J. Exp. Biol. (1971), **55**, 165–176
With 1 plate and 5 text-figures
Printed in Great Britain

THE RESPIRATORY SYSTEM OF THE EGG-SHELL OF *HOMOROCORYPHUS NITIDULUS VICINUS* (ORTHOPTERA, TETTIGONIIDAE)

By J. C. HARTLEY
Department of Zoology, University of Nottingham

(*Received* 31 *December* 1970)

INTRODUCTION

Much has been written in recent years on the structures of insect egg-shells and this is the subject of a review by Hinton (1969). Most of the structures described are considered to have a respiratory function and many are termed plastrons. However, there has been relatively little experimental work on the precise way in which some of these structures function. Accurate oxygen-monitoring equipment and the resolution afforded by the scanning electron microscope have made it possible to analyse these systems in greater detail than hitherto.

In this paper the respiratory system of a tettigoniid egg is examined. The structures of the egg-shells of some tettigoniids were the subject of a previous paper (Hartley, 1964), but because of difficulties with diapause at that time it was not possible to attribute functions to their types of shell structures with complete certainty. Laboratory rearing of *Homorocoryphus nitidulus vicinus* Walker (Hartley, 1966) has made it possible to study the developmental biology of the egg stage of one species. It is particularly suitable for this study as the egg-shell has not an obviously straightforward plastron system as found in the eggs of some Diptera (Hinton, 1960 *a*, *b*). Nevertheless, the experimental work and discussion in this account are applicable to most egg respiratory systems.

MATERIAL

Eggs were collected daily from the cultures of *Homorocoryphus*. They were washed and placed on damp cotton wool in Petri dishes. These in turn were kept on open shelves in the same insectary as the adults and were thus subject to the same lighting rhythm for the whole of the incubation period.

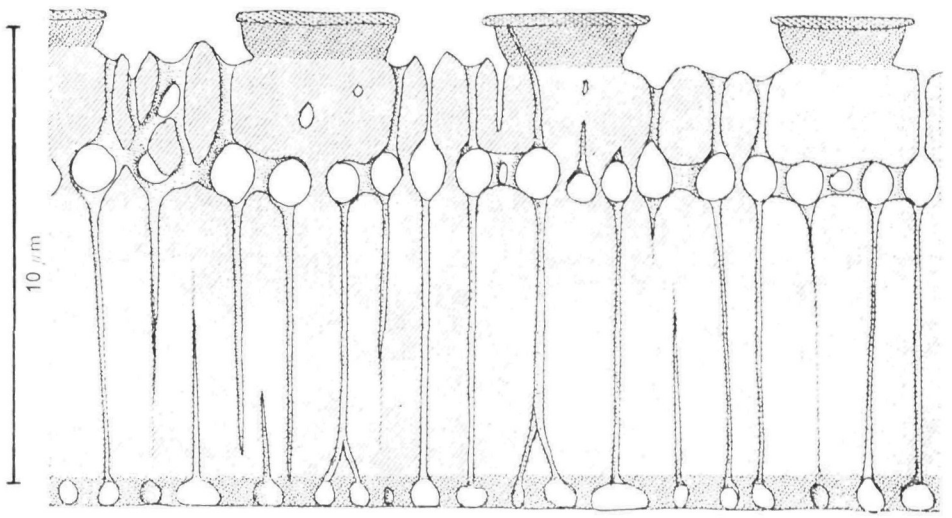
The new-laid egg is about 5 mm long by 0·7 mm wide and is of an elongate cylindrical form tapering slightly to rounded ends. Its initial weight is in the order of 1·7 mg. During development water is absorbed and the weight increases by 150%. This is allowed by an anisometric stretching of the shell with the length increasing by 14% and the girth by 50%. The shell is unpigmented and the embryo can be observed through the shell. This is greatly facilitated by immersing the whole egg in xylene, a procedure which has proved to have no harmful effect on the embryo even after 12 h immersion.

Development under normal conditions in the insectary takes 19 ± 1 days, with the insects hatching at dawn.

SHELL STRUCTURE

The egg-shell has a whitish tinge due to the air spaces within it. The poles of the egg and a large part of the dorsum are distinctly whiter. This whiter dorsal region, which comprises about one-third of the shell area, is for convenience here referred to as the *saddle region*.

The structure of the shell differs according to region, but the basic structure is as shown in Text-fig. 1. The inner part, as in other Tettigoniidae (Hartley, 1964), is a solid layer 6·5 μm thick traversed by numerous fine pores. At their inner ends the pores terminate in bulbous dilations which do not pierce the inner surface of the shell. There is little evidence of any lateral interconnexion, although some pores branch before reaching their end swellings. At their outer ends these pores open out into the



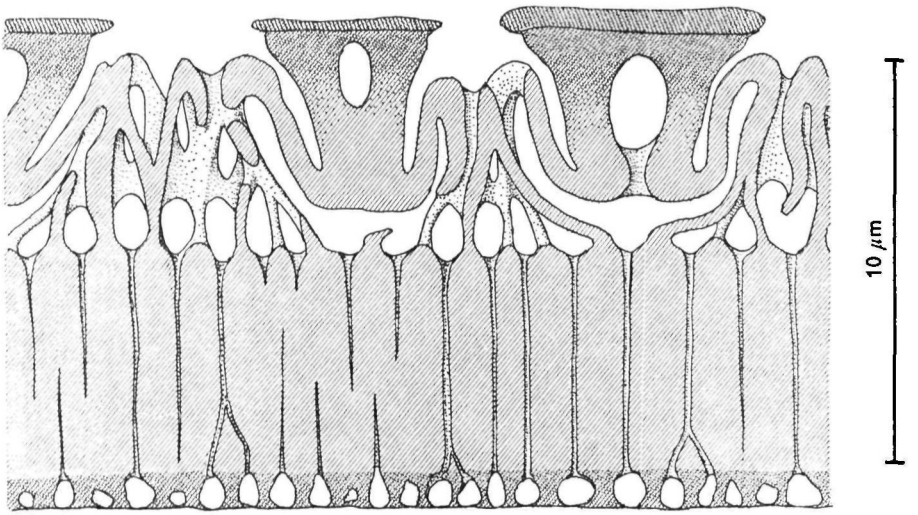
Text-fig. 1. Section of the egg-shell of *Homorocoryphus* in the mid-ventral region.

spaces of the pillar zone, which separates the inner and outer parts of the shell with an air layer 1 μm thick. The outer zone of the shell is 2·5 μm thick and appears as an outward extension of the pillars which have become very much dilated and are fused together with pores continuing through. Islands of solid chorion (Pl. 1, fig. 1) rise above this region by a further 1 μm and these tend to obliterate the pores in the outer zone beneath them, but have no effect on the pores of the inner layer of the shell.

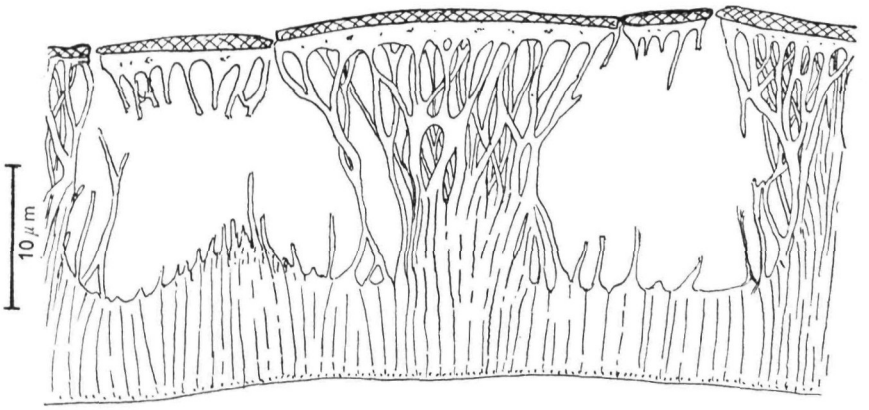
The pores of the inner shell have an apparent diameter of between 0·2 and 0·3 μm and an estimated density of some 250/20 μm square so that the pores represent between 1·9 and 4·4 % of the cross-sectional area of this part of the shell. The pores in the outer part appear to be continuous with the pores of the inner zone, but they have a greater diameter of between 0·5 and 0·6 μm. Since the islands occupy 36 % of the surface area, the approximate pore density of the outer zone is only 160/20 μm square. Thus the effective porosity of this zone is 7·9–11·4 %.

In the part of the shell referred to as the saddle region, modifications have occurred in the outer layer (Text-fig. 2; Pl. 1, fig. 2). The islands are taller and are supported by

flexed extensions of the pillars, the pillar system beneath being displaced laterally. The rest of the outer layer presents a more fibrous appearance and probably has a greater porosity. The islands in this region have a slightly concave cap, and cement applied to the shell adheres to their surfaces. The eggs are normally laid cemented together in flat ribbon-like groups with the long sides touching. The adhesion is very high, and unless they are soaked in water for many hours separation is not possible without damage to the shell. When the eggs swell during development some tearing across the pillars of this region often occurs; this, and the greater flexibility of these islands' supports, allows swelling to occur without the development of sharp angles and hence lines of weakness.



Text-fig. 2. Section of the egg-shell in the mid-dorsal or saddle region.



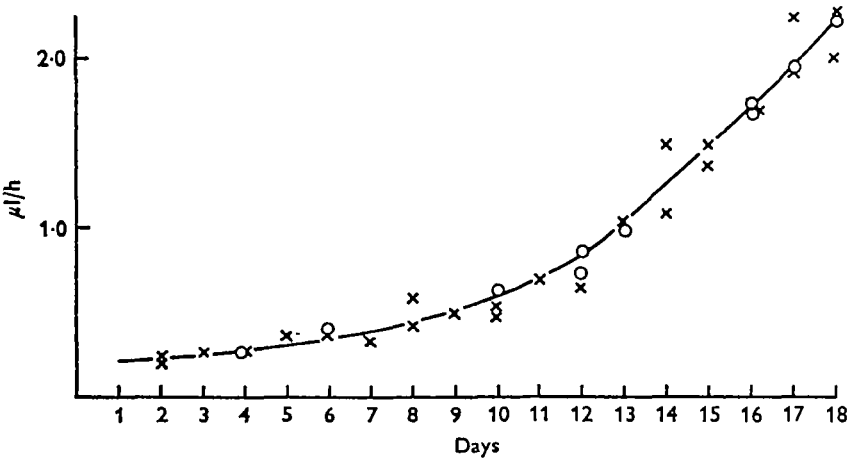
Text-fig. 3. Section of egg-shell near anterior pole.

The chorion of the poles of the egg also differs by modification of the outer layers. The pillars have become longer and are drawn together in clumps (Text-fig. 3). The lumen around these clumps is continuous with the spaces of the pillar zone elsewhere on the egg.

EXPERIMENTAL STUDIES ON THE RESPIRATORY SYSTEM

Both direct and indirect methods have been used to determine the effect of the shell structure on respiration. Direct measurements of the rate of oxygen consumption of eggs in air have been made with differential capillary microrespirometers of modified Scholander and Stern-Kirk patterns, giving an accuracy of about 0·1 μl/h. Measurements on eggs under water have been made with YSI Biological Oxygen Monitor, the eggs being held in a cage built into the stirrer. Indirect measurements arise from the time taken by the egg to develop under different conditions.

The pores and spaces in the shell normally contain air. When the egg is immersed in water the outer layer may be partially flooded trapping air in the pillar zone and below. This shows quite distinctly as a whiter layer. The air held in the pillar zone forms the principal air layer in the shell and this is referred to below as the air layer. It can be displaced by immersion of the egg in a wetting agent, which is then thoroughly washed out in water. Teepol has been used for this purpose, and as it only slowly displaces the air it is possible to check the process so that the shell is only part-flooded. More rapid and complete flooding can be produced by the use of alcohol as a wetting agent. This has the advantage that it can be washed out more rapidly, but preliminary experiments indicated that industrial alcohol had a harmful effect, reducing viability by rather more than 50%. Butanol was used instead without any obvious disadvantages. Flooded shells were kept under water or in contact with damp filter paper to prevent drying out during experiments.



Text-fig. 4. Graph of rate of oxygen uptake of dry eggs against age. Temperature 30 °C. ×, Points from single eggs; O, points from matched pairs of eggs.

The normal pattern of oxygen uptake by eggs in air during development is shown in Text-fig. 4. This is based on readings over periods 1–3 h on one or two eggs at a time with dry shells, taken from stock cultures of known age. No allowance has been made for differences in weight. It is evident, however, that the rate of oxygen consumption increases about tenfold during development, whereas weight increases only two and a half times.

The rates of oxygen uptake by eggs under experimental conditions are given in Table 1. The efficiencies are calculated by dividing the rate under experimental contions by the corresponding rate in air. As most of them are based on the expected rate in air they should be regarded as having a possible error of up to 10%.

In this table the efficiency of the egg is taken as the rate of oxygen consumption under experimental conditions over the rate in air.

Table 1. *Respiratory rates*

(Oxygen flow in μl/h/egg.)

		In air		Under water				
		Shell condition				Efficiency, e		
Egg age (days)	No. in batch	Air F_a	Flooded F_{fa}	Air F_s	Flooded F_{fw}	e_{fa}	e_s	e_{fw}
5	6	0·3*	—	0·18	—	—	0·60	—
5	6	0·3*	—	0·16	0·16	—	0·54	0·54
15–16	3	1·7*	—	1·40	1·20	—	0·82	0·71
	4	1·7*	—	1·41	0·95	—	0·83	0·56
16	2	1·8	—	1·48	—	—	0·82	—
	2	1·8*	—	—	1·12	—	—	0·62
17	4	1·9*	—	1·56	1·20	—	0·82	0·63
	4	1·9*	—	1·60	1·28	—	0·84	0·67
	2	2·0	1·4	1·68	—	0·7	0·84	—
	2	2·0	1·4	—	1·28	0·7	—	0·64
	4	2·0*	—	—	1·30	—	—	0·65
18	1	2·2	—	1·83	1·43	—	0·83	0·65

* Estimated reading based on Text-fig. 4, for known age of egg. It is probably accurate to within ± 0·1.

The effect of various treatments on the time of development is given in Table 2. The procedure was to take eggs of known age X and incubate them under different conditions until hatching after Y days. Submergence implies eggs under water but containing air in the shell, contrasting with flooded, where the air has been previously displaced. Underwater incubation was carried out in 100 ml conical flasks containing 90 ml water stirred and aerated by bubbling air. Other incubation was carried out on moist filter paper in Petri dishes. Previous experiments with eggs immersed under 2–3 mm of water, unstirred, in Petri dishes produced a much slower developmental rate and under these conditions no eggs hatched. Eggs under aerated water hatched without difficulty and the nymphs were able to shed their embryonic cuticle and float to the surface without drowning.

DISCUSSION

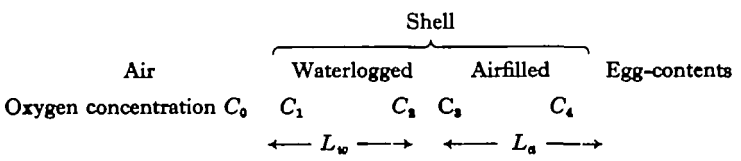
Certain features of the respiratory system of this type of egg cannot be observed directly. This is particularly so when the egg is underwater. However efficiently stirred, there is bound to be a layer of stagnant water around the egg, neither is it possible to observe accurately the extent to which water penetration of the outer layers occurs. The respiratory system of this type of egg, however, can be subjected to a mathematical analysis as given below. It is assumed that respiration occurs only through the pore system of the shell. It then becomes possible to calculate the unknown features of the system, and also to check that the interpretation of the system is correct.

Table 2. *Observed and expected development times for eggs incubated under different conditions*

Experimental treatment	X	Y	$N-X\pm1$	e_o	Number hatched	Air in shell (%)	e_p	Y_p	Total hatch
Submergence	6	17	13	0·71–0·83	2	100	0·84	15–17	
		17		0·71–0·83	2	90	0·82	15–17	
		17		0·71–0·83	2	80	0·80	15–18	
		18		0·67–0·78	2	80	0·80	15–18	
		18		0·67–0·78	3	60	0·76	16–19	
		19		0·63–0·74	3	60	0·76	16–19	
		19		0·63–0·74	1	40	0·72	17–20	
		20		0·60–0·70	2	40	0·72	17–20	
		21		0·57–0·67	2	20	0·68	18–21	
		25		0·48–0·56	1	30	0·70	18–20*	19/20
Submergence	9	11	10	0·82–1·0	2	100	0·84	11–13	
		12		0·75–0·92	1	80	0·80	11–14	
		13		0·69–0·85	3	100	0·84	11–13	
		13		0·69–0·85	2	90	0·82	11–14	
		14		0·64–0·79	2	90	0·82	11–14	
		15		0·60–0·73	1	80	0·80	11–14*	
		16		0·56–0·69	1	70	0·78	12–15*	12/12
Flooded in air	9	12	10	0·75–0·92	2	0	0·70	13–16	
		13		0·69–0·85	3	0	0·70	13–16	5/5
Flooded and submergence	9	14	10	0·64–0·79	2	0	0·64	14–18	
		15		0·60–0·73	3	0	0·64	14–18	
		17		0·53–0·65	3	0	0·64	14–18	
		18		0·50–0·61	2	0	0·64	14–18	
		20		0·45–0·55	1	0	0·64	14–18*	
		21		0·43–0·52	5	0	0·64	14–18*	
		22		0·41–0·50	2	0	0·64	14–18*	18/20
Submergence	14	6	5	0·67–1·0	5	100	0·84	5–8	
		7		0·57–0·86	4	100	0·84	5–8	
		8		0·50–0·75	2	60	0·76	6–8	11/12
Flooded and submergence	14	9	5	0·45–0·67	3	0	0·64	7–10	
		12		0·34–0·50	2	0	0·64	7–10	
		13		0·31–0·46	2	0	0·64	7–10	7/10
Flooded in air	15	4	4	0·75–1·2	2	0	0·70	5–8	
		5		0·60–1·0	5	0	0·70	5–8	
		6		0·50–0·84	3	0	0·70	5–8	10/10
Flooded and submergence	15	7	4	0·43–0·71	11	0	0·64	7–10	
		8		0·38–0·63	10	0	0·64	7–10	
		9		0·33–0·56	18	0	0·64	7–10	
		10		0·30–0·50	10	0	0·64	7–10	
		11		0·27–0·45	3	0	0·64	7–10	54/60

X, Days before start of incubation under experimental conditions.
Y, Days under experimental conditions until hatching.
$N-X\pm1$, Expected number of days until hatching, after the start of experiment, for untreated eggs in air.
e_o, Observed efficiency given by $e_o = N-X/Y$.
e_p, Predicted efficiency from Table 1.
Y_p, Predicted number of days until hatching.

Since the thickness of the shell is much less than the radius of the egg, the problem can be treated as equivalent to that of diffusion along a column, area A, where A is equal to the area of the shell. Consider the case where oxygen diffuses from ambient air through a shell to the egg-contents as shown at top of facing page.



At the air/water interfaces equilibrium is assumed so that $PC_0 = C_1$ and $C_2 = PC_3$ where P is the partition coefficient water/air. D_x is the appropriate diffusion coefficient.

The oxygen flow F is proportional to C_4 so that

$$\frac{F}{A} = \frac{C_4}{R}, \tag{1}$$

where R is the overall internal resistance of the egg.

At a steady state this flow must be the same as that of diffusion across the layers, so that

$$\frac{F}{A} = \frac{D_w}{L_w}(C_1 - C_2) = \frac{D_w P}{L_w}(C_0 - C_3), \tag{2}$$

$$\frac{F}{A} = \frac{D_a}{L_a}(C_3 - C_4). \tag{3}$$

Rewriting (1), (2), (3) and adding

$$\frac{F}{A}\left(R + \frac{L_w}{D_w P} + \frac{L_a}{D_a}\right) = C_4 + C_0 - C_3 + C_3 - C_4$$

$$= C_0,$$

$$\frac{F}{A} = \frac{C_0}{R + (L_w/D_w P) + L_a/D_a)}. \tag{4}$$

It is apparent from (4) that the flow per unit area is directly proportional to the external concentration of oxygen and inversely proportional to the sum of the resistances in series and that sequence of these does not matter. The diffusion coefficients for oxygen at 30 °C in air and in water are 0·2 and $2{\cdot}7 \times 10^{-5}$ cm²/s respectively (Table 3), and it becomes obvious that L_a/D_a is insignificantly small compared with $L_w/D_w P$ and can be ignored; this even applies to the egg in air as shown in the example below. With the egg in air $L_w = 0$, so

$$F_a = \frac{C_0 A}{R + (L_a/D_a)} \quad \text{or} \quad R = \frac{C_0 A}{F_a} - \frac{L_a}{D_a}.$$

Taking an oxygen consumption of 2 μl/h, $F = 5{\cdot}6 \times 10^{-7}$ ml/s, a shell thickness $L_a = 10{\cdot}5 \times 10^{-4}$ cm, and the effective area A_x as being the open area of the pores through the inner part of the shell, with a value in the order of 3 % of the total area, i.e. $1{\cdot}7 \times 10^{-1} \times 3 \times 10^{-2}$ cm²,

$$R = \frac{0{\cdot}2 \times 1{\cdot}7 \times 3 \times 10^{-3}}{5{\cdot}6 \times 10^{-7}} - \frac{10{\cdot}5 \times 10^{-4}}{0{\cdot}2}$$

$$= 1{\cdot}83 \times 10^{3} - 5 \times 10^{-3}.$$

Table 3. *Data used in the calculations*

Physical data

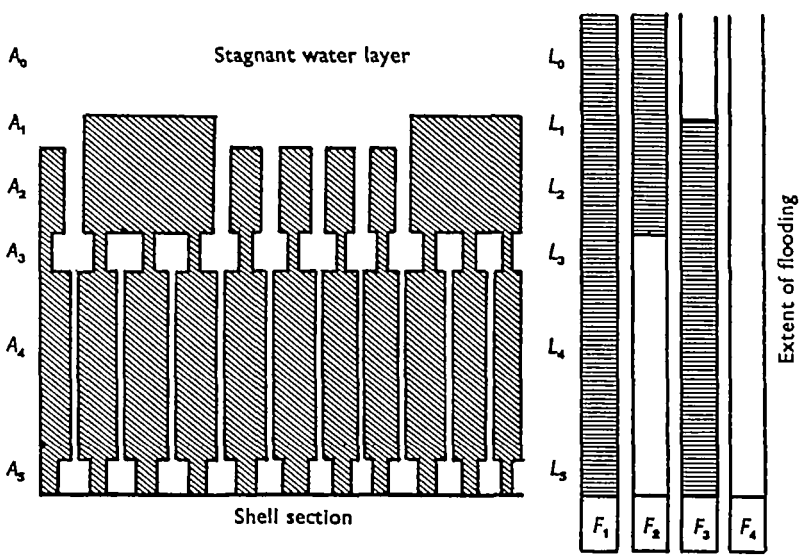
Temperature 30 °C. (All data below related to this temperature.)

Concentration of oxygen in air	$C_0 = 0{\cdot}2$ ml/ml
Concentration of oxygen in water	$C_w = 5{\cdot}3 \times 10^{-3}$ ml/ml
Partition coefficient	$P = C_0/C_w = 2{\cdot}65 \times 10^{-2}$
Diffusion coefficient oxygen in air	$D_a = 0{\cdot}2$ cm²/s
Diffusion coefficient oxygen in water	$D_w = 2{\cdot}7 \times 10^{-5}$ cm²/s*

Observed data (means for 17-day eggs)

Rates of oxygen consumption:	
In air	$F_4 = 5{\cdot}6 \times 10^{-7}$ ml/s
Submerged, not flooded	$F_3 = 4{\cdot}7 \times 10^{-7}$ ml/s
Submerged and flooded	$F_2 = 3{\cdot}6 \times 10^{-7}$ ml/s
Flooded in air	$F_1 = 3{\cdot}9 \times 10^{-7}$ ml/s
Shell thickness (L) and effective area (A):	
Outer shell, island caps	$L_1 = 1{\cdot}0 \times 10^{-4}$ cm $A_1 = 64\,\%\ A_0$
Outer shell, coarse pore zone	$L_2 = 2{\cdot}5 \times 10^{-4}$ cm $A_2 = 7{\cdot}9$–$11{\cdot}4\,\%\ A_0$
Pillar zone	$L_3 = 1{\cdot}2 \times 10^{-4}$ cm $A_3 = 80\,\%\ A_0$
Inner shell, fine pore zone	$L_4 = 5{\cdot}5 \times 10^{-4}$ cm $A_4 = 2$–$4\,\%\ A_0$
Terminal dilations	$L_5 = 0{\cdot}8 \times 10^{-4}$ cm $A_5 = 50\,\%\ A_0$
Total shell thickness	$L_t = 11 \times 10^{-4}$ cm
Total surface area	$A_0 = 1{\cdot}7 \times 10^{-1}$ cm²

* This is taken from Himmelblau 1964, fig. 5, which is itself a composite figure based on several authors.



Text-fig. 5. Diagrammatic representation of the shell structure with the effective area A_n and thickness of the different layers L_n. The extent of flooding is indicated on the right with resultant oxygen flow F_n under each condition.

Thus the rate of oxygen consumption would have to be in the order of 10^5 times greater for a shell of this thickness to produce any appreciable effect, as long as the problem is oxygen diffusion through air in the shell. Alternatively, the effective area of the shell, that is the pore area, would have to be reduced by a factor of 10^5.

Therefore, the air resistance in this system can be neglected altogether, and the equation for the egg underwater now becomes

$$F_w = \frac{C_0 A}{R+(L_w/D_w P)},$$

$$\frac{L_w}{D_w P} = \frac{C_0 A}{F_w} - R = C_0 A\left(\frac{1}{F_w}-\frac{1}{F_a}\right),$$

$$\frac{L_w}{A} = C_0 D_w P\left(\frac{1}{F_w}-\frac{1}{F_a}\right). \tag{5}$$

This can be expanded to allow for the different effective areas within the shell.

Text-fig. 5 is a diagrammatic representation of a shell of the *Homorocoryphus* pattern, showing effective area A_x, thickness L_x, extent of flooding with corresponding oxygen flow F_x. The following equations can be derived

$$\frac{L_0}{A_0}+\frac{L_1}{A_1}+\frac{L_2}{A_2}+\frac{L_3}{A_3}+\frac{L_4}{A_4}+\frac{L_5}{A_5} = C_0 D_w P\left(\frac{1}{F_1}-\frac{1}{F_4}\right), \tag{6}$$

$$\frac{L_1}{A_1}+\frac{L_2}{A_2}+\frac{L_3}{A_3}+\frac{L_4}{A_4}+\frac{L_5}{A_5} = C_0 D_w P\left(\frac{1}{F_3}-\frac{1}{F_4}\right), \tag{7}$$

$$\frac{L_0}{A_0}+\frac{L_1}{A_1}+\frac{L_2}{A_2} = C_0 D_w P\left(\frac{1}{F_2}-\frac{1}{F_4}\right), \tag{8}$$

(6−8)
$$\frac{L_3}{A_3}+\frac{L_4}{A_4}+\frac{L_5}{A_5} = C_0 D_w P\left(\frac{1}{F_1}-\frac{1}{F_2}\right), \tag{9}$$

(6−7)
$$\frac{L_0}{A_0} = C_0 D_w P\left(\frac{1}{F_1}-\frac{1}{F_3}\right), \tag{10}$$

(7+8−6)
$$\frac{L_1}{A_1}+\frac{L_2}{A_2} = C_0 D_w P\left(\frac{1}{F_2}+\frac{1}{F_3}-\frac{1}{F_1}-\frac{1}{F_4}\right). \tag{11}$$

These equations can be used both as a check on the results of respiration experiments and as a means of calculating the effective open areas within the shell and the extent of water penetration.

Using the data in Table 3, the following solutions can be obtained. The stagnant water layer L_0 is given by equation (10) and has a value of 53 μm. The porosity of the outer shell and the extent of water penetration is given by equation (11). For the data obtained $L_2/A_2 = 3{\cdot}08 \times 10^{-3}$ cm^{-1}; and with $L_2 = 2{\cdot}5$ μm, A_2 becomes 8·1 %. It, therefore, must be assumed that in submerged eggs water penetrates the outer shell as far as the pillar zone, that the effective porosity is minimal, and that the greater apparent porosity of the saddle region is counteracted by the slightly greater thickness of this region.

The porosity of the inner shell derived from equation (9) gives A_4 as 3·5 % A_0 which is well within the estimated range.

Although these results can only be considered approximate since the data they are derived from was obtained from comprehensive studies on only four eggs, the figures obtained above are sufficiently close to observed values for there to be little doubt that the respiratory system functions in the way assumed, namely, that oxygen diffuses into the egg by way of the shell pores and spaces.

The efficiency of the respiratory system under different conditions ($e = F_x/F_a$) varies slightly for different eggs (Table 1). This variation is mostly within the experimental error. But what is important here is that the efficiency remains relatively constant with increasing oxygen demand, except in young eggs with a very low oxygen demand.

The young unswollen eggs do present a problem. Not only is their efficiency lower, but the shell resistivity, equation (5), is about 7·5 times greater than in 17 day eggs. The shell is unstretched and thicker if one assumes that the volume of the shell remains constant. An increase in area by 1·7 times, which is approximately the change that occurs if the eggs are treated as cylinders, means that L/A will decrease by $1{\cdot}7^2$, i.e. 2·9. This is rather less than a third of the difference observed, and, therefore, other explanations must be sought. Shell expansion may not be an equal stretching process of chorion and pores alike, but rather an expansion of the pores. Indeed, many pores may not be open and functional until stretching occurs. This is further supported by the lack of difference between the respiratory rates submerged and flooded, suggesting diffusion through the substance of the chorion rather than through pores.

Incubation of eggs under water prolongs their development time. This delay may be the result of a lowered metabolic rate caused by a lowered rate of oxygen uptake, in which case there should be a close correlation between the respiratory efficiency and the delay. This can be tested in two ways. The observed efficiency can be found by the expression

$$e_o = \frac{N-X}{Y},$$

where N is the normal development time of 19 ± 1 days, X the number of days before treatment, and Y the number of days under experimental conditions until hatching. The expected efficiency derived from the data in Table 1, is given by

$$e = \frac{F_n}{F_a},$$

where $F_n = F_s m + F_{fw}(100\text{-}m)$ and m is the percentage of non-flooded air layer in the shell. This relationship can be used to calculate Y_p the expected number of days before hatching under experimental conditions:

$$Y_p = \frac{N-X}{e_p}.$$

The eggs, however, normally always hatch around daybreak, so that an egg expected to hatch in, for example, 11·25 days will hatch on the morning of the 12th day and not during the 11th day. Therefore Y_p has been calculated to the next whole number. These values, e_o, e_p, Y_p, have been inserted in Table 2. Most of the results come within the predictions, those marked with an asterisk having a delay longer than expected. This has occurred in eggs where the air layer has been partially or completely lost and

e eggs have been immersed in water for a long time. A slight decrease in the efficiency of stirring or a slight build-up of surface contamination would produce further delay. It is indeed very unlikely that the stirring efficiency in the flasks where the eggs are free to be carried in the water currents would be as good as that in the oxygen monitor.

It seems likely, therefore, that the delay is directly due to a reduced metabolism caused by reduced availability of oxygen. It is suggested that the egg requires a finite quantity of oxygen for its development and that if the rate of oxygen uptake is reduced then the time is extended until this finite quantity is attained, provided it does not fall below a viable level.

The air layer in the shell, that is, the air in the pillar zone and below, does not give the system a very great advantage over the completely flooded situation. It is not, therefore, a plastron according to the definition of Thorpe & Crisp (1947) as found in the Diptera (Anderson, 1960; Hinton, 1960*a*). The principal air layer in *Homorocoryphus* is in the wrong place for good plastron respiration as the outer layer of the shell is in the way. There is, of course, no reason why *Homorocoryphus* should have a plastron system, for although its rate of development is faster than that of any other known tettigoniid, the egg is in a protected situation, namely the sheathing bases of grass leaves, where it is unlikely to be flooded for long. Nor does it matter to this sort of insect whether hatching is delayed by a few days, unlike some flies where the food source is of a transient nature. It must also be remembered that water uptake is important and that water could be in short supply. A shell that retains a water layer may be more important than one that allows unhindered respiration.

The term 'respiratory efficiency' as used here should not be confused with the plastron efficiency of Crisp (1964) and Hinton (1969). In this paper the respiratory efficiency is considered as the ability of the egg to obtain oxygen from the environment, whereas the term plastron efficiency is used to cover the extent of usage of the plastron surface. For example, a plastron that has twice the area that is necessary for respiration under the conditions set by Crisp (1964) is given an efficiency of one half. However, there appears to be no allowance for differences in the layer of stagnant water (L_0) according to external conditions. This factor is going to be very different for an egg in running water as opposed to an egg in standing water and it is felt that this aspect should be considered when considering plastron efficiency.

SUMMARY

1. An account is given of the structure of the egg-shell of *Homorocoryphus nitidulus vicinus* based on conventional and scanning electron microscopy.

2. The respiratory rates of the egg in air and under various degrees of flooding have been measured.

3. The problems of oxygen diffusion through egg-shells of this type have been subject to mathematical analysis.

4. The hypothetical and observed results for oxygen diffusion compare favourably, confirming that the respiratory system functions as suggested.

5. The eggs of *Homorocoryphus* cannot be said to have a plastron, and it is suggested that water retention may be more important than a high respiratory efficiency under water.

6. A distinction is drawn between respiratory efficiency of an egg under water and plastron efficiency.

I am very grateful to Dr G. S. Hartley for his assistance in the analysis given in the Discussion, and to him and Dr J. C. Mecklenburgh for general discussions on problems of oxygen diffusion. I am also indebted to Miss Pauline Horwill for some of the data in Text-fig. 4, and to Mr A. W. R. McCrae who provided the original nucleus of the *Homorocoryphus* culture.

REFERENCES

ANDERSON, D. S. (1960). The respiratory system of the eggshell of *Calliphora erythrocephala*. *J. Insect Physiol.* **5**, 120–8.

CRISP, D. J. (1964). Plastron respiration. *Recent Prog. Surface Sci.* **2**, 377–425.

HARTLEY, J. C. (1964). The structure of the eggs of the British Tettigoniidae (Orthoptera). *Proc. R. ent. Soc. Lond.* A **39**, 111–17.

HARTLEY, J. C. (1966). Laboratory culture of a tettigoniid, *Homorocoryphus nitidulus vicinus* (Wlk.) (Orthoptera). *Bull. ent. Res.* **57**, 203–5.

HIMMELBLAU, D. M. (1964). Diffusion of dissolved gases in liquids. *Chem. Revs.* **64**, 527–50.

HINTON, H. E. (1960*a*). Plastron respiration in the eggs of Blowflies. *J. Insect Physiol.* **4**, 176–83.

HINTON, H. E. (1960*b*). The chorionic plastron and its role in the eggs of the Muscinae (Diptera). *Q. Jl. microsc. Sci.* **101**, 313–32.

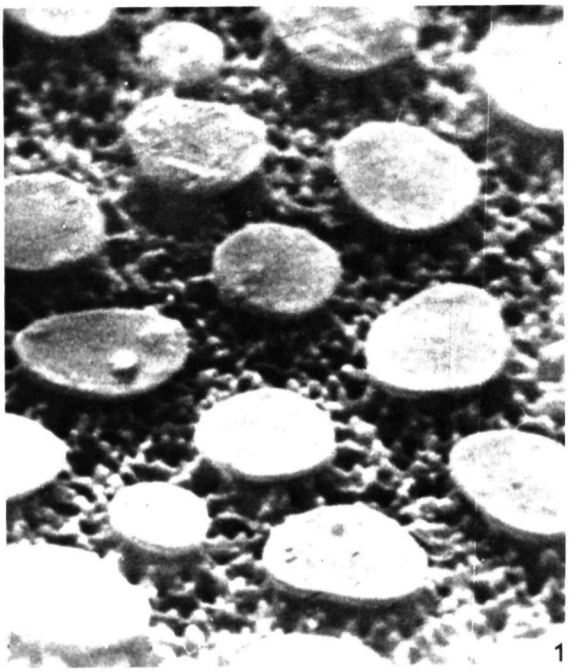
HINTON, H. E. (1969). Respiratory systems of insect egg shells. *A. Rev. Ent.* **14**, 343–68.

THORPE, W. H. & CRISP, D. J. (1947). Studies on plastron respiration. I. The biology of *Aphelocheirus* (Hemiptera, Aphelocheiridae (Naucoridae)), and the mechanism of plastron retention. *J. exp. Biol.* **24**, 227–69.

EXPLANATION OF PLATE

Fig. 1. Oblique Stereoscan picture of the surface of the egg-shell. × 6000.

Fig. 2. Oblique Stereoscan picture of the edge of a transversely broken egg-shell, saddle region. × 6000.


1


2