THE STRUCTURE OF THE INSECT EGG-SHELL IN RELATION TO THE RESPIRATION OF THE EMBRYO

By P. H. TUFT, Department of Zoology, University of Cambridge

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(With Two Text-figures)

Many insect eggs are able to avoid mechanical injury and desiccation because they are protected by an egg-shell or chorion which is tough, rigid and relatively water-proof. These properties tend to make it impermeable to gases, and since the egg contains a living embryo, there must be provision for the diffusion of an adequate volume of oxygen into the egg. The way in which this is achieved has never been satisfactorily explained. Specialized respiratory structures have been described in many species of insect egg, especially in those of parasites (Maple, 1937), and a respiratory function has sometimes been ascribed to the system of micropylar canals that penetrate the shell in hard-shell eggs, but until now there has been no direct evidence for the respiratory function of these structures. Indeed it seems unlikely, at first sight, that sufficient oxygen could diffuse through such small areas of the shell to satisfy the needs of the embryo, although in some species there can be little doubt that oxygen uptake is restricted to a small part of the shell; in certain species of capsid, for instance, the eggs are embedded in the tissues of a plant with only the caps exposed to the air.

What follows is an account of an investigation of this problem in the eggs of the reduviid bug, *Rhodnius prolixus* (Stahl). Beament (1947) has shown that the egg-shell in this species is composed of several layers, and its waterproofing properties are due to a thin continuous layer of wax on its inside surface. He has also shown that at the cap end of the egg the shell is penetrated by a series of micropyles and pseudomicropyles. I have been able to show that most of the oxygen consumed by the egg enters through the micropyles and pseudomicropyles, and that the relative impermeability of the rest of the shell is due, not to the wax layer, but to the components that give the shell its mechanical strength. From the theoretical point of view it can be shown that sufficient oxygen can only enter the egg through these pores if there is a continuous gas phase under the shell. A gas space can be demonstrated in the *Rhodnius* egg, and its minimum dimensions can be calculated.

EXPERIMENTS TO DETERMINE THE SITE OF OXYGEN UPTAKE

The oxygen consumption of groups of eggs was measured before and after selected areas of the shells had been covered with wax. To ensure that the effects were due to the smothering action and not to the toxic properties of the wax, control experiments were carried out using shellac, vaseline and gelatine. Eggs of different ages were studied, Beament having shown that the composition of the chorion changes during development.
Experimental procedure. In each experiment three groups of eggs of the same age (maximum variation 24 hr.) incubated at 25° C. were used, 70 % R.H. The number of eggs in a group varied in different experiments and is recorded in the table of results (Table 1).

Table 1. Results of smothering experiments

<table>
<thead>
<tr>
<th>No. of exp.</th>
<th>Age of eggs (days)</th>
<th>No. of eggs</th>
<th>mm.3O2/egg/hr.</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Un-treated</td>
<td>Treated</td>
</tr>
<tr>
<td>78 i</td>
<td>3-4</td>
<td>6</td>
<td>0.082</td>
<td>0.018</td>
</tr>
<tr>
<td>ii</td>
<td></td>
<td>4</td>
<td>0.092</td>
<td>0.020</td>
</tr>
<tr>
<td>iii</td>
<td></td>
<td>6</td>
<td>0.080</td>
<td>0.088</td>
</tr>
<tr>
<td>79</td>
<td>6</td>
<td>6</td>
<td>0.088</td>
<td>0.020</td>
</tr>
<tr>
<td>81 i</td>
<td>7</td>
<td>3</td>
<td>0.101</td>
<td>0.011</td>
</tr>
<tr>
<td>ii</td>
<td></td>
<td>3</td>
<td>0.106</td>
<td>0.014</td>
</tr>
<tr>
<td>iii</td>
<td></td>
<td>3</td>
<td>0.107</td>
<td>0.021</td>
</tr>
<tr>
<td>97 i</td>
<td>10</td>
<td>3</td>
<td>0.181</td>
<td>0.028</td>
</tr>
<tr>
<td>ii</td>
<td></td>
<td>3</td>
<td>0.152</td>
<td>0.020</td>
</tr>
<tr>
<td>iii</td>
<td></td>
<td>3</td>
<td>0.145</td>
<td>0.025</td>
</tr>
<tr>
<td>83 i</td>
<td>11</td>
<td>4</td>
<td>0.150</td>
<td>0.020</td>
</tr>
<tr>
<td>ii</td>
<td></td>
<td>4</td>
<td>0.136</td>
<td>0.018</td>
</tr>
<tr>
<td>iii</td>
<td></td>
<td>4</td>
<td>0.117</td>
<td>0.021</td>
</tr>
<tr>
<td>84 i</td>
<td>12</td>
<td>4</td>
<td>0.134</td>
<td>0.115</td>
</tr>
<tr>
<td>ii</td>
<td></td>
<td>4</td>
<td>0.164</td>
<td>0.158</td>
</tr>
<tr>
<td>iii</td>
<td></td>
<td>4</td>
<td>0.131</td>
<td>0.122</td>
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<td></td>
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</tbody>
</table>

The oxygen consumption of the eggs was measured at 25° C. by means of a new type of microrespirometer which is to be described elsewhere, capable of measuring the rate to the nearest 0.001 mm.³. The rate was recorded over a period of 1 hr., then the eggs were removed from the respirometers and a selected area of the shell covered with wax (m.p. 30° C.) by means of an electrically heated microcautery. The eggs were gripped in a pair of fine forceps controlled by a screw clamp. A drop of wax, held on the wire loop of the cautery, was brought up to the egg and heated until it just melted. It was then touched against the egg and quickly withdrawn. The wax adhering to the egg solidified immediately. To ensure that the seal was complete the edges of the wax drop were carefully remelted and again allowed to solidify.

After treatment, the groups of eggs were returned to the same respirometers from which they had been removed, and the oxygen consumption measurement was repeated.

In the control experiments the vaseline was placed on the egg in the same way as the wax, the shellac was put on with a camel hair brush and the gelatine covers by holding the eggs in gelatine solution until it solidified, and then cutting out the block containing the egg with a sharp scalpel.

Results. The experimental results are shown in Table 1. It will be seen that whatever compound was used for covering the egg, the results were essentially the same. The low melting-point wax was, however, the more convenient material and was used in most of the experiments.
Structure of insect egg-shell in relation to respiration of embryo

Blocking the cap end of the egg reduces the oxygen consumption to a value of about 0.02 mm.³/egg/hr., irrespective of the initial rate or age of the eggs, whereas if the posterior end was covered the rate of oxygen consumption was not affected (Exp. 84). There was an exception, however; young eggs, less than 5 days old, show a reduction in rate if the posterior end of the egg is covered with wax (Exp. 79). These young eggs are delicate and the reduction in the respiratory rate is probably the result of injuring the egg and not to the suffocating effect of the wax. It was found that this treatment caused the death of the 3-day-old eggs, but waxing the posterior end of the older eggs had no effect on hatching. The remarks which follow refer to eggs which are more than 6 days old.

It was clear from these results that although oxygen is absorbed through the whole surface of the shell, most of it enters through the cap end. The value obtained for the rate at which the gas is absorbed by eggs in which the cap has been covered by wax probably represents the limiting rate of oxygen diffusion through the egg membrane at the partial pressure of oxygen in the atmosphere.

It is interesting that this value does not change appreciably as the egg gets older. The additional wax which, according to Beament (1946), is laid down during development does not seem to affect the permeability to oxygen.

Table 2. Results of smothering experiments, in which different parts of the cap were covered with wax

<table>
<thead>
<tr>
<th>No. of exp</th>
<th>Age of eggs (days)</th>
<th>No. of eggs</th>
<th>mm.³O₂/egg/hr.</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>115</td>
<td>11</td>
<td>3</td>
<td>0.149</td>
<td>Centre of cap covered, rim partially covered</td>
</tr>
<tr>
<td>116</td>
<td>12</td>
<td>3</td>
<td>0.145</td>
<td>Centre of cap covered, rim clear</td>
</tr>
<tr>
<td>117</td>
<td>12</td>
<td>3</td>
<td>0.154</td>
<td>Centre of cap covered, rim clear</td>
</tr>
</tbody>
</table>

In the foregoing experiments the wax cover enveloped the whole of the anterior part of the egg. A further set of experiments was therefore carried out, in which the effect of covering the centre of the cap was compared with the effect of covering the rim (Exps. nos. 115, 116, 117, Table 2). The results of these experiments show clearly that it is in the region of the rim that oxygen passes into the egg.

Beament has shown that round the rim of the cap there are two series of small canals passing through the egg-shell; the pseudomicropyles and the true micropyles. The position of these structures is shown in Figs. 1 and 2.

It will be seen that the pseudomicropyles are closed at both ends except for minute pores at the outer end which penetrate the exochorion. At the inner end, the pseudomicropyles do not penetrate the inner wax layer, but end a short distance from it. There are some 200 of these structures and the number is approximately constant in eggs of all ages (Beament, 1947).

The true micropyles are much larger and they are open at the outside end, but like the pseudomicropyles they do not penetrate the inner wax layer. They are few in number, averaging about 13 per egg, and, moreover, the number may be considerably reduced in eggs laid by older females. The oxygen consumption of eggs
Fig. 1. The egg of *Rhodnius prolixus*.

Fig. 2. A diagram of a longitudinal section through the part of the shell indicated by the circle in Fig. 1 (after Beament).
laid by old females does not differ appreciably from that of eggs laid by younger ones. This suggests that it is the pseudomicropyles that are the main site of gaseous diffusion into the egg.

THE DIFFUSION OF OXYGEN INTO THE EGG

It is difficult to see how an adequate supply of oxygen can reach the embryo through such a small area of the egg surface. No precise analysis of this problem is possible because the shape of the egg and the canals are such that the mathematical treatment would be too complicated. But it is possible to consider a simplified system, to which Hills's equations (Hill, 1928) for the penetration of oxygen into respiring tissues can be applied, which gives some idea of the problem and its solution.

Consider a sheet of tissue consuming oxygen at the same rate per unit volume as the *Rhodnius* egg; then assuming a concentration of oxygen at the free surface equal to that in air, and assuming a diffusion coefficient for the tissues it is possible to calculate the depth at which the oxygen concentration is zero. Thus

$$b' = \sqrt{\frac{2ky_0}{a}},$$

where \( k \) = diffusion coefficient, \( y_0 \) = partial pressure of oxygen at surface, \( a \) = rate of oxygen consumption in c.c. of \( O_2 \)/min.

Let \( k = 1.48 \times 10^{-5} \) (i.e. \( k \) for muscle at 20° C.), \( y_0 = 0.21 \) atm., \( a = 3.47 \times 10^{-3} \) (rate on sixth day); then \( b' = 0.042 \) cm.

Similarly, if the diffusion coefficient for water is substituted for that of muscle, the value of \( b' \) is 0.064 cm.

The egg is 0.17 cm. long and so there would be no oxygen available more than half way down the egg even if the cap were removed and the end of the yolk exposed to the air.

This treatment assumes that the whole of the egg contents consume oxygen uniformly throughout. If we assume that the yolk does not consume oxygen, which is unlikely, and that the embryo is consuming oxygen at \( 1 \times 10^{-4} \) c.c./hr. at a point 0.1 cm. from the cap, that is about half-way down the egg, then we can calculate the apparent permeability coefficient \((P)\) of oxygen through the yolk. Thus

$$\frac{dm}{dt} = P_{yolk} A \Delta p \frac{\Delta x}{\Delta t},$$

area of cap = \( \pi \times 9 \times 10^{-4} \) cm.²,

\( \Delta p_{O_2} = 0.21 \) atm.,

\( \frac{dm}{dt} = \frac{1 \times 10^{-4}}{3600} = 2.78 \times 10^{-8}, \)

\( \Delta x = 0.1 \) cm.,

therefore

$$P_{yolk} = \frac{dm}{dt} \frac{\Delta x}{\Delta p A} = 2.78 \times 10^{-8} \times 0.1 \times 0.21 \times \pi \times 9 \times 10^{-4} \times 4.6 \times 10^{-6}.$$
Thus the permeability coefficient of the yolk would appear to be some ten times greater than that for water $P_{\text{water}} = 5.8 \times 10^{-7}$.

There is no data for the permeability of oily solutions to $O_2$, and in view of the greater solubility of $O_2$ in oils it might be thought that, as the yolk contains oil, a greater permeability coefficient would be expected. But it must be remembered that the case considered here is that of an egg with the cap removed and the surface of the yolk exposed to the air, whereas in the normal egg the air enters through a very small pore round the cap.

If, however, we assume that the yolk is exposed to air on all sides, Hills's equation for the limiting thickness of a cylinder of respiring tissue can be applied (Hill, 1928, equation 25). Substituting the same constants as before in this equation:

$$T_0 = \frac{4k_0}{\sqrt{a}} = \frac{4 \times 1.47 \times 10^{-5} \times 0.21}{3.4 \times 10^{-3}} = 0.060 \text{ cm.}$$

The radius of the Rhodnius egg is $0.03 \text{ cm.}$, which is well within the limit for the complete permeation of a cylinder of tissue respiring at this rate in air.

At first all attempts to demonstrate a layer of air inside the shell were unsuccessful. Small bubbles of air could sometimes be seen when the eggs were squashed under water, but it was not easy to see where they came from. Eggs were also placed in a container of boiled water and subjected to a low pressure, when it was found that bubbles, large enough to make the eggs rise in the water, appeared round the rim of the cap. These bubbles might have been due to air trapped in the groove round the rim, although the eggs had previously been wetted with alcohol. These experiments showed that even if the air was present under the shell it was in very small amounts.

An air space under the shell was eventually demonstrated by illuminating an egg with strong transmitted light under a binocular microscope, and observing the changes in the appearance of the shell when drops of odourless kerosene were placed, first on the cap rim and then on the shell. In both cases the spreading of the oil can easily be seen because it decreases the light scattered by the shell. When the oil is placed on the outside it spreads unevenly in all directions and evaporates, but when it is placed on the rim of the cap it immediately encircles the egg and the smooth boundary of the oil can be seen moving towards the tail end of the egg. It is clear that in this case the oil is passing down under the shell, because it does not evaporate, and the two boundaries can be seen to pass each other if drops are placed simultaneously on the cap rim and on the surface of the shell. It only takes 4 sec. at room temperature for the oil to reach a point half-way along the egg so that there is little doubt that it is moving through a gas-filled space between the shell and the serosa. An attempt is now being made to determine the dimension of this space, which is probably very small. A space only $1 \times 10^{-5} \text{ cm.}$ width, however,
Structure of insect egg-shell in relation to respiration of embryo

Is sufficient to provide a diffusion path wide enough to ensure an adequate supply of O₂ to the whole of the egg surface.

There must be some means of preventing this gas space from collapsing, therefore it will probably be found to consist of a labyrinth of small interconnected pores, rather than a continuous space. Furthermore, if these pores occur in, or are lined with, the waxy substance that Beament has found under the chorion, then they would be able to prevent the invasion of the gas space by water. This is an important factor because the life of the embryo depends on the gas space being uninterrupted.

OXYGEN CONSUMPTION AND WATER LOSS IN THE RHODNIUS EGG

Having described the relationship between the structure of the egg-shell and the respiration of the egg, it is now possible to consider the problem of how the waterproofing properties of the shell are reconciled with the need for an adequate supply of oxygen.

Beament has shown that the waterproofing properties of the Rhodnius egg are due to a thin layer of wax laid down on the inside of the shell (Beament, 1946). But although this enables the egg to develop in atmospheres of low relative humidity, it does not prevent some loss of water. He reports that at 0 % R.H. the egg loses 0.09 mg. water per cm.² of surface per hr.; this represents the passage of some 3 mm.³ of water vapour per hr. through the egg-shell. It will be recalled that the maximum rate at which oxygen will diffuse through the general surface of the egg at atmospheric pressure is about 0.02 mm.³/hr. This relatively low permeability to oxygen cannot, however, be due to the wax layer since this is continuous over the whole of the shell and is not penetrated by the micropylar canals. The wax must in fact be very permeable to oxygen since it has been shown experimentally that more than 0.2 mm.³/hr. can pass into the egg through a very small area of wax at the ends of the pseudomicropyles. Therefore the impermeability to oxygen must be due to other components of the shell, and it would seem that an adequate waterproofing mechanism is in no way incompatible with the absorption of a sufficient supply of oxygen, because the Rhodnius egg can survive the loss of several mm.³ of water vapour per hr. but absorbs not more than 0.3 mm.³ of oxygen per hr. It is probably the component of the shell that gives strength rather than its waterproofing properties that restricts the diffusion of oxygen. The two are, however, not completely unrelated, for as Beament points out, the waterproofing wax has to have a high melting-point if it is to be efficient at high temperatures; such wax layers are probably brittle, and require a mechanical support (Beament, 1946). Thus the thick rigid egg-shell of the Rhodnius egg fulfils a very important function by providing a firm support for the wax layer, and as the sites at which oxygen is able to enter the egg are restricted to small pores in the egg-shell, only small areas of the wax layer are unsupported.

The Rhodnius egg thus maintains a cleidoic habit in conditions of high temperatures and low humidities, by reason of its tough rigid shell which supports a waterproof layer of high melting-point wax on its inner surface. The mechanical component of the shell restricts the uptake of oxygen through the general surface, but
oxygen is able to reach the embryo through a series of small pores in the rim of the cap—the micropyles and pseudomicropyles. These pores communicate with a gas space under the chorion which ensures that all parts of the egg can obtain an adequate supply of oxygen.

SUMMARY

1. The site of gaseous exchange in the eggs of *Rhodnius prolixus* (Stahl) is shown to be the rim of the cap which covers the anterior end of the egg. Most of the oxygen consumed by the embryo enters the egg through the micropyles and pseudomicropyles which penetrate the shell in this region.

2. The physical conditions necessary for the passage of sufficient oxygen through these pores is discussed. A continuous gas space under the shell—the presence of which can be deduced on theoretical grounds—is shown to exist in the *Rhodnius* egg.

3. The relationship between waterproofing and the permeability of the shell to oxygen is discussed.

My thanks are due to members of the Zoology Department who have helped in various ways during the course of this work and especially to Dr Thorpe and Dr Crisp for helpful criticism and suggestions, to Dr Townsend of the Cavendish Laboratory for help with the physical problems involved in interpreting the results.

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REFERENCES