



## FUNCTION OF SHELL STRUCTURES OF PIG LOUSE AND HOW EGG MAINTAINS A LOW EQUILIBRIUM TEMPERATURE IN DIRECT SUNLIGHT

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**Abstract**—The functional significance of the structures of the eggshell of the pig louse, *Haematopinus suis*, and those of some other Anoplura and some Mallophaga, is described.

The shell of the pig louse has an outer air-filled meshwork. The air in this meshwork is isolated from that in the respiratory system. This layer does not function as an insulator. Calculations show that the temperature drop across the layer is only about 1/75°C.

The air-filled outer meshwork functions as a reflector. The eggs are often exposed to direct sunlight because of the wide spacing of the pig's bristles. The outer reflecting layer has an enormous selective advantage for the egg in two quite different ways: (1) it slows down the rate at which equilibrium temperature is reached, and (2), much more important, it enables the egg to lower equilibrium temperature.

Assuming that reflectance = 0.9 and sunlight =  $10^3 \text{ Wm}^{-2}$ , the heat input is  $3.1 \times 10^2 \text{ J/sec}$ , and the time required for a rise of 20°C, assuming no heat loss, is 4.3 min. Assuming a more realistic energy flux (sunlight =  $500 \text{ Wm}^{-2}$ ) for the latitudes in which most pigs are bred, and even assuming a reflectance of only 20%, the time required for a rise of 20°C in direct sunlight, with no heat loss, is increased by 1 min 4 sec.

Lowering the equilibrium temperature is of much greater selective advantage than merely slowing down the rate at which it is reached. Reflectance + emissivity = 1. Nevertheless, the egg can achieve a reflectance of, say, 0.8 at the same time as it achieves an emissivity of 0.8 from the energy absorbed by the chorion and especially the aqueous embryo or pharate first instar larva, either of which will function as a black body and absorb all of the infra-red not absorbed by the chorion. Calculations show that if a heat input of  $200 \text{ Wm}^{-2}$  is assumed, at an ambient temperature of 27°C, the equilibrium temperature can be maintained at 47°C.

The chorionic hydropyle of the pig louse, like that of apparently all other Phthiraptera, is most unusual in that the hygroscopic spumaline secreted by the accessory glands flows through the hydropyle canals of the chorion into the interior of the egg while the egg is still in the common oviduct. Molecules as large as those of fast green (mol. wt 809) pass through the spumaline into the interior of the egg and then pass through the serosal hydropyle. The amount of spumaline secreted greatly exceeds that necessary to glue the egg to the bristle. Once absorption is seen as another function of the spumaline, its amount no longer becomes difficult to understand: under many natural conditions it will enable the egg quickly to replace losses of water that result from exposure to high temperatures in direct sunlight.

The egg of the pig louse, like those of other species of *Haematopinus*, has a very well-developed plastron or permanent physical gill. A number of the aeropyles are covered by spumaline, but even assuming that 30% of them are so covered, the water-air interface for small eggs is  $3.5 \times 10^6 \mu\text{m}^2/\text{mg}$  and for large eggs  $2.6 \times 10^6 \mu\text{m}^2/\text{mg}$ . Thus the relation of water-air interface to weight of tissue is the most favourable so far reported in insects.

### INTRODUCTION

TO BE ATTACHED to a pig's bristle exposes an egg to environmental stresses of a kind that few other insect eggs experience: because the pig has widely spaced bristles that do not always provide shade, the egg may be directly exposed to the sun at one moment only to be plunged into mud or water at another. Thus a period of flooding and a period of baking in the sun may alternate at fairly frequent intervals throughout the day as the pig wallows between spells of grubbing for food.

Because the egg of the pig louse is exposed to hazards not often encountered by other eggs, it was supposed that the shell might have some exceptional features. A study of its structure was undertaken for it seemed that recognition of structures that enable the egg to tolerate the sharply contradictory features of its environment might well result in the recognition of similar but less well-developed structures in other eggs exposed to similar but less violent extremes. And such features were indeed found.

The most unusual structure of the egg, and one hitherto completely unknown, was an outer gas-con-

taining chorionic meshwork that was isolated from the inner respiratory air layer and from the aeropyles. This outer layer lowers the rate at which equilibrium temperature is reached, and, together with the black body (aqueous embryo or pharate first instar larva) that absorbs infra-red, enables the egg simultaneously to maintain a high reflectance and a high emissivity, although at any one wave length reflectance + emissivity = 1. The ability to maintain simultaneously a high reflectance and a high emissivity enables the egg to have a much lower equilibrium temperature than it would otherwise have when exposed to direct sunlight, as it often is. Another unusual feature of the egg is its chorionic hydropyle, a structure that occurs in all Phthiraptera. Chorionic hydropyles were first described in the eggs of bugs of the family Nepidae (HINTON, 1961). Another feature, also of great interest, is a system of ridges on the inner side of the thick outer chorionic sheet and the outer side of the thin inner sheet. These ridges reduce friction when the inner layer of chorion slides over the outer layer in the main body of the shell. The ridges also prevent the inner respiratory air layer from being displaced. I know of no other order of insects that has eggs in which there is an inner layer of chorion that slides freely over the outer layer of chorion.

## GENERAL STRUCTURE OF SHELL

### *Structure of main body of shell*

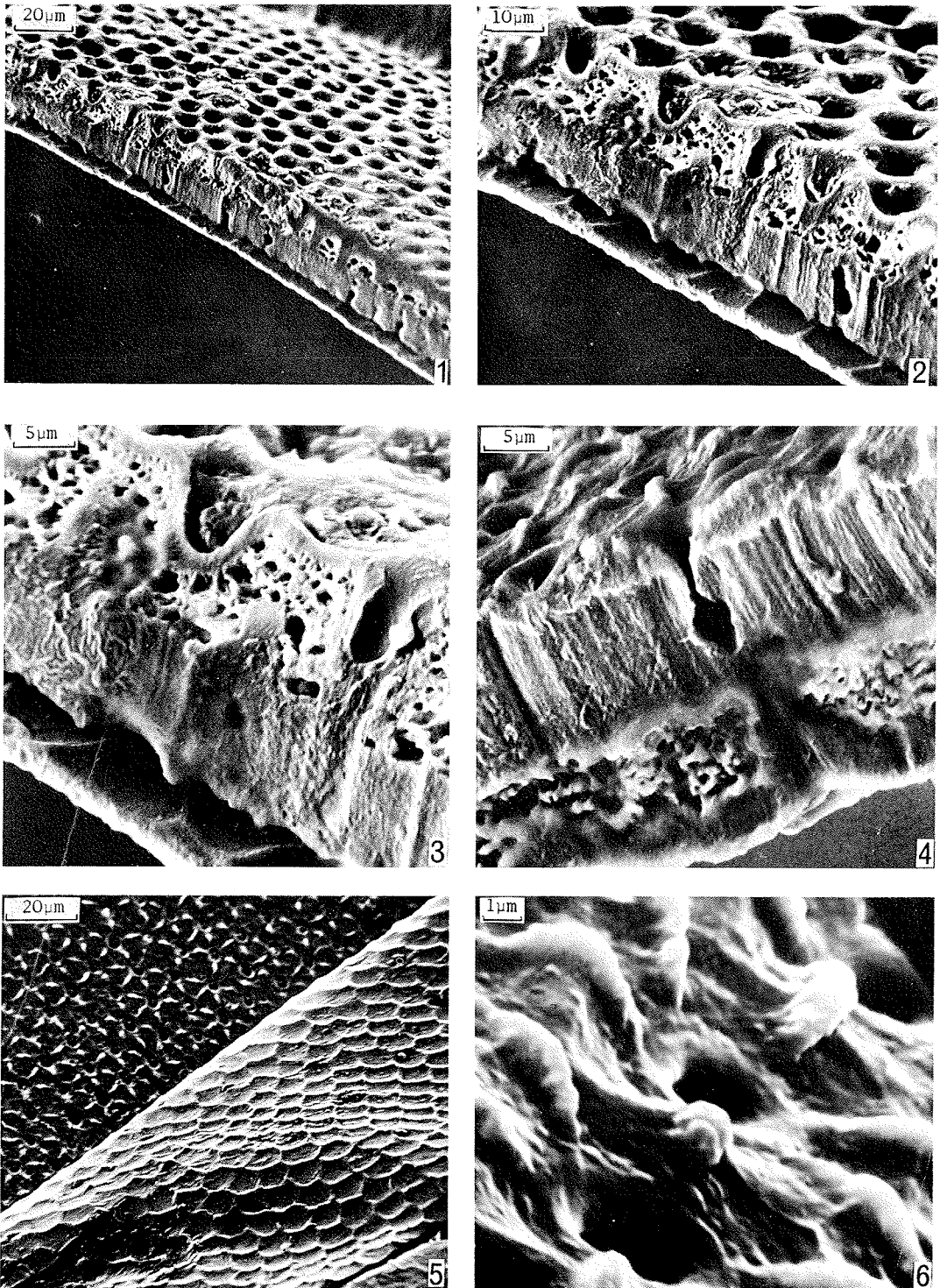
As shown in Figs. 1 to 7, the shell consists of two sheets of chorion. These sheets are joined to each other at the preformed line of weakness of the operculum and again before the hydropyle. Elsewhere the two sheets of chorion are quite separate. The outer sheet of chorion is usually 25 to 30  $\mu\text{m}$  thick. It consists of (1) a transparent outer layer about 2  $\mu\text{m}$  thick. (2) There is an outer air-containing meshwork about 10  $\mu\text{m}$  thick. This layer will be called the reflecting layer. The gas held in the meshwork of the reflecting layer does not communicate with the respiratory air layer or with the aeropyles. Using alcohols and oils of different mobilities it is possible to flood the inner respiratory layer without for a time flooding the outer reflecting layer, although if the egg is submerged for long enough the gas in the reflecting layer is also displaced by oils and alcohols. (3) Below the gas-containing reflecting layer there is a layer of 'solid' chorion about 10  $\mu\text{m}$  thick. The aeropyles extend through all of the layers of the thick outer sheet. They convey gases in and out of the respiratory air layer between the thick outer sheet and the thin inner sheet. The inner sheet is about 2  $\mu\text{m}$  thick. The upper side of this inner sheet has a system of ridges arranged in polygons (Fig. 5). These ridges slide over the ridges, usually 5 to 6 (Figs. 5 to 6) that radiate from the

inner openings of the aeropyles of the outer sheet. This system of ridges subserves two functions: (1) it prevents the inner respiratory air layer from being displaced, and (2) it reduces the contact area and therefore friction when the inner sheet slides over the outer sheet, as it seems it must do when the egg is being hydrated or dehydrated.

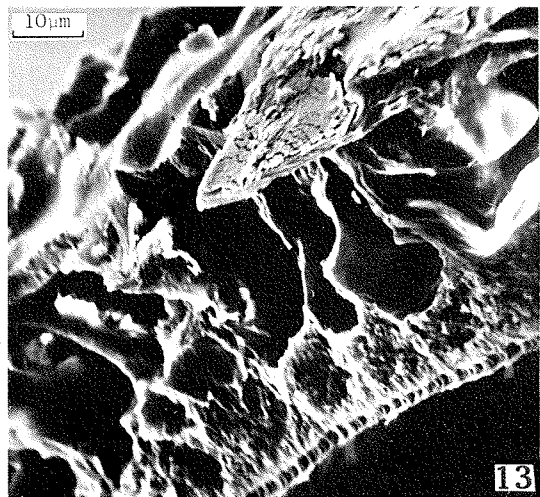
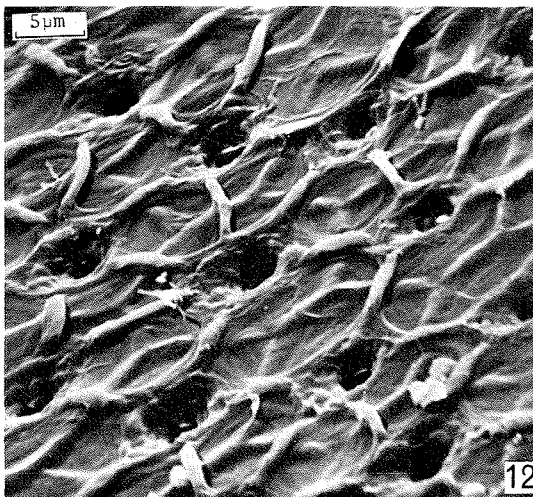
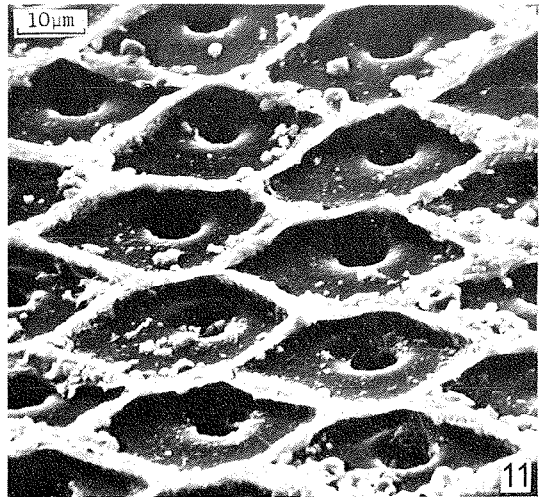
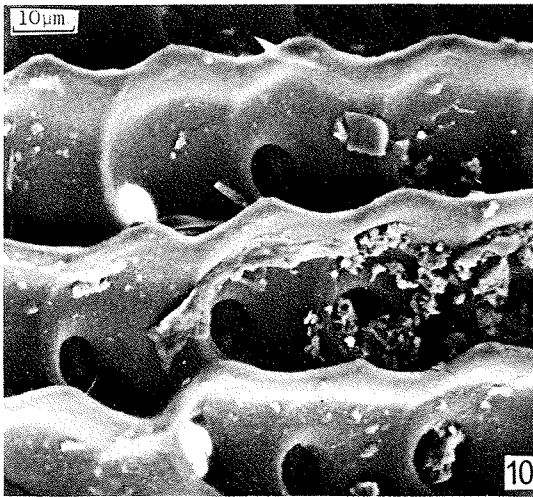
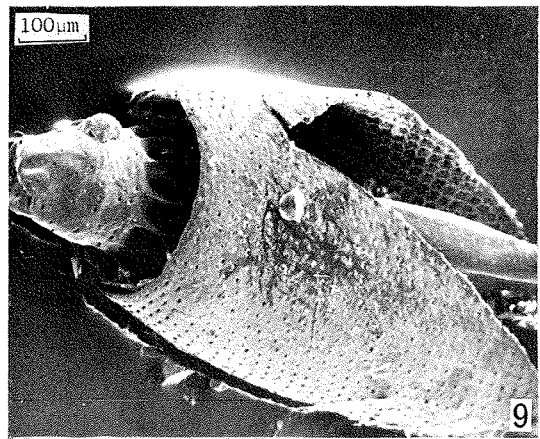
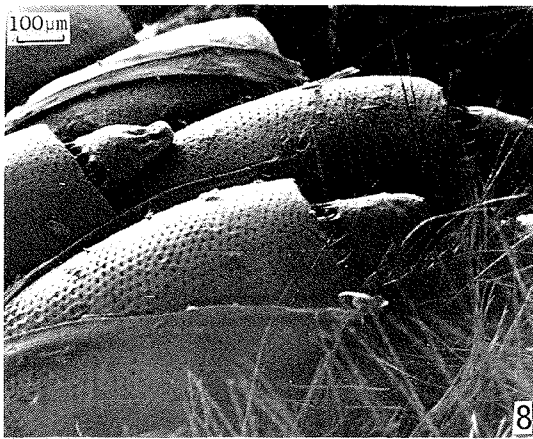
Among the lice that have been examined carefully, an outer air layer in the outer sheet of chorion isolated from the respiratory air has only been found in the genus *Haematopinus*. In this genus it has been found in all species examined. In all Phthiraptera of the three suborders, the thin inner sheet of chorion of the main body of the shell is quite separate from the thick outer sheet except before the operculum and before the hydropyle. In other Phthiraptera the ridges on the inner side of the thick sheet may be polygons, as in the biting louse *Bucerocephagus* (Fig. 11).

### *Structure of operculum*

The operculum consists of large air-filled chambers, the walls of which are meshworks containing air (Figs. 14 and 15). Each of the micropyles opens into such an air-filled chamber, the external openings of which are shown in Fig. 17. The inner openings of the micropyles are not plugged with spumaline or any other substance, as they are in nearly all other insects. The functional significance of the open inner ends of the micropyles is dealt with in the Discussion. The innermost layer of the operculum has a very distinct air layer supported by vertical columns. This layer is clearly shown in micrographs of *H. suis* (Fig. 15) and *H. asini* (Fig. 13). Experiments with oils and alcohols show that there is no communication between the air cavities of the operculum and those of the main body of the shell: all of the air in the operculum can be displaced without displacing any of the air in the main body of the shell. In order to emerge from the shell, the larva increases internal pressure until the operculum is broken along all or part of a preformed line of weakness (Figs. 16, 18, and 19). Thus when the operculum is burst open part of the line of weakness may serve as a hinge to the lid, or the operculum may fall off completely. Egg-bursters have been described and illustrated on the head of the embryonic cuticle of some lice by writers who supposed they functioned in cutting the chorion (e.g. WIGGLESWORTH, 1932; WEBER, 1939), but it seems more likely that the structures described by these and other writers function in cutting the tough serosal cuticle: I do not know of a single operculate egg with an embryonic egg-burster that is used to rupture the chorion. The eggs of many Heteroptera-Pentatomomorpha have a pseudoperculum, but, as noted by COBBEN (1968), electron micrographs do not show a line of weakness between the main body of the shell and the pseudoperculum. In these Hemiptera the chorion is normally ruptured with the aid of an embryonic egg-burster.

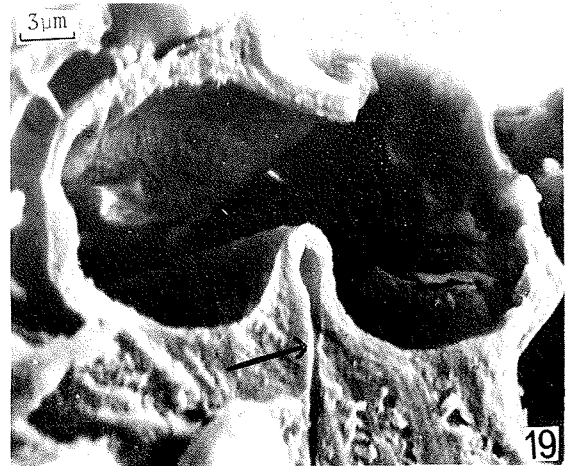
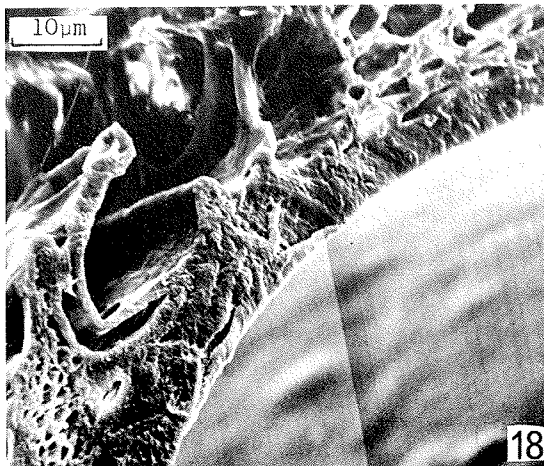
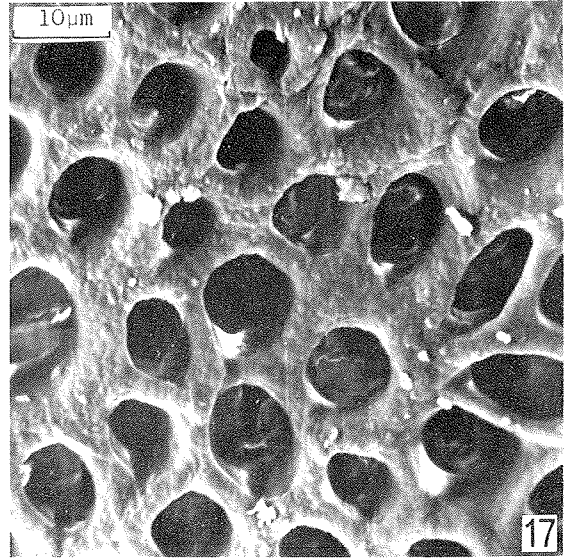
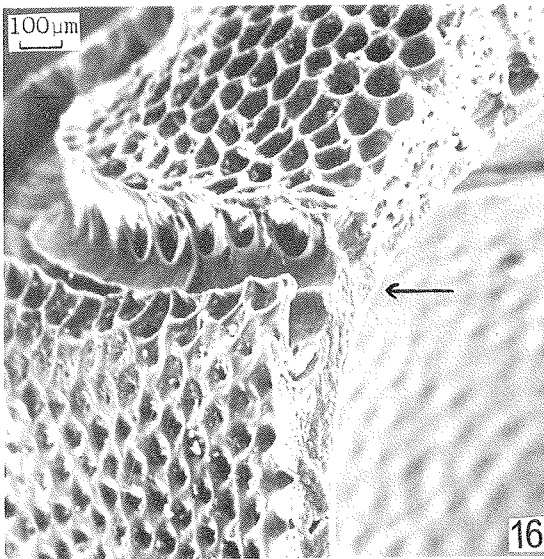
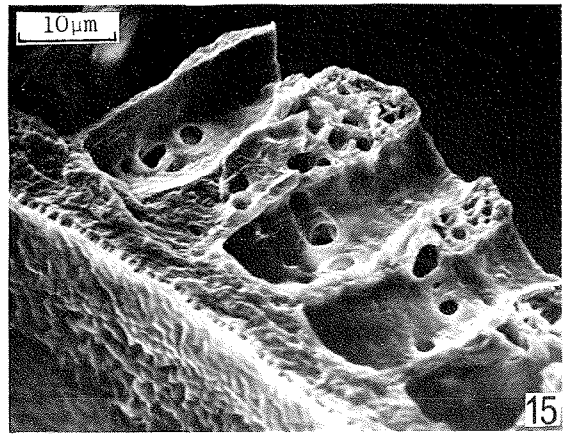
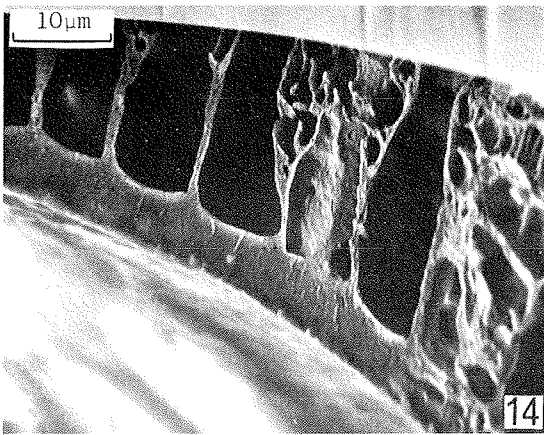


Figs. 1-6. Scanning electron micrographs of middle sides of shell of the pig louse, *Haematopinus suis* (L.). (1-3) Various views of shell showing aeropyles, outer reflecting layer, and space between the thick outer and the thin inner sheets of chorion. (4) Part of middle of shell with inner side uppermost so that the relation between the reflecting layer, solid middle part of outer sheet, and ridges radiating from the aeropyles are clearly seen. (5) Inner view of openings of aeropyles to show the ridges radiating from them. Inner sheet rolled back to show ridges that ride upon the ridges radiating from the inner openings of the aeropyles. (6) Inner opening of an aeropyle.



Figs. 8–13. Scanning electron micrographs. (8) Eggs of *Bucerophagus productus* (Burm.) from the ground hornbill. (9) Shell of *B. productus* with shell broken so that the inner openings of the aeropyles are visible. Each aeropyle opens into a polygon bounded by ridges. (10) *Bucerophagus africanus* Bedford from the African hornbill. Aeropyles of middle of shell. (11) Inner openings of aeropyles of *B. africanus*. Each aeropyle opens into a polygon bounded by ridges. (12) Inner openings of the aeropyles of *Haematopinus asini* (L.). (13) Operculum of *H. asini* broken to show the innermost air layer supported by vertical columns.





Figs. 14–19. Scanning electron micrographs of the shell of *Haematopinus suis*. (14–15) Views of air chambers in operculum. (16) Longitudinal section through top part of shell showing hatching line (arrow) between operculum and main body of shell. (17) Openings of air chambers above micropyles. (18) Area around hatching line. (19) Hatching line (arrow).

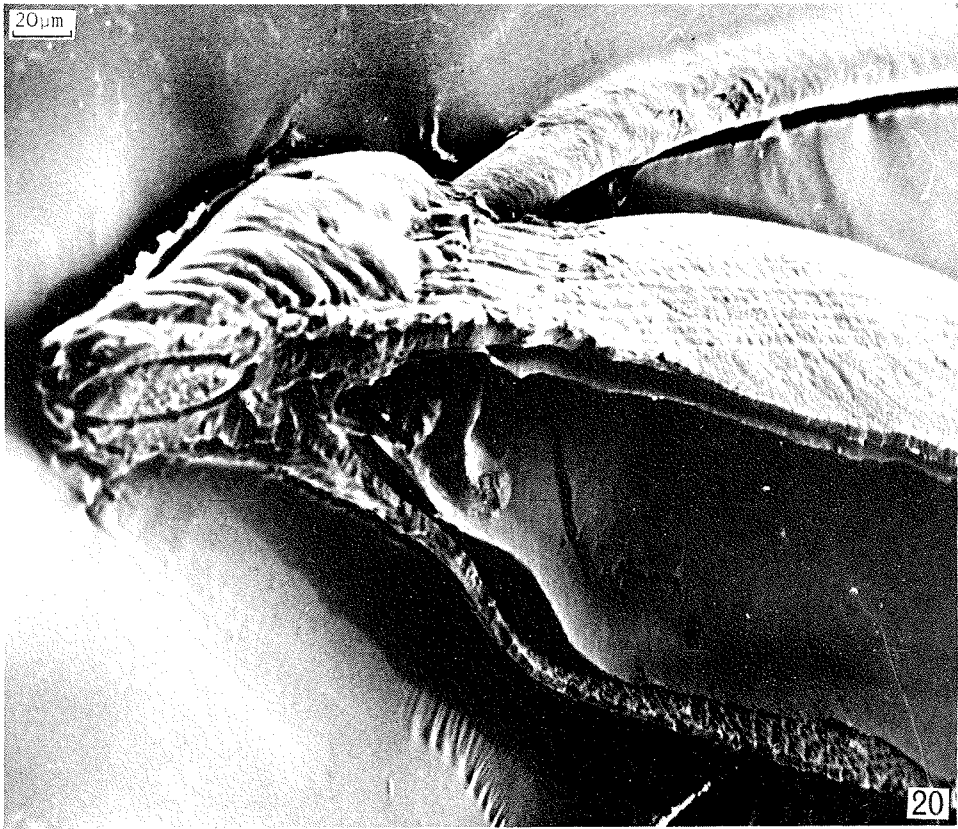


Fig. 20. Scanning electron micrograph of egg of *Haematopinus eurystenus* (Nitzsch). The egg has been cut longitudinally near the hydropyle in order to show the large collar of spumaline deposited round the hair of a cow.

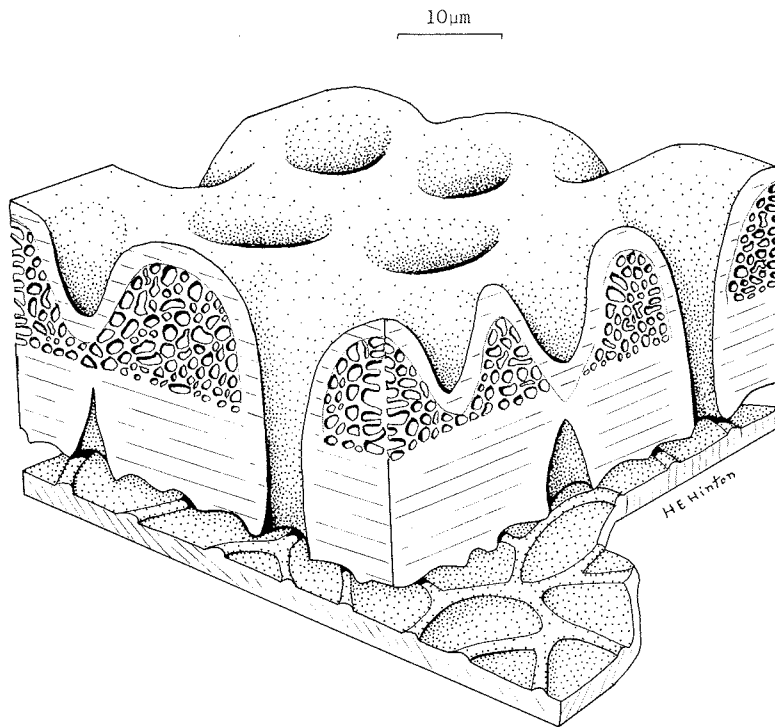


Fig. 7. *Haematopinus suis*, diagram of the middle portion of the shell to show relation of parts.

#### WHITE COLOUR OF EGG NOT A DISADVANTAGE BECAUSE ITS PREDATORS DO NOT HUNT BY SIGHT

When the colour of the egg is compared with colours of papers of known reflectance, it seems that about 90%, or a little more, of the visible light is reflected from all parts of the egg except the base and parts of the base covered with spumaline. The egg is thus very conspicuous when seen against the much darker bristles or skin of the pig. It might be thought that there would be a heavy selective pressure against being so conspicuous. However, the pig louse, like other species of *Haematopinus* and many other kinds of lice, is not attacked by predators, apart from mites that do not find them by sight. The birds that hunt for ticks and lice on horses, cattle, water-buffalo, and other animals are hunting for ticks and lice that are very large in comparison to the egg, and they do not, to the best of my knowledge, attack eggs. Thus, despite its colour the egg escapes attention from large predators because of its small size and also because it is very firmly glued to the base of the bristle near the skin of its host. Predators, apart from mites that do not hunt by sight, small enough for the egg to provide them with a quantity of food that would make the expenditure of energy necessary to break into the egg worthwhile are not found on pigs, cattle, or other large animals attacked by species of *Haematopinus*.

#### INSULATION, REFLECTANCE, AND EMISSIVITY

##### *Outer meshwork of egg as an insulator and a reflector*

The egg appears to be matt white, and there is little specular reflection from it. Immediately below a transparent outer layer about  $2\text{ }\mu\text{m}$  thick there is an air-containing meshwork about  $10\text{ }\mu\text{m}$  thick (Fig. 7). The air-containing meshwork is an optically heterogeneous system with many of the interstices or holes about a wave length wide so that visible light is scattered. Most of it (ca. 90%) is reflected out of the egg. The conductivity of air ( $0.026\text{ W/}^\circ\text{K}$ ) is much less than that of the struts of chorion that form the meshwork. There are no figures for the conductivity of chorion. Figures exist for the conductivity of leather ( $0.0005\text{ W/}^\circ\text{K}$ ) and similar materials, and, although some of these are cross-linked with quinones rather than S-S bonds, I assume that the conductivity of chorion will be roughly the same and certainly within the same order of magnitude. It is thus clear that the air-containing meshwork must be considered both as an insulator and a reflector.

##### *Effect of reflecting layer as an insulator*

Even assuming that air is a perfect insulator, which it is not, a meshwork of half air and half chorion would only insulate as well as a layer of chorion twice

as thick. Thus the temperature drop (assume input =  $200 \text{ Wm}^{-2}$ ) across a  $20 \mu\text{m}$  meshwork is about  $20/500 \times 1/3 = 1/75^\circ\text{C}$  (temperature drop across an egg  $500 \mu\text{m}$  wide is  $1/3^\circ\text{C}$  when input is  $650 \text{ Wm}^{-2}$ ). Thus the insulating effect of the meshwork is negligible. The insulating effect is slightly better than in the calculation given above because the struts are not straight pillars but curved and twisted so that from the point of view of heat transfer the layer of chorion is slightly thicker than assumed in the calculation. However, even taking into account the increased path length the insulating effect is still entirely negligible.

*Effect of reflecting layer on time required to reach equilibrium temperature*

The vast majority of all pigs are today reared between  $25^\circ$  and  $50^\circ$  North. Relatively few pigs are reared on or close to the equator. Thus few pigs will be exposed to a sun with an altitude of  $90^\circ$ . At  $90^\circ$  on the equator at sea level the direct radiation from the sun measured on a horizontal surface is just over  $900 \text{ Wm}^{-2}$ . This energy flux increases with altitude so that at, say,  $3,000 \text{ m}$  on a mountain, it is just under  $1,200 \text{ Wm}^{-2}$  when the sun is at zenith. For these reasons, if we assume that sunlight =  $1,000 \text{ Wm}^{-2}$ , we have assumed an energy flux unfavourable to our argument, which is as it should be.

Any structure that slows down the rate at which the egg reaches equilibrium temperature is of selective advantage when there is much sunshine. The thermal capacity of the egg (using cm as a basic unit for radius) =  $\frac{4}{3}\pi r^3 \times \text{specific heat} = \text{calories degree } ^\circ\text{C}^{-1}$ . Assuming specific heat = 1 and density = 1, we have  $16r^3 \text{ J/}^\circ\text{C}$ . Assuming that reflectance = 0.9 and sunlight =  $1,000 \text{ Wm}^{-2}$ , the heat input will be  $100 \times \pi r^2 / 10^4$  or  $3.1 \times r^2 / 100 \text{ J/sec}$ .

The time required for a temperature rise of  $1^\circ\text{C}$  is  $16r^3 / (3.1/100 r^2) \text{ sec} = 1600 r / 3.1 = 1600/3.1 \times 40 = 12.9 \text{ sec}$  for a rise of  $1^\circ\text{C}$  with no heat loss. For a rise of  $20^\circ\text{C}$  the time is therefore 258 sec. When the same calculation is made assuming a reflectance of only 20% (sunlight =  $1,000 \text{ Wm}^{-2}$ ) instead of 90%, the time required for a rise of  $20^\circ\text{C}$  is only 32 sec instead of 4.3 min. Similar calculations have been made assuming the sunlight to be  $500 \text{ Wm}^{-2}$ , and, as shown in Table 1, at a reflectance of 90% a rise of  $20^\circ\text{C}$  with no heat loss requires 8.9 min, whereas under the same conditions a reflectance of 20% results in a rise of  $20^\circ\text{C}$  in only 1 min 4 sec.

The selective advantage normally considered to be sufficient to preserve and develop any attribute of an animal is the Haldane-Fisher figure of 0.01%. It is thus not necessary to dwell upon the colossal selective advantage of the reflecting layer when the egg is exposed to direct sunlight. In this section only the energy (ca. 62%) in the visible part of the spectrum has been considered. Most of the remainder of the energy from the sun is in the infra-red. The air-containing meshwork is undoubtedly transparent to infra-red, but the contents of the egg—first the

embryo and later the pharate first instar larva—are mostly water and so will act as a black body. The effect of this black body in enabling the egg at one and the same time to maintain a high reflectance and a high emissivity is considered in another section.

*How the egg keeps its equilibrium temperature low*

At any wave length reflectance + emissivity = 1. It is thus not possible at any particular wave length to have both high reflectance and high emissivity. However, reflectance and emissivity are different for different wave lengths. Thus, for an egg exposed to the hazard of overheating in sunlight, there is great selective pressure to arrange its structures so that they achieve both high reflectance for sunlight and high emissivity for infra-red. If the egg can do this, it can stay much cooler.

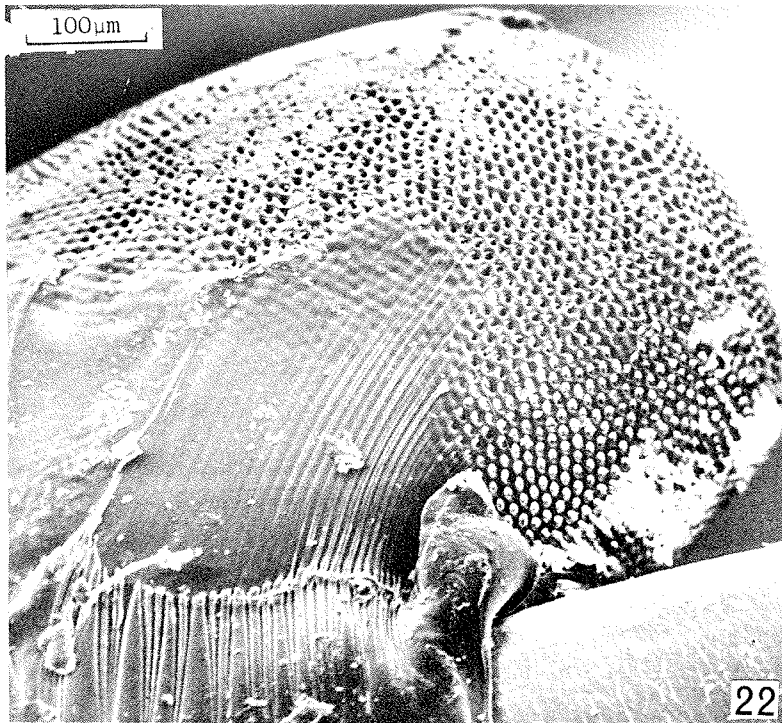
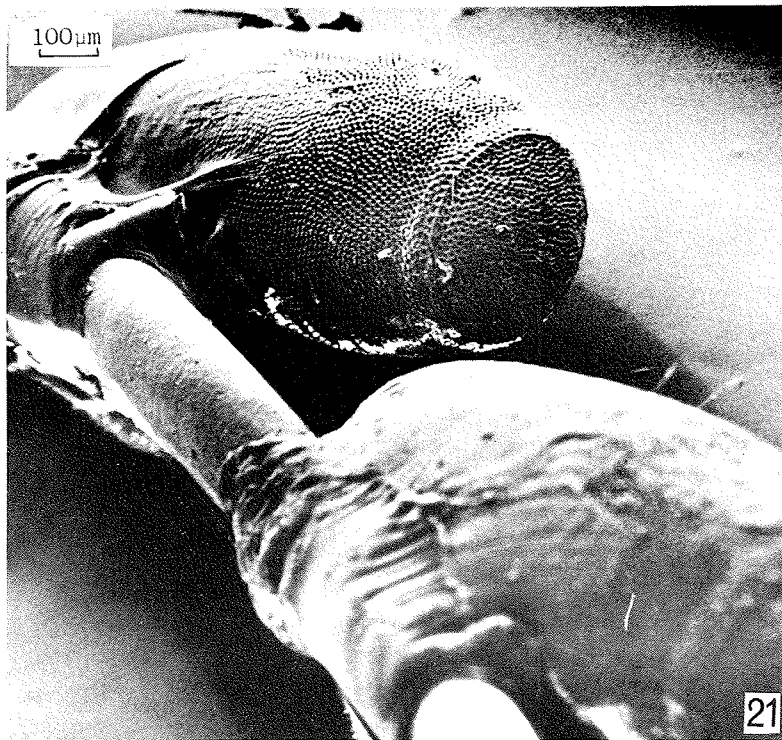
In another section we have seen how effective the outer air-containing layer is in slowing down the rate at which equilibrium temperature is reached. For instance, considering only the energy flux in the visible part of the spectrum, we have seen that the egg reflects 90% of visible light. Thus, when sunlight =  $1,000 \text{ Wm}^{-2}$ , it would require 4.3 min to raise its temperature from  $27^\circ\text{C}$  to  $47^\circ\text{C}$  (Table 1).

However, reducing the equilibrium temperature is a far more effective solution than merely slowing down the rate at which it is reached. As noted above, in any one part of the spectrum reflectance + emissivity = 1. Nevertheless, the egg can achieve a reflectance of, say, 0.8 at the same time as it achieves an emissivity of 0.8 from the energy absorbed by the chorion and especially the aqueous embryo or larva. About 62% of the energy of the sun is in the visible part of the spectrum, a very little in the ultra-violet, and less than 38% in the infra-red. The air layers are transparent to infra-red. Some infra-red will be absorbed by the chorion. Figures for transmission of infra-red by dry cross-linked proteins, e.g. the elytra of the Hercules beetle, show that such proteins absorb much of the infra-red (HINTON and JARMAN, 1973). What is certain is that the aqueous embryo or pharate first instar larva will act as a black body and absorb all of the infra-red not absorbed by the chorion. The relative percentages of infra-red absorbed by the chorion and black body do not concern us at this time:

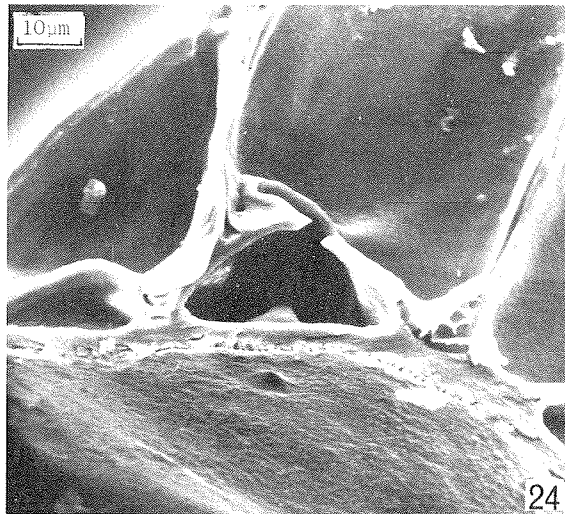
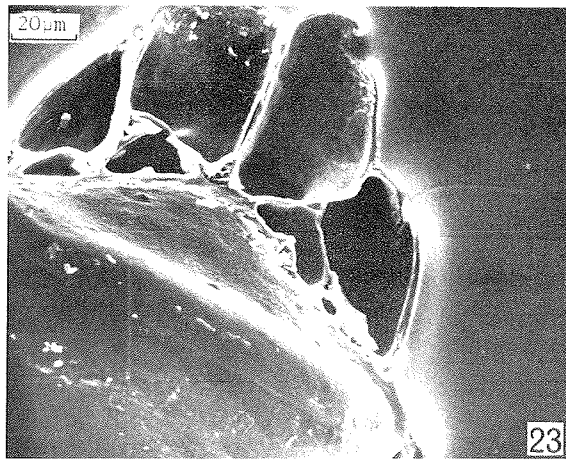
Table 1. The effect of the outer air-containing meshwork layer of the egg of *Haematopinus suis* on the time required for a temperature rise of  $20^\circ\text{C}$  with no heat loss when different percentages of the visible spectrum are reflected and sunlight =  $1,000 \text{ Wm}^{-2}$  or  $500 \text{ Wm}^{-2}$

Watts/m <sup>2</sup>	Percentage reflectance	Seconds
1,000	0.9	258
1,000	0.2	32
500	0.9	533
500	0.8	258
500	0.2	64

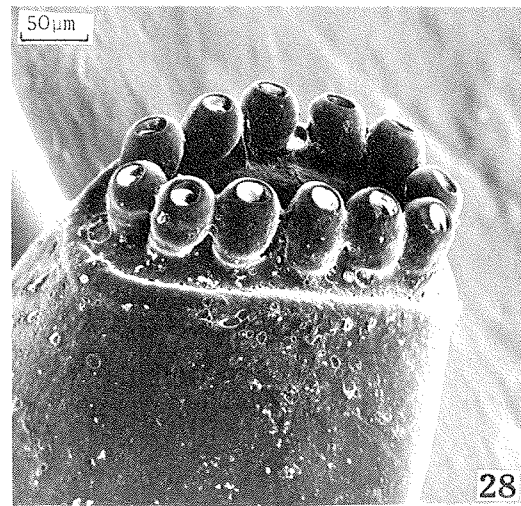
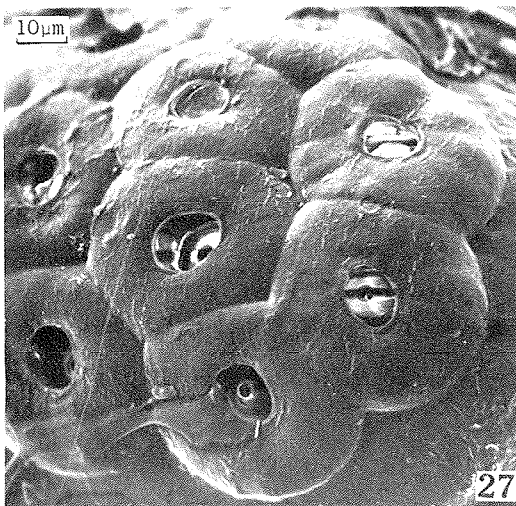
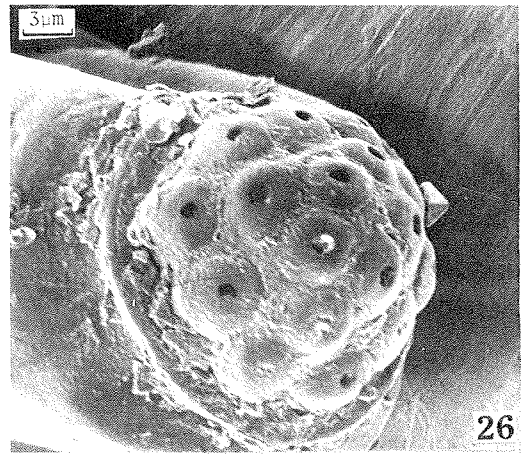
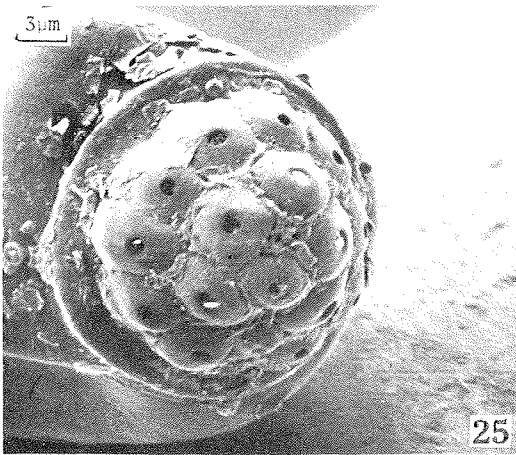




Figs. 21–22. Scanning electron micrographs of *Haematopinus suis*. They show the way the egg is attached to a pig's bristle. They also show how much spumaline covers the posterior part of the egg.



Figs. 23–24. Scanning electron micrographs of the opercular air chambers above the micropyles of *Phthirus pubis* (L.), the pubic louse.



Figs. 25–28. Scanning electron micrographs of openings of air chambers above micropyles. (25–26) *Phthirus pubis*, the pubic louse. (27) *Pediculus humanus corporis* Deg., the human body louse. (28) *Lepidophthirus macrorhini* Enderlein, the louse of the southern elephant seal.



both heat up the egg and increase radiation from it. The following calculations show how effectively the egg keeps equilibrium temperatures low:

Assume heat input =  $200 \text{ W m}^{-2}$

Heat loss at equilibrium temperature

$$= 0.8 \times 56 \times 10^3 ((T_1/10^3)^4 - (300/10^3)^4) + 1.5 \times (T_1 - 300)^{4/3}$$

Assume  $T_1 = 47^\circ\text{C}$

$$\therefore \text{Loss} = 45 \times 10^3 (320^4 - 300^4/10^{12}) + 1.5 \times 20^{4/3}$$

$$= (45 \times 10^3 \times 4 \times 2.4 \times 300^3/10^{12}) + 1.5 \times 55$$

$$= 4.5 \times 4 \times 2.4 \times 2.7 + 82.5$$

$$= 4.5 \times 2.6 \times 10 + 82.5$$

$$= 117 \text{ (radiation loss)} + 82.5 \text{ (convection loss)}$$

$$= 199.5$$

$$\therefore T_1 = 47^\circ\text{C}.$$

From what has been said in the preceding pages there seems to be little doubt that the pig louse egg not only slows down the rate at which equilibrium temperature is reached, but, much more important, by being able to maintain a high reflectance and a high emissivity at the same time, is able to keep its equilibrium temperature much lower than it would otherwise be. I suspect, but I have no firm evidence, that eggs exposed to direct sunlight for long periods are probably chiefly killed by losing too much water rather than by over-heating. Figures given by MURRAY (1963) have shown that for the biting louse, *Damalinia equi* (Denny), there is no difference in hatch when the eggs are exposed for one hr at temperatures between  $42^\circ\text{C}$  and  $49^\circ\text{C}$ , when the hatch is 38 to 39%. When exposed for 2 hr to  $49^\circ\text{C}$  no eggs hatch. At  $45^\circ\text{C}$  for 1 hr 38% hatch, and even when exposed to  $45^\circ\text{C}$  for 6 hr 23% hatch.

### CHORIONIC HYDROPILE

A chorionic hydropyle was first recognized as such in the eggs of aquatic bugs of the family Nepidae (HINTON, 1961). However, long before this chorionic hydropyles had been described and illustrated for various species of biting and sucking lice. Some 19th century writers thought the hydropyle canals were micropyles, whereas many writers up to recent times (e.g. WEBER, 1939) thought the hydropyle was an attachment disk. GRABER (1872) thought it was an apparatus for aerating the shell and called it an *Eistigma*, a misleading term that has persisted in the literature.

GROSS (1906), who also used the term *Eistigma* and supposed the chorionic hydropyle to be an attachment disk, has given very good descriptions and illustrations of those of the pig louse and two species of biting lice. The chorionic hydropyle of the pig louse has 24 to 26 canals about  $4 \mu\text{m}$  wide at their outward openings. Each of the canals is filled with spumaline. The spumaline enters the hydropyle canals while the

egg is still in the common oviduct, as shown for the elephant louse by WEBER (1939). Weber claimed that the chorionic hydropyle of the elephant louse was an attachment disk because the surface of the chorion was too smooth for the spumaline (= *Kittsubstanz*) to stick to it, which seems absurd in view of the fact that spumaline sticks very firmly to eggs with surfaces much smoother than those of lice.

Three kinds of hydropyles are known in insect eggs: (1) serosal hydropyles; (2) serosal cuticle hydropyles; and (3) chorionic hydropyles. In some insects, e.g. grouse locusts (Tetrigidae) all three kinds of hydropyles coexist (HARTLEY, 1962). Serosal and chorionic hydropyles may coexist without (apparently) the presence of a serosal cuticle hydropyle, e.g. many Heteroptera-Amphibicorisae in which the serosal cuticle consists of a single thin membrane, and in which, even before blastokinesis, the serosal hydropyle sometimes becomes detached from the remainder of the serosa. I do not know if there is a serosal hydropyle in the pig louse egg. In the later stages of development of the pig louse egg, the serosal cuticle immediately opposite the chorionic hydropyle is thickened. The fine structure of the serosal cuticle hydropyle is unknown, but experiments show that it has canals wide enough to pass quite large molecules.

Eggs of the pig louse were cross-sectioned with a razor blade. The remains of the pharate first instar larva were then removed, leaving the serosal cuticle intact. The cut edges of the shell were sealed with vaseline. The shells were then suspended in an aqueous solution of fast green. Within a minute or so, the spumaline in the canals of the chorionic hydropyle was coloured green. After about 15 to 20 minutes, some of the fast green accumulates in the bottom of the egg. It is thus clear that the stain passes through the canals of both the chorionic and serosal cuticle hydropyles. The solution of fast green passes into the eggs without penetrating into the outer reflecting air layer or into the inner respiratory air layer. These experiments were repeated by one of my students (G. D. NEWMAN) in 1971 with similar results.

The experiments described above were made in 1969 to discover if urea from the pig's urine could enter through the hydropyle. The molecular weight of fast green is 809, whereas that of urea is 60. Both are polar molecules. There can therefore be no question that if urea comes into contact with the spumaline it will enter the egg. No experiments were made to determine the toxicity of urea when it comes into contact with the spumaline and is translocated into the egg. It may be that eggs exposed to much urea will be killed, but they may perhaps tolerate or detoxify the amounts that are a normal hazard of their environment.

In the genus *Haematopinus* the spumaline is wrapped completely around the bristles or hairs. This is clearly shown in Fig. 20, which is a micrograph of the egg of the short-nosed cattle louse, *H. eurystenus* Denny, cut along its major axis close to the



hydropyle. The amount of spumaline greatly exceeds that necessary to glue the eggs to the bristles not only in the genus *Haematopinus* but also in many other kinds of sucking lice and in the biting lice glued to feathers. However, once the absorption of water is seen as another function of the spumaline, it becomes easier to understand its amount. When any part of the spumaline is in contact with water, water is transported directly into the interior of the egg along the stalks of spumaline that fill the hydropyle canals. These extensions of spumaline in the hydropyle canals have been called (BALTER, 1968b, p. 170), "...protoplasm connecting stigma to the endochorion." But of course there is no protoplasm on the outside of the serosal cuticle. Theoretically, there is no reason why the hygroscopic spumaline should not be used to collect atmospheric water when the relative humidity is high, a suggestion that has previously been made by BALTER (1968a).

On page 797 figures have been given for the temperature tolerance of eggs of *Damalinia equi*. These figures suggest that louse eggs may be killed by dehydration rather than by direct damage to the tissues by overheating. As shown in another section of this paper, *Haematopinus suis* has a special reflecting air layer that slows down the rate at which equilibrium temperature is reached. More important, the egg also keeps the equilibrium temperature low by having simultaneously a high reflectance and a high emissivity. When, say, the ambient temperature is 27°C but an egg exposed to direct sunlight can keep its equilibrium temperature at, say, 47°C or below, there will be a great loss of water. There will thus be a heavy selective pressure to develop structures that will replace such losses of water as rapidly as possible when the opportunity arises.

#### Plastron

The egg of the pig louse, like those of other species of *Haematopinus*, has a very well developed plastron. The plastron functions when it is raining or when the pig is wallowing in water. However, if the pig wallows in mud or dirty water in which the oxygen pressure is less than that of the embryo or larva, the plastron will function in reverse.

The water-air interface is established, at any rate at very low hydrostatic pressures, near the top of the outer rim of each aeropyle because water does not spread on the surface. If the area of the cross section of each aeropyle is taken as that of a cross section near the top of the outer rim, about 50% of the surface area is water-air interface when it is submerged. The amount of the posterior part of the egg covered by spumaline varies greatly, but in most eggs it would be about 20% (Figs. 21 and 22). Even if it be assumed that as many as 20% of the aeropyles are covered by spumaline, the water-air interface is still in the region of 40% of the total surface area. This figure becomes 35% in the unlikely event that 30% of the aeropyles are covered by spumaline.

The eggs are 1.5 to 1.75 mm long and 0.5 to 0.8 mm wide. The egg is a prolate spheroid. Using tables that are more extensive than those previously published (HINTON, 1969), the percentage of the surface area that has to be water-air interface for there to be  $10^6 \mu\text{m}^2/\text{mg}$  is between 8% and 12% for small eggs and 16% for the largest eggs. The shell has a total surface area greater than the metabolically active part of the egg by a factor of the square of the ratio of the linear dimensions. The percentage of the shell that must be water-air interface is therefore smaller by this factor. To correct this, the percentages given above must be multiplied by  $(r/R)^2$ , where  $r$  is the radius of the egg without the shell and  $R$  is the radius of the egg including the shell. Making such a correction, the percentages of the surface of small eggs that have to be water-air interface for there to be  $10^6 \mu\text{m}^2/\text{mg}$  is between 7 and 10% and for large eggs 13%. Thus the relation of water-air interface to weight of tissue is one of the most favourable known in insects. It has previously been shown that some insects satisfy their oxygen requirements when in well-aerated water with a water-air interface of  $1.5 \times 10^4 \mu\text{m}^2/\text{mg}$  (HINTON 1968). The figure for the pig louse egg is about  $3 \times 10^6 \mu\text{m}^2/\text{mg}$ . Thus even assuming the parameters I have used are fairly wide of the mark, the plastron of the egg is an effective one. It seems probable that the most important error that may have been made is the assumption that the water-air interface is established under natural conditions near the outer rim of the aeropyles. For instance, if the water-air interface is established below this across the parallel part of the aeropyles, the total surface area would be in the neighbourhood of  $30\% \times 0.83 = 25\%$  (— allowance for spumaline) = 20% water-air interface, which is half again the water-air interface required even for large eggs for  $10^6 \mu\text{m}^2/\text{mg}$ .

## DISCUSSION

### *Functional significance of open micropyles*

Because the inner ends of the micropyles are not plugged after fertilization as they are in most insects, they provide a route for oxygen from the ambient air to pass directly into the subchorionic air space. In early embryonic stages, slight dehydration may cause a subchorionic air space to appear between the vitelline membrane and the chorion. In later stages when there is a serosal cuticle, the subchorionic space is between the serosal cuticle and the chorion. That the subchorionic space is filled with air has been observed by BALTER (*in litt.*). The ingenious suggestion has been made (BALTER, *in litt.*) that in both the Mallophaga, e.g. *Pitrufulgenia coyus* (Marelli), and the Anoplura, e.g. *Pediculus humanus* L., the open micropyles play an important rôle in respiration when the egg is submerged in water. Absorption of water by the hydropyle, and the consequent swelling of the embryo, or pharate first instar larva, reduces the sub-

chorionic air space and forces air out of the micropyles, which increases the pressure of the air already in the chambers above the micropyles (Figs. 23 and 24). This eventually forces a bubble partly out of the air chamber above each micropyle.

Bubbles of air attached to the outer openings of the chambers above the micropyles, such as those shown in Figs. 25 to 28, would function as physical gills of the compressible or shrinking type. In this type of physical gill, as oxygen is withdrawn from the bubble the partial pressure of oxygen in the bubble falls and the partial pressure of nitrogen rises. Oxygen therefore diffuses into the bubble and nitrogen diffuses out of it. However, because oxygen is much more soluble in water than nitrogen, the water-air interface of the bubble is much more permeable for oxygen than for nitrogen. Because of this difference in the solubilities of the two gases, there will be a tendency for equilibrium to be restored by oxygen diffusing into the bubble rather than by nitrogen diffusing out of it. Nevertheless, some nitrogen is continually leaking out of the bubble so that it is continually becoming smaller and less effective until it is renewed. It has been shown (EGE, 1915) that using compressible air bubbles the backswimmer, *Notonecta*, can extract from the ambient water as much as 13 times the amount of oxygen that the bubble originally contained. Other writers have shown that at low temperatures, when activity is reduced and not much oxygen consumed, a respiratory bubble may last for several days. It would thus seem that for both *P. coypus* and *P. humanus* the selective advantage of the bubble is great in preventing drowning when the egg is submerged because neither the coypu nor humans normally remain submerged for long periods.

#### *Adaptations for survival in direct sunlight*

When the pig louse egg was first examined, simple experiments with oils at once showed that the air in the outer meshwork of what I now call the reflecting layer was not immediately replaced like that of the respiratory air layer between the outer and inner sheets of chorion. At first it seemed that such an air layer must be an insulating layer. But the temperature drop across the layer was calculated to be only about  $1/75^{\circ}\text{C}$ . Thus the reflecting layer has an entirely negligible effect as an insulator. Once it was realised that the reflecting layer was not an insulator of any kind, attention was focused on its reflectance. To be brief, it was eventually realised that the egg was keeping its equilibrium temperature low by reflectance in one part of the spectrum and emissivity in another. It is possible that many insect eggs that are laid in places where they are exposed to direct sunlight for part of the day have structures that function in the same way. For instance, there seems to be no necessary reason why the air in the reflecting layer should be isolated from that in the respiratory system. The problem of how some eggs keep a low equilibrium

temperature would probably never have occurred to me but for the discovery that the air in the reflecting layer was isolated from that in the respiratory system.

The figure usually accepted for the selective value necessary for some attribute to be preserved and developed is  $1/10^4$ . If a rise of  $20^{\circ}$  over ambient temperature, say from  $27$  to  $47^{\circ}\text{C}$  is considered, calculations show that, assuming no heat loss and a reflectance of 90%, the delay in a rise of  $20^{\circ}\text{C}$  when sunlight =  $10^3 \text{ Wm}^{-2}$  is 4.3 min. If sunlight =  $500 \text{ Wm}^{-2}$  the delay is 8.9 min. Even in a similar egg reflecting only 20%, a rise of  $20^{\circ}\text{C}$  (sunlight =  $500 \text{ Wm}^{-2}$ ) would be delayed by 1 min 4 sec.

In a previous section of this paper it was noted that (1) as many eggs of the biting louse, *Damalinia equi* hatch when exposed to  $49^{\circ}\text{C}$  for 1 hr as when exposed to  $42^{\circ}\text{C}$  for 1 hr; and (2) that there is reason to believe that when eggs die that are exposed to long periods at incubator temperatures between  $42^{\circ}\text{C}$  and  $49^{\circ}\text{C}$ , death is probably caused by dehydration rather than by irreversible heat injury to the tissues. In most circumstances, especially when the energy flux is high, it is very hard to imagine a pig remaining so still that an egg at the base of a bristle is exposed to direct sunlight for continuous periods of as long as 1 hr. The figures given in the preceding paragraph clearly show the enormous selective advantage of the reflecting layer in slowing down the rate at which the egg reaches a critical temperature.

But, as has been shown, there is a much greater selective advantage in keeping the equilibrium temperature low than in slowing down the rate at which it is reached. The pig louse keeps equilibrium temperatures low by a high reflectance in one part of the spectrum and a high emissivity in another. Calculations show that at an ambient temperature of  $27^{\circ}\text{C}$  and an energy input of  $200 \text{ Wm}^{-2}$ , assuming a reflectance of 0.8, the egg can keep its equilibrium temperature at  $47^{\circ}\text{C}$ .

Lowering equilibrium temperatures may be achieved in a similar way in some snakes and in some agamid, teiid, and scincid lizards with scales that reflect much of the visible part of the spectrum. In the tropics this would enable them to hunt a little longer in direct sunlight before having to seek shade.

#### *The hydropyle*

I do not know of any other insect in which spumaline is associated with the chorionic hydropyle, as it is in all lice. The hygroscopic spumaline secreted by the accessory glands enters the hydropyle canals while the egg is in the common oviduct. During the time that the egg is being laid, spumaline is deposited around the bristle (Figs. 21 and 22) and over about 20 to 30% of the posterior part of the shell. The spumaline provides, so to speak, a large catchment area for the collection of any free water in the vicinity. It also provides a large surface for the extraction of water from the atmosphere when the ambient humidity is high. In some bird lice, e.g. *Laemobothrium*

*tinnunculi* (L.) of the kestrel, spumaline almost completely covers the egg.

Reasons have already been given for believing that under natural conditions eggs exposed to direct sunlight are perhaps more likely to die from dehydration rather than from irreversible heat injury. If this be true, it further emphasizes the selective advantage of the very unusual hydropyle of lice in which hygroscopic strands of spumaline pass water through the chorion directly into the interior of the egg. The egg of the pig louse is often laid very near the base of a bristle. Thus, when the pig is in direct sunlight and sweating, some of the sweat may run up the bristle, depending on the position of the pig. If so it would be absorbed by the spumaline and enter the egg. The possibility that a small percentage of eggs exposed to direct sunlight for long periods survive because they gain moisture in this way remains to be investigated.

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