Beament (1946) has already described in detail the properties, formation and permeability of the chorion of the heteropteron, Rhodnius prolixus Stähl. The unspecialized shell consists of seven layers, and the properties attributed to them enable such materials to be detected in more complex regions of the shell. It was, moreover, shown that in three specialized areas of the shell (the cap surface, the neck and the rear end) modifications were produced by variation in the relative thickness of the component layers and not by the secretion of new components.

It is the object of this paper to investigate the structure of the micropyle and the associated structures of the junction between the shell and its cap, in terms of the component layers of other regions of the shell, and by following the secretions of the follicle cells. In this way it is hoped to obtain a structural basis for the importance of such a complex in the physiological relationship of the egg.

THE LAYERS OF THE RHODNIUS EGG-SHELL

Beament (1946) has shown that the unspecialized part of the shell of the Rhodnius egg is formed in the following way (Fig. 1).

The follicle cells in a deeply staining phase secrete the endochorion layers. These consist of:

1. A series of tanned granules of protein, 2μ in diameter, with a high polyphenol content. This is the inner polyphenol layer.

2. A layer of tanned protein, 1–2μ thick, and resistant to enzymes and to acids and bases, called the resistant protein layer.

3. The outer polyphenol layer, which is similar to the inner layer but in which the granules are much smaller.
(4) The amber layer, which is the only coloured layer of the shell. It is formed by the addition of oil to tanned protein after the latter has been secreted. The oil is released from this product only after the action of the strongest hot acid.

(5) The soft protein layer forming the greater part of the thickness of the endochorion. Its material is similar to that of the resistant protein layer, but is more readily attacked by acids and bases. It contains diffusely scattered polyphenol elements. This layer will also be referred to as the soft endochorion, as opposed to the resistant endochorion, made up of the other four layers.

The exochorion is secreted after the follicle cells have undergone a transition to a non-staining phase. It consists of two layers of a lipoprotein (‘chorionin’) which is formed in the cells before secretion (cf. the amber addition product).

Fig. 1. Diagrammatic representation of a fragment of the *Rhodnius* egg-shell from the unspecialized region of the chorion, showing the polygonal markings of the surface (pol.mk.) and the follicular pits (f.pt.) at their centres. Inset: the layers of the resistant endochorion. amb. amber layer; i.pl.l. inner polyphenol layer; o.pl.l. outer polyphenol layer; p.can. pore canals; r.end.pr. resistant endochorion protein layer; r.exo. resistant exochorion layer; s.end. soft endochorion layer; s.exo. soft exochorion layer; l.wax. primary wax layer. (After Beament, 1946 b.)

(6) The soft exochorion is a thick layer, which is mainly responsible for the sculptured appearance of the egg. It is impermeable to all but small ions and water, and is soluble in potash and in strong nitric acid, releasing the oily component of the lipoprotein.

(7) The resistant exochorion forms the outermost, thin layer of the chorion and is a more resistant form of chorionin.

Each binucleate follicle cell leaves a polygonal outline on the shell and there is a deep pit, the follicular pit, in the centre of each polygon, but confined to the exochorion layers. Pore canals are present in the exochorion layers only, running from the inside of the pits towards the endochorion; they do not penetrate to the
Micropylar complex in egg-shell of Rhodnius prolixus

soft endochorion (Beament, 1946b) (Fig. 1). According to these characteristics the shell may be divided into a number of blocks of material, each the product of an individual follicular cell, and this method of analysis will be used in the following work.

The rims and seal complex

Fig. 2 shows a longitudinal section through the region where the rim of the cap is sealed on to the rim of the unspecialized shell. The main parts referred to below will be described briefly.

Fig. 2. Longitudinal section through the seal between the rims of the cap and main shell. amb. amber layer; exp.r.end. expanded resistant endochorion layer; f.pt.A., f.pt.B. follicular pits of the A and B cells; f.pt.cp. follicular pit of the main cap surface; f.pt. res. residues of follicular pits in the rim of the shell; h.ln. hatching line; i.rs.sl. inner recess of the seal; nk. neck of the shell; o.rs.sl. outer recess of the seal; p.can.cp. pore canals of the rim of the cap; p.can.psm. pore canals of the pseudomicropyles; p.can.sh. pore canals of the rim of the shell; psm. pseudomicropyle; r.end.sh. resistant endochorion layers of the shell; r.exo. resistant exochorion layer; sec.h.ln. secondary hatching line; s.end.cp. soft endochorion layer of the cap; s.end.sh. soft endochorion layer of the shell; s.exo.cp. soft exochorion layer of the cap; s.exo.sh. soft exochorion layer of the shell; sl.b. sealing bar; sl.ct. sealing catch.
The circumference of the cap is expanded to form the rim of the cap. This expansion is produced mainly by an increased thickness of soft exochorion and secreted round a ring of very long, but otherwise normal follicular pits. On the inside of this rim the amber layer is folded inwards and thickened, but the soft endochorion protein layer and the resistant endochorion layers are extremely thin. The rim of the cap is therefore forked in section; the sealing bar which connects the rim of the cap to the shell is attached to the inside of this fork. It abuts on to the amber layer, and its junction with the cap is marked by the line of weakness, or hatching line along which the shell breaks at eclosion. The sealing bar is the thinnest part of the shell; it consists of a very thin inner resistant endochorion, together with a very thick amber layer—the exochorion layers are missing.

Below the sealing bar, the rim of the shell is again formed by an expansion of the soft exochorion, while all the inner layers are very thin. This rim is secreted around four rings of long follicular pits. The posterior three are filled in during secretion, but the anterior ring, set in the upper part of the rim of the shell, remain. At first, these appear (Fig. 11) to be normal follicular pits of exceptional length; there are some two hundred around the rim in *Rhodnius*. They are, apparently, present in most hemipteron eggs, and have been the subject of considerable controversy and misstatement.

Leuckart (1855) and others have stated that these pits are all micropyles, and the name ‘samenbecher’, or seminal cups, has been applied to them, especially in some other species of Heteroptera where they may be produced into processes rising from the rim. On the other hand, Gross (1901) and Heymons (1926) suggested that these were for aeration, though no other structures for the entry of spermatozoa were described. Johnson (1934), describing the rim of the egg-shell in *Notostira erratic*ica, depicted tubes at this level opening on to the inside of the shell by a funnel 5\(\mu\) in diameter, but could not find any trace of their external opening and supposed that they were connected with the large cavities in the cap. He stated that twenty to forty may occur, scattered irregularly around the rim. Other distributions of ‘micropyles’ in hemipteron eggs are quoted by Heidemann (1911): eggs of *Brochymena* may have thirty to forty, *Euschistus* sixty or more, and *Belonochilus numenius* as few as five. Apparently the numbers are variable, and the distribution is irregular in all hemipteron eggs that have been described.

It will be shown below that the majority of the pits in the shell rim are not open to the external surface of the shell, neither do they penetrate to the surface of the egg cell. These cannot satisfy the function of micropylar apparatus, since they cannot permit the passage of spermatozoa through the shell for fertilization. (It is known (Beament, 1946\(d\)) that the *Rhodnius* egg is fertilized after the shell is complete, and, therefore, the existence of some form of micropyle is apparently imperative.) These closed tubes will be referred to as *pseudomicropyles*. However, a modification of about fifteen of these pits results in the formation of the true micropyles (Fig. 13). Each opens on the outer side of the egg-shell by a funnel-shaped orifice of about 2\(\mu\) diameter and in the groove which occurs below the dorsal point of the rim. This groove can, therefore, be called the *spermatic groove*.
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(Figs. 11, 13). On the inner surface, the micropyles penetrate the resistant endo-
chorion, opening by a small aperture with a diameter of less than 1μ (Fig. 12). The
inner openings are on the same level as the blind ends of the pseudomicropyles,
but the remainder of the tubes lie in a plane approximately 5μ posterior to them.

METHODS

(1) Cutting sections

Most completed egg-shells, including that of Rhodnius, are too brittle to give serial
sections by standard methods of procedure. Hence, for most of this study the
method of observing the cut edge of shells cut longitudinally while frozen has been
used (Beament, 1946a). However, for the elucidation of the formation and structure
of the rims, seal and micropylar structures, it was essential to obtain a suitable
technique. All stages of oocyte development and shell formation up to the time
when the exochorion layers are being secreted can be cut by normal methods
(e.g. wax and celloidin embedding after fixation). On the other hand, the exochorion
material hardens soon after secretion, especially with dehydration, and the shell
then fragments when sectioned.

It has been shown (Beament, 1946b) that although potash solutions attack and
dissolve the exochorion material, previous immersion in picric acid solution will
prevent this. However, though solution does not take place, the shell substance is
considerably softened by such action and remains so after drying. For cutting
sections of eggs with any exochorion, ovarioles were fixed in aqueous Bouin’s
solution for 3 days and then dissected in the fixative. Incomplete oocytes were
returned to the fixative, and those which had apparently reached full size were
punctured in the side with a fine needle to ensure the complete fixation of the egg
contents. After a further 3 days the complete oocytes were treated as follows:

The rear end of each egg was cut off about 0.5 mm. from the end, and mounted
in glycerine, whence the degree of formation of the exochorion could be distin-
guished. Eggs without exochorions, or with less than about 2μ of exochorion, were
embedded and sectioned in the normal way, while those with thicker chorions were
immersed in cold normal potash for about 10 min. and then washed in water and
embedded as before. The picric acid in the Bouin’s solution apparently ‘fixed’ all
the proteins in the follicle cells as well as in the shell layers so that these sections
appeared to be quite normal. They were, however, compared with similar (though
fragmented) sections which had not been treated with potash, in order to confirm
the histology of the follicle cells, and to ensure that the morphology of the minute
structures in the rims was unaltered.

(2) Staining in inorganic solvents

Much of the information on the structure of the seal has been obtained by
observing the action of solvents on the various components of this region and
comparing their properties with those already determined for components of the
unspecialized part of the shell. For this purpose it was desirable to have some part
of the material under observation coloured by staining, and to use this as a reference point. All usual stains are broken down by such solvents as concentrated potash and strong nitric acid, while the only stain which remains coloured in most solvents (picric acid) has already been shown to alter the properties of the layers considerably. For this particular purpose it was found that if half-shells were soaked in a Universal Indicator (B.D.H.) for 2 hr., and then dried in the air before placing in the solvent, some protein layers retained a marked colour in any of the solvents used, and this considerably facilitated the elucidation of the complex.

THE FORMATION AND DETAILED STRUCTURE OF THE SEAL

The shell can be considered as a series of units, each the product of a single follicle cell; the formation of the rims and seal will now be followed in terms of the activity of each specialized follicle cell in this region. It will be found that, in the completed shell, the units are more distinctly marked in this part of the shell than in any other.

Figs. 3–5 represent longitudinal sections through the cells of the follicle over the region where the seal is to be formed. These cells will be referred to by a series of letters (passing from the cap to the shell) as follows:

- **Z**: the cells of the central cap region.
- **A, B, C, D, E, F, H, K**: the specialized cells of the seal.
- **M1, M2, M3, M4**: the cells of the shell rim.
- **N1, N2, N3**: the cells of the neck.

Since the shell is radially symmetrical it must be remembered that each of these cells is representative of an annulus of similar cells around the oocyte at that particular level, and that secretions will take the form of annuli, produced, for example, by the ring of **B** cells.

Before secretion, the cells **A–K** do not differ in histology or morphology; they are apparently differentiated from the unspecialized cells during a series of movements associated with the production of a slight fold in the vitelline membrane at the site of the seal (Figs. 4, 5). These movements take place principally around the rings of cells **A**, and **K** and **M1**, which are displaced inwards towards the yolk, while the central cells (**E**) are raised slightly (Fig. 5). The movement of the **K** cells relative to their original position is of the order of 20\(\mu\) along the radius of the annulus, so that this ring of cells is compacted (see p. 228). At the same time, the cells **B, C, D, F, and H** lose all but fragmentary contact with the surface of the oocyte (Fig. 5).

It is during these movements that shell secretion starts, and it appears that this activity takes place only in those cells which are in contact with the surface of the oocyte. Cells **B, C, D, F, and H** are therefore dormant at this stage, but as the shell is secreted, the increase in surface area allows them to resume contact with their substrate and to secrete (see below).

Together with all the other cells of the follicle, the **A, E, K, and M** cells secrete the inner polyphenol layer and the protein of the resistant endochorion layer.
Micropylar complex in egg-shell of Rhodnius prolixus

(Fig. 5). This is followed by the outer polyphenol layer in the case of all cells but the $K$ series. The $K$ ring of cells will eventually form the pseudomicropyles. The process of pit formation (with the exception of pore canals) by the $K$ cells is similar to follicular pit formation in the unspecialized shell, but it starts in the depth of the resistant protein layer which is slightly thickened opposite the cells $K$ and $M$ (Figs. 5, 6). The blind ends of the pits formed by the $K$ cells are separated from the surface of the oocyte by a distance of about 0.5$\mu$, and at this point the substance of the resistant endochorion protein layer is permeated by minute polyphenol granules, contrasting strongly with the composition of this layer in all other parts of the shell. On the other hand, over the areas secreted by the $E$ and $A$ cells, the resistant endochorion is very thin and the two layers of polyphenol granules cannot be readily resolved.

In following the subsequent secretions of these specialized follicle cells, it can be seen that their displacement from 'normal' positions corresponds to gross disturbance of their activity, compared with the unspecialized cells. All other materials which form separate layers in the unspecialized shell, are secreted as such. However, in some places they are compounded to form new substances, and polyphenols are often produced at an unusual stage (Table I).

The $A$ and $E$ cells now secrete protein material similar to the cap cells, while the $K$ cells produce soft lipoprotein identical with the material of the soft exochorion (Figs. 6, 10). The $M$ cells, together with those of the neck, form a submicroscopic layer of amber material, and then lay down protein of the soft endochorion type; these cells have identical staining reactions with the generalized cells of the shell at this time. Very shortly after the production of the innermost secretions of the $A$ and $E$ cells, the $B$ cells start to secrete. Their product is also a protein material, but it differs from the substance secreted by the $A$ and $K$ cells, for it has a slightly greater affinity for water-soluble stains (probably indicating a less degree of tanning and associated processes). Small quantities of unfixed polyphenol material are also present (these $B$ cells did not contribute polyphenol to the resistant endochorion).

While the $A$, $B$ and $E$ cells are secreting protein, they accumulate numerous oily droplets in the region farther from the shell, very similar to the oily droplets found in the cap cells (Beament, 1946b). Towards the end of this secretory phase (Fig. 6), the oil disappears and the layer of protein already secreted is transformed to amber material. It is interesting to note that the material secreted by the $E$ cells (the sealing bar) constitutes the thinnest region of the whole shell. This is perhaps counterbalanced by its composition, for it consists almost entirely of the extremely resistant amber material which is thicker here than anywhere else in the shell. The only exception to lipidization in the sealing bar is the resistant endochorion.

The region formed by the $B$ cells up to this stage is less lipidized, and more soluble in chlorated nitric acid, than the surrounding amber material. The rate of secretion of these cells is not uniform, for while the cap cells have produced a membrane about 2$\mu$ thick, the $A$ cells have secreted about 4$\mu$, and the $E$ cells approximately 7$\mu$ of amber material. However, this extremely rapid rate of secretion
on the part of the $E$ cells completes their active life, for they cease secretion after the sealing bar is formed.

The predetermined line of weakness, or hatching line, is already present and is marked by the site of junction first of the cells $A$ and $E$ and later $B$ and $E$. It is,

Fig. 3. Diagrammatic representation of a longitudinal section through the follicular cells at the site of the seal and rims complex (each cell represents an annulus around the oocyte long axis). $Z$, cell forming the cap; $A$-$K$, cells forming the rims and seal; $M$, cells of the rim of the shell; the arrows indicate the determination movements.

Fig. 4. Diagramatic representation of follicle cells of the seal during determination movements. Note. Cells $B$, $C$, $D$ and $F$, $H$ have moved out of contact with the oocyte surface. The secretion of the chorion starts at approximately this point.

Fig. 5. Secretion of the seal region. Determination movements are now complete and the cells have secreted the initial layers of the shell. Cells $Z$, $A$, $E$, $M_{1-4}$ and $N_{1-4}$ (of the neck) secreting the outer polyphenol layer, cells $B$, $C$, $D$ and $F$, $H$ not active, and the $K$ cell (pseudomicropyle cell) is secreting the expanded granular resistant layer.

Fig. 6. Secretion of the seal region. The $B$ cell is now active, and together with the $A$ cell is secreting protein ($amb$. $pr.$) to be lipidized to amber material. The $E$ cell has already formed about half the thickness of the sealing bar, while the $M$ and $N$ cells are completing the reduced soft endochorion ($s$. $end.$). The $K$ cell has completed the expanded resistant endochorion leaving the inner end of the pseudomicropyle and is secreting lipoprotein. The paths of retreat of the inactive cells ($C$, $D$, $F$, $H$) are marked by the primary ($h$. $ln.$) and secondary ($sec$. $h$. $ln.$) hatching lines.
therefore, the path of retreat of the tips of the non-functional cells B, C and D and later C and D (Figs. 5, 6), whose presence appears to weaken the co-ordination between the secretions of the two active cells. Because the B cells become active shortly after the cells A and E, there is a small projecting wedge on the sealing bar which fits closely into a groove on the boundary of the material secreted by the A

Fig. 7. Secretion of the seal region. The endochorion layers (end.) are now complete, and almost all the cells are secreting the soft exochorion (s.exo.). Note the inner end of the long follicular pit of the A cell. The C and D cells have now started to secrete and are forming the overhanging rim of the cap (rm.c.). The E cell is completing the lipidisation of the sealing bar, while the M cells are forming the rim of the shell with its long follicular pits (rm.sh.). The K cell now has a very long villus in the cavity of the pseudomicropyle (psm.). The F and H cells are not yet active. amb. the amber layer.

Fig. 8. Secretion of the seal region. The exochorion layers are half completed. The F and H cells are the only ones not secreting, while the E cells, cut off by the outer ends of the D and F cells, are now dying. The rims are assuming their final form. psm. pseudomicropyle; tr.f.pt. transitory follicular pits.

cells. This is important in the mechanism of hatching (Fig. 10). It is also noteworthy that a secondary predetermined line of weakness is produced along the line of retreat of the other set of non-functional cells F and H (Fig. 6). Occasionally, after the front ends of incomplete shells had been boiled in chloroform, pressure on the cap from within resulted in splitting along this secondary line of weakness instead of the normal one. However, such a phenomenon was not found in any of the naturally vacated shells.
With the completion of the amber layer, the cells undergo a transition phase during which the cells $A$ and $B$ produce a minute amount of soft protein material similar to that present in the cap (Fig. 2 and see Beament, 1946b). It is again, perhaps, a vestigial form of the processes involved in the production of the un-specialized shell. The cells $A$, $B$ and $C$ and those of the rim and neck, $M$ and $N$, assume the characteristics of chorionin secretion (p. 214) and now produce soft lipoprotein (Fig. 7). The $C$ cells, using the thin layer of soft protein as a substrate, produce a long overhanging lip. At the same time, the $D$ cells, starting on the amber material secreted by the $B$ cells, produce a trace of soft protein, and then also secrete soft lipoprotein which fills the recess of the overhanging lip formed by the $C$ cells (Figs. 7, 8).

During this time, the $K$ cells continue to secrete soft exochorion material, leaving their long cytoplasmic processes which penetrate into the resistant layer of the endochorion. Similarly, the rim cells $M$ have left long processes in the soft lipoprotein (Fig. 8). It is about half-way through their phase of soft lipoprotein production that the unspecialized cells over the main shell change to the secretion of the exochorion (Beament, 1946b). The rates of secretion in all parts of the shell are apparently so co-ordinated that, although at any time during the secretion of the inner layers, different rings of cells may be secreting different materials, all regions complete their soft exochorion layer and arrive at the anteterminal phase at the same time. Immediately before this, the $M$ cells in the rim withdraw their villi and fill the cavities of their follicular pits with an extremely soft vacuolated lipoprotein material which has greater solubility in aqueous potash than any other part of the shell. The sites of these pits are marked in the completed shell by their large content of chloride (Fig. 2); they are the only parts of the shell to stain jet black after treatment with 5% silver nitrate solution in the presence of nitric acid, followed by exposure to light. This would indicate that cellular debris is left when the villi are withdrawn.

In the final stage (Fig. 9) the resistant exochorion is secreted over the whole shell with the exception of the outer surface of the sealing bar and a small region of the initial amber material secreted by the $B$ cells. The lack of resistant layer here is due to the fact that this surface is covered by the necrotic remains of the $E$ cells (Fig. 8), and therefore no secretion takes place. It is during this final phase that the long cytoplasmic villi are withdrawn from the pseudomicropyles, and the outer ends sealed, with the exception of a small bunch of pore canals (Figs. 2, 11) which give communication with the surface of the shell. The pseudomicropyles are not lined with resistant lipoprotein like the other follicular pits of the shell and cap, since the villi are withdrawn before the terminal phase of secretion. When placed in potash they dissolve away completely, instead of leaving the normal thin layer of resistant material. Only the outermost part of the cavity lies in the resistant layer formed by the $K$ cells, and after immersion in potash solution, this remains as a lamina containing small cavities at the sites of the pseudomicropyles.

It is during this final phase that the two remaining groups of cells ($F$ and $H$) pour out their secretions (Fig. 9). They produce resistant lipoprotein over the soft
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Fig. 9. Secretion of the seal region, showing the areas of the complex which are secreted by the various annuli of cells. This should be compared with Fig. 2 where the actual delineation of areas is shown. Note. While the cells in this figure represent the final appearance of the follicle cells, the shading of areas merely represents a cell and its respective secretion. It has no relation to the key used for Figs. 3–8.

Table 1

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<td>S</td>
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a, polyphenols; b, protein; c, soft lipoprotein; d, resistant lipoprotein; o, lipidization.

The secretions of cells in the specialized areas of the shell (and see Beament, 1946b), compared with the cycle of secretions from the unspecialized cells. The arrows indicate the points at which a cell starts to secrete, using the secretion of another cell as its substrate. Z, cap cells; A–H, specialized cells of the seal; K, pseudomicropyle cells; M, cells of the rim of the shell; N, cells of the neck; S, cells of the unspecialized shell.
lipoprotein units containing the pseudomicropyles, and so give a resistant layer to
the upper surface of the rim of the shell. The resistant layer which they secrete, and
that formed by the C cells, is extremely porous and heavily impregnated with
polyphenols (Fig. 2), so that when the completed egg is immersed in ammoniacal
silver nitrate, these are the only areas of the surface which stain with the typical
pink-brown coloration (see micropyles, p. 226). The pore canals formed by the F
and H cells are, of course, confined to the layer which they secrete, and do not
penetrate to the lumen of the pseudomicropyles; the canals are of the larger variety
found in thickened resistant exochorion in other parts of the shell (Beament, 1946b).

Variations in the pseudomicropyles

Not all the pseudomicropylar pits are open to within about 1μ of the surface of
the shell. In a few cases these pits end at points opposite the junction between the
regions secreted by the F and H cells. The remainder of the path which would
have been described by the normal follicular pit is filled in with soft lipoprotein
material containing polyphenols, and it is by staining in this way with ammoniacal
silver nitrate that the subsequent path of the follicular villi can be traced (Fig. 11).
The filling of these pits is similar to the closure of the pits secreted by the M cells
of the rim of the shell.
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The function of the pseudomicropyles

No obvious role can be allotted to the pseudomicropyles in connexion with fertilization; it is, however, important to consider them further, since they may possibly convey liquids and gases into close proximity with the embryo (Fig. 10).

EXPERIMENTAL

Whole waterproof eggs (Beament, 1946a, 1946c), removed from the ovaries and dried for several hours, were immersed in aqueous stains for 24 hr., and the rims were then dissected from the eggs and observed in liquid paraffin (to prevent the diffusion of any aqueous stain in the shell cavities). The pseudomicropyles were unstained. Other eggs of the same stage were immersed in a solution of Sudan III in light petroleum, and observation of their rims (mounted in glycerol) showed that the lumen of the pseudomicropyles was filled with stain after 24 hr. immersion. Similar results were obtained with suitably coloured benzene and xylene. It therefore appears that oily liquids can penetrate the shell and fill the pseudomicropyles, and it was important to discover whether the oil was penetrating through the lipophilic chorionin, or along the small group of pore canals which make connexion with the shell surface.

In order to clarify this point, eggs were immersed in stain in an evacuated vessel, by using the apparatus described by Beament (1946b, 1946c). In this way air

Fig. 11. Plan of arc of the rim of the shell, seen from above, showing the path of two micropyles. F and H, very porous lipoprotein secreted by the F and H cells respectively; i.hy.m. inner part of micropyle lined with hydrophylic material; i.o.m. inner opening of the micropyle; o.li.m. outer part of micropyle lined with lipophylic material; o.o.m. outer opening of micropyle; p.can.psm. pore canals leading to pseudomicropyle; psm. normal pseudomicropyle; sh.psm. short variety of pseudomicropyle; sl.b. sealing bar; sp.g. spermatic groove; ves.f.pt. 'vestigial' follicular pits, possibly formed by the F and H cells.
spaces in the egg were emptied, and the readmittance of air into the vessel might force liquid through the pores into the pseudomicropyles. Under these conditions the cavities of the pseudomicropyles were filled with both aqueous and oily stains almost immediately.

CONCLUSION

It appears from these experiments that, after waterproofed eggs have been removed from the ovaries and desiccated sufficiently to remove water from the shell, there is free passage for air from the surface of the shell along the pore canals. These pore canals are, presumably, lined with chorionin and will therefore be tiny hydrophuge capillaries, so that aqueous material will not pass along them at all easily. On the other hand, their surface will be wet readily by oily material, and this will traverse the pores, providing the air in the pseudemicropylar cavity can be displaced. When eggs are merely immersed in oils, the air must either be dissolved in the oil or taken into the egg cavity, possibly by respiration; it is a slow process, however, and, from the above experiments, one which does not take place in water. Since air is more soluble in oil than in water, the air in the lumen may be dissolved by the oil as it enters, but neither respiration nor solution seems to produce sufficient drop in pressure to suck water along the lipophilic capillaries, and the entry of oil must depend primarily on its ability to wet the surfaces of the pore canals.

It is, however, apparent that the pseudomicropyles could, and probably do, act as respiratory structures and are the points in the shell where air can penetrate most readily, but it is doubtful if they could supply the whole oxygen needs of the egg.

The true micropyles

It has been stated above (p. 217) that the true micropyles lie slightly posterior to the plane of the pseudomicropyles for most of their length (Fig. 13). Each is the product of a single follicle cell, and the micropylar tube appears to be a modified follicular pit corresponding to a specialized cell at that particular site in the follicle. It may be noted at this stage that the position of these micropyle-forming cells does not correspond to either the $K$ or $M_1$ ring. The cells would, therefore, appear to be isolated units occupying an intermediate position as opposed to being units of a uniform annulus of cells. The activity of these cells during secretion differs from the normal $K$ cells in the following details:

A 'follicular pit' is present from the initial stage in shell secretion, so that the innermost polyphenol and resistant protein layers are perforated by a funnel-shaped opening about $1\mu$ in diameter. The granular resistant endochorion is formed as in the case of the pseudomicropyles, and the cells then produce soft lipoprotein in every way similar to the normal $K$ cells, so that the lumen of the micropyle at this stage is similar in size to that of the pseudomicropyles. However, as the long follicular villus is withdrawn, it lines the innermost half of the tube with a proteinaceous material containing polyphenol granules. This material stains moderately with ammoniacal silver nitrate solutions, and slightly with water-soluble stains and protein indicators. The lumen of the inner portion of the micropyle is thus reduced to a tube of approximately $0.5\mu$ in diameter (Figs. 11–13). The outer region of the tube
Micropylar complex in egg-shell of Rhodnius prolixus

Fig. 12. Longitudinal section through the inner portion of the seal region, passing through a true micropyle. gr.pr.m. granular protein lining of the micropyle; i.o.m. inner opening of the micropyle; lu.m. lumen of the micropyle.

Fig. 13. The rim of the shell, with the cut edge passing through a true micropyle. h.ln. hatching line; i.o.m. inner opening of the micropyle; m. lumen of the micropyle; o.o.m. outer opening of the micropyle; psm. outer ends of the pseudomicropyles; pth.psm. path of a pseudomicropyle; sl.b. sealing bar; sp.g. spermatic groove.
is lined with resistant exochorion lipoprotein, secreted at the same time as this product over the rest of the shell, but the formation of this compound does not result in the constriction of the outer half of the tube. The completed pit is not sealed, but opens on to the spermatic groove by a funnel-shaped orifice at a level approximately 5µ below the ring of pseudomicropyles. These tubes are the only structures which allow free access between the surface of the oocyte and the external environment.

![Diagram 14](image1.png)

**Fig. 14.** Diagrammatic impression of the movements in the follicle cells around the rims, which may determine the micropyle forming cells. *a,* the $K$ (pseudomicropyles forming cells) and $M_1$ (rim forming cells), in two rows around the oocyte, before determination movements or secretion; *b,* the compression on the $K$ ring during the folding movements have displaced one cell (*x.*) into an intermediate position between the $K$ and $M_1$ cells; this is the micropyle-forming cell.

![Diagram 15](image2.png)

**Fig. 15.** The distribution of the micropyles around the rim of the shell, showing the irregular pattern adopted. *a* and *b,* normal distribution of micropyles; *c,* a typical distribution of micropyles in sterile egg laid by an aged female, showing the small number of micropyles found only in one side of the rim.

### The determination of micropyle-forming cells

During the formation of the initial fold in the oocyte membrane (p. 218) and the secretion of the inner polyphenol layer, the $K$ ring of cells is apparently suppressed radially through a relative distance of about 20µ. There are approximately 200 cells in this ring which describes a circle of about 250µ in radius. Supposing no shrinkage takes place in the $K$ cells, there will be room for 20–250 fewer cells in the smaller ring. In other words, approximately sixteen cells will be displaced from the $K$ ring. If this displacement took place towards the posterior end of the shell, the displaced cells would be in the positions which are occupied by the micropyles. A diagrammatic impression of such movements is given in Fig. 14. Micropyle-forming cells, of course, might be so derived from the slight compression of the $M_1$ ring, or they may be present in the micropylar position from the time when the follicle cells are first arranged in a single layer around the oocyte, but evidence can be advanced for the above theory of determination as follows:
(1) The shell units containing the micropyles are morphologically more akin to the pseudomicropylar units than they are to any other segments of the shell.

(2) The inner ends of the micropyles open on to the shell surface at almost the same level as the inner ends of the pseudomicropyles, suggesting that the micropyle cells are only slightly displaced from the plane of the K ring at this stage in shell secretion.

(3) The micropylar tubes, in contrast to the pseudomicropyles, do not follow a straight path through the shell. The tube is straight in its outer portion, but where it passes into the endochorion, its path may be quite irregular. It may thus show all variations between a slightly undulating course, and a path which bends sharply in the endochorion, passing beneath two pseudomicropyles, and often returning before resuming a course radial to the shell. Such a path would be readily explained if the micropylar cells were being squeezed into its final position during the secretion of this part of the tube.

<table>
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<tr>
<th>First egg batch</th>
<th>After 4 weeks egg-laying</th>
<th>Sterile eggs from old females</th>
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<td>Av. 15.8</td>
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(4) The number of micropyles is not constant: it varies between six and twenty in the shells examined, and Table 2 shows that the average number per shell is fifteen—a remarkably good confirmation of the above calculation. The proposed mechanism for the determination of micropyles explains this variation in numbers, which would depend on the amount of shrinkage in the K ring. It also provides a reason for the irregular distribution of these tubes; there would be no reason for cells to be displaced at regular intervals (see Fig. 15), or determined by some inherent plan of radial or bilateral symmetry (which appears to be the manner in which most biological structures are determined).

(5) The eggs laid by young females contain, on the average, more micropyles than those produced by females after several weeks of egg production (Table 2). If the displacement of the micropylar cells depends on the general activity of the follicle cells, then the cells of older females must be less 'virile'. It has been found (Beament, 1946d) that about $\frac{1}{2}$% of the eggs from the cultures were sterile, though
produced by fertilized females. It was found that these were the product of aged females, and an examination of the rims showed that the average number of micropyles present in these sterile eggs was four (Table 2 and Fig. 15c), though the pseudomicropyles and all other parts of the shell appeared to be normal. Where greater numbers of micropyles were present they appeared to be crowded into half or two-thirds of the circumference of the rim. These sterile eggs show an extreme case of the decrease in micropylar numbers with the ageing of the female. During the passage of these eggs through the lower female genital ducts, one side of the spermatic groove will be presented to the opening of the spermathecae, and will receive a small quantity of spermatozoa to fertilize the egg. Where there are few micropyles, or where they are crowded into one side of the rim, the sperm may not be able to migrate round the groove sufficiently to find and enter a micropyle. It is therefore possible that these eggs from old females may be sterile on account of the lack of micropyles.

DISCUSSION

A consideration of the materials secreted by the specialized follicle cells of the seal region (and see Table 1 and Fig. 9; compare Figs. 2 and 10) shows that all the cells secrete modifications of a proteinaceous substance, and that the formation of the complex does not involve the production of any compound other than those secreted by the cells forming the unspecialized portions of the shell (Beament, 1946b). Moreover, the order of secretion appears to be almost the same as that for the main shell and cap, though phases of the secretory cycle may be omitted, and with the exception that polyphenols may appear at any time in the cycle of secretion of a specialized cell. Where the specialized cells do not start to secrete until after adjacent cells have produced some portion of the shell, there is some correlation between the material with which they commence secretion, and the substrate on which they are placed at that time.

Thus, while the secretory cycle of the $A$ cells follows the normal pattern, the $B$ cells do not produce polyphenol or resistant protein layers; the substrate on which they start secretion is protein (subsequently lipidized by the $A$ and $E$ cells), and they start secretion with protein which is lipidized, and the normal cycle is then completed. The substrate on to which the $C$ cells start secretion is the very thin layer of soft endochorion protein by the $B$ cells. Their initial product is soft lipoprotein—the next phase in the normal cycle. The $C$ cells produce polyphenol in the pore canals of their last product, the resistant exochorion; this is an example of a product being produced out of the normal position. The $D$ cells start secretion on a substrate of amber material ($B$) and produce first protein and then the normal exochorion layers.

The $E$ cells are normal in their activity up to the lipidization of their amber layer. But with the completion of the sealing bar, secretion ceases, possibly due to the fact that they lose contact with the tunica propria covering the ovariole, and are cut off by the outer ends of the $D$ and $F$ cells. The secretions of the $F$ and $H$ cells consist solely of resistant lipoprotein containing polyphenol material, and the
substrate for this secretion is soft lipoprotein, so that they form merely the last phase of the sequence.

The chain of secretions from the $K$ cells represents a special point of interest. The initial polyphenol and protein layers are normal, but the subsequent phases are lacking and the cells pass straight to the secretion of soft lipoprotein. In doing so, the substrate on to which the soft lipoprotein is deposited is a protein layer as in all other regions of the shell, and in omitting three of the secretory phases, the attachment of lipoprotein to protein has been retained. The remainder of the sequence is normal.

Apart from the apparent correlation between the phase of secretion and the substrate at that time, the rigid cycle of secretions may be adhered to for purposes of obtaining a complete adhesion between the respective layers of the shell, and so the shell retains strength with a degree of flexibility.

The formation of any form of follicular pit appears to be correlated with the existence of an initial depression at the point in the shell where it will eventually be formed, and, also, with the secretion of the exochorion layers by the follicle cells. It has already been pointed out by Beament (1946b) that this relationship exists in the less specialized portions of the chorion. In the main part of the shell, undulation of the endochorion surface produces regular depressions at the bases of the follicular pits, but the folds which precede the secretion of the rims of the shell and cap are themselves depressions in the substrate on to which secretion takes place. On the other hand, these folds also produce convexities which appear to have a retarding effect on the production of pits. It was found (Beament, 1946b) that pit formation was also slightly retarded over the convexity of the rear end of the shell.

It is therefore interesting to note that the deepest pit in the cap, that formed by the $A$ cells, is formed in the anterior depression of the initial fold, and that it lies entirely within the exochorion, which is very thick at this point. However, the $B$ cells, secreting over the convex portion of the fold, produce a relatively short pit, while the $C$, $D$, $F$, and $H$ cells do not form any kind of pit. In the other depression, opposite the $K$ cells, the micropyles and pseudomicropyles are formed. While both these types of 'pit' start in the endochorion layers, and so form exceptions to the correlation mentioned above, it may also be pointed out that in the shell units which contain these specialized cavities, three of the endochorion layers are missing, and so the formation of the pits may be aided by the transition of the cells to exochorion formation at a very early stage in shell secretion.

**SUMMARY**

An investigation has been made of the junction between the shell and cap in the egg-shell of *Rhodnius prolixus*. This complex region consists of the thickened rim of the cap connected by a thin sealing bar to the rim of the shell. The secretion of this part of the shell has been followed and compared with the formation of less specialized portions of the shell.

The shell has been divided into units, each the product of an individual follicle cell. It has been found that all the seven layers which make up the unspecialized
parts of the shell are present in the seal complex; that these consist of five endochorion layers and two exochorion layers in their normal order.

The exochorion is secreted around long villi, one from each follicle cell. These give rise to follicular pits in the shell.

In this complex region, cells start to secrete at various stages in the seven-phase cycle; their initial secretion is apparently related to the material with which they make contact at that time. After secretion has started, each cell completes the remainder of the cycle.

The rim of the cap is the product of four rings of follicle cells; the additional thickness is achieved by an increase in the exochorion layers, secreted around a series of very long follicular pits.

The sealing bar, which is produced by one ring of follicle cells, is composed of the inner four layers of the chorion only; the cells do not produce soft endochorion, or exochorion layers.

At the cap end of the sealing bar there is the predetermined hatching line. It is apparently produced by the presence in the follicle of cells which are inactive during the secretion of the inner layers, and so prevent co-ordination between the active cells on either side. A weak point is also present at the base of the sealing bar, at the site of other inactive cells, though this fissure is not used at hatching.

The rim of the shell is similarly produced by an expansion of the exochorion layers secreted around four rings of follicular villi. Of these, three rings of pits are filled in towards the end of secretion, but the fourth, lying on the upper portion of the rim, remains. These pits become the micropyles and associated structures.

There are 200 pits in the completed rim, divided into two groups. About fifteen are micropyles; the remainder are cavities closed at each end, and to which the name 'pseudomicropyle' has been given.

The pseudomicropyles are formed in a similar way to normal follicular pits, but start in the resistant protein layer, 0.5 µm from the inside of the shell. They end in the resistant exochorion, where they are connected to the external surface by small bunches of pore canals. They probably play some part in the respiration of the embryo.

The true micropyles form the only free path through the shell. The inner portion of each tube is lined with hydrophilic protein, and the outer portion, which lies slightly posterior to the pseudomicropyles, is composed of hydrophobic lipoprotein.

The number of true micropyles is not constant, there being between ten and twenty scattered irregularly around the rim. However, eggs produced by older females contain fewer micropyles; this may account for a higher rate of sterility among these eggs.

The cells which form the micropyles and pseudomicropyles are the only ones which do not adhere to the typical cycle of seven secretory products. But in omitting three phases, the attachment of the exochorion to a protein layer is retained.

Evidence suggests that the cells forming the micropyles are determined in the earliest stages of secretion by being squeezed out of the pseudomicropylar ring of cells.
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