The microstructure of avian eggshells, adaptive significance and practical implications in aviculture

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Introduction

In a discussion of 'problems concerned with eggs', Lack (1968) concluded that the following features of eggshells were probably adaptations for waterproofing: the chalky films on eggshells of grebes (Podicipedidae) and cormorants (Phalacrocorciidae); the powdery cover on those of flamingoes (Phoenicopteridae); the greasy surface of the duck's (Anatidae) egg, and the polished one of the lily-trotter (Jacanidae). The reasons for waterproofing were not discussed. The application of electron optics has shown that all but one of these eggshells share a common morphological feature, a layer of spheres on their outer surfaces (Board 1980; Board *et al.* 1977). The exception, the Lily-trotter *Micropara capensis*, has a dense crystalline outer border to the calcitic shell and the bell-shaped outer orifice of the pore canal is roughly plugged with amorphous material rich in sulphur (Board & Perrott 1979*a*). Although spheres are common to the other groups, chemical differences do occur. The spheres on grebes' eggshells contain amorphous calcium phosphate (Board *et al.* 1981) whereas those on the cormorant's shell contain an unusual form of calcium carbonate, vaterite (Tullett *et al.* 1976; Board & Perrott 1979*b*). It will be noted below that flamingo eggshells are covered with spheres containing amorphous calcium phosphate and a material rich in sulphur.

This communication has three objectives: (i) to discuss the problems faced by eggs laid in wet or muddy nests, (ii) to encourage field studies of the interplay between the microstructure of eggshells, the nest environment and bird behaviour, and (iii) to alert aviculturists to the possible danger in adopting the wrong methods for cleaning and disinfecting eggshells.

Problems faced by eggs

Independence of a need for external water can be considered the last major step in the evolution of the cleidoic eggs of birds (Needham 1931). Although such an egg is self-contained in respect of available water and nutrients for embryo development, its shell must be porous in order that the embryo can exchange respiratory gases with the nest environment. As the oxygen molecule is larger than that of water, the pores allow diffusion of water vapour and thereby depletion of the reservoir of water present when laid. Drent's (1975) review of field observations indicates that 16%, on average, of the reservoir is lost during incubation. The classic studies of Rahn and his collaborators (e.g. Ar & Rahn 1978) have shown that this loss is the outcome of an interplay between shell conductance (porosity), egg mass, nest humidity, incubation period and barometric pressure at the nest site. Indeed the weight of evidence can easily lead to the conclusion that avian eggshells are precisely adapted for water conservation, and that an embryo's requirements for oxygen are of secondary importance. The emphasis given to water conservation has been challenged by Simkiss (1980) who successfully hatched chicks of the domestic hen even though the allantois had been drained about two-thirds the way through incubation.

The cross-sectional area of an eggshell pore canal is never more than a few square microns and the majority of pores must remain open throughout incubation if the embryo's demands for oxygen are to be satisfied. A problem of equal importance to that of water conservation, therefore, is how the pore canals do not become blocked by mud, preening oils, nest debris or the dust arising from attrition between eggs. In this context, waterproofing would be but one of the prerequisites of a shell. Indeed there appears to be no record of waterlogging of shells leading to the asphyxiation of embryos, but the experiences of the poultry industry leave no doubt that the flooding of a few pores with contaminated water is the first step in the process leading to the addling of eggs (Board 1980; Board & Halls 1973). Moreover glycoproteins in the cuticle on hens' eggshells are colonized and digested by bacteria if the storage conditions are very humid (Board *et al.* 1979).

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Thus it would seem reasonable to assume that adaptations of eggshells will be influenced by the nest environment.

The nest of a flamingo, a mud platform alongside water rich in minerals, would offer a harsh environment to eggshells lacking appropriate adaptations. For example, if the developing embryo were contained in a calcitic shell in which the open orifice of the pore canal was flush with the shell's surface, as is in the pigeon *Columbia livia* (Board 1974), then blockage of the pores with mud, debris or the crystals remaining after evaporation of liquid brought to the nest by the parents would be a distinct possibility. The following discussion of the microstructure of flamingo eggshells supports the contention that they are adapted to counter these inimical features.

Flamingo eggshells

Examination of the eggshells of all extant species of flamingoes has shown that they share a common structure. The outer surface of the eggshell and the outer orifice of the pore canal are covered (Figure 1) with a thick layer of spheres (Figure 2); the transition between the calcitic shell and spheres is abrupt (Figure 3). Electron probe analysis has shown that the spheres are rich in sulphur and phosphorus (Figure 4), infra-red analysis that calcium phosphate is a component of the spheres, and X-ray diffraction studies that this phosphate is non crystalline.

If the structure shown in Figure 1 is considered as a diffusion pathway for res-

Figure 1. The radial face of a piece of snapped shell of Greater Flamingo as seen with a scanning electron microscope. Bar marker, 1000μm. S, layer of spheres; P, pore canal; C, cone layer, and SM, shell membranes. The numbers refer to probe sampling sites, see Figure 4.

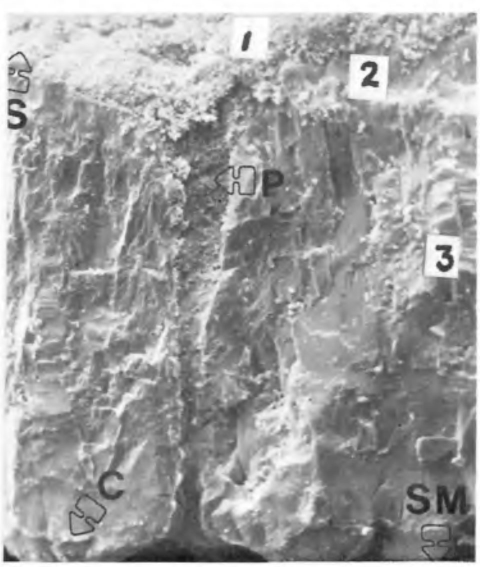


Figure 2. Details of the spheres occurring on the outer surface of the shell of Greater Flamingo. Bar marker, 2·5μm.

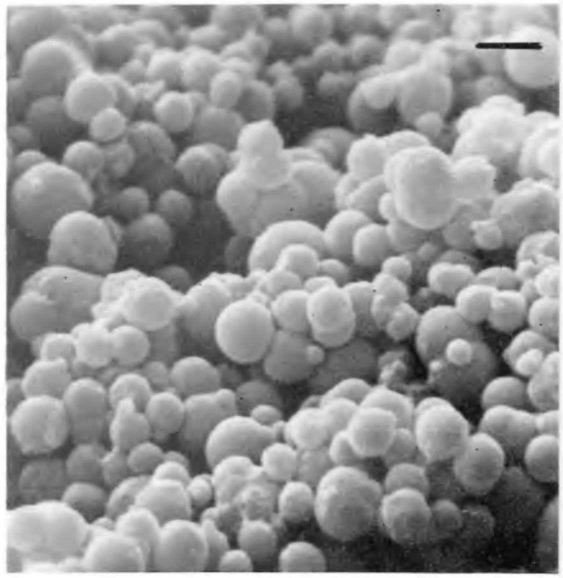
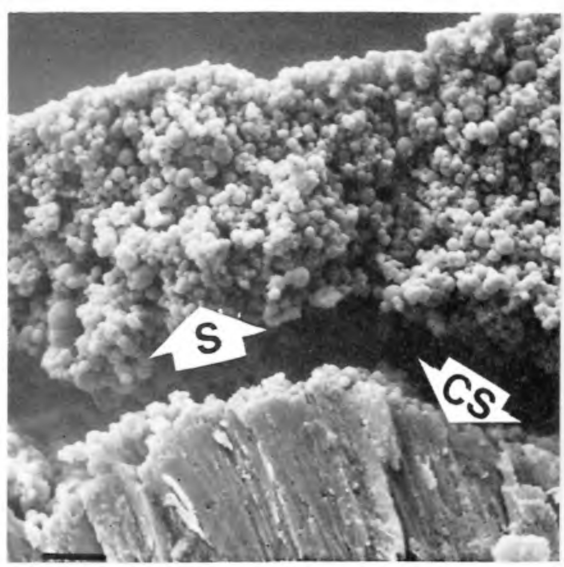


Figure 3. A scanning electromicrograph showing the abrupt change from the calcitic shell (CS) to the outer layer of spheres (S) on the eggshell of Greater Flamingo. Bar marker, 1μm.



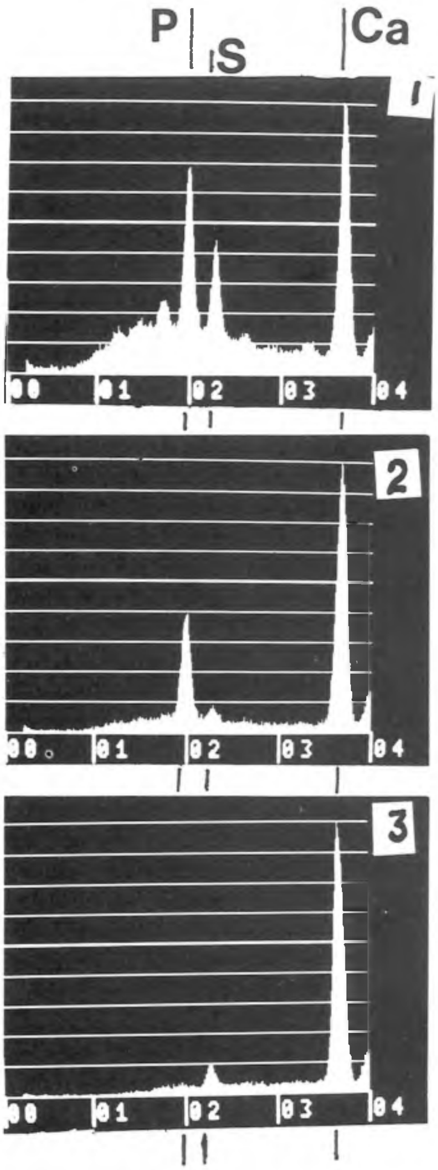


Figure 4. Elemental analysis of the eggshell of Greater Flamingo by electron probe analysis. Point analyses were done at the sites noted in Figure 1. The spheres contained relatively large amounts of calcium, sulphur and phosphorus (1). The outer edge of the calcitic shell contained calcium, phosphorus and a trace of sulphur (2) whereas the central region (in radial plane) of the shell contained no demonstrable amount of phosphorus, a small amount of sulphur and a large amount of calcium (3).

piratory gases and water vapour (Figure 5), then a series of resistances to the inward flux of oxygen can be identified: R_1, the layer of spheres on the shell surface; R_2, the pore canal, and R_3, the shell membranes. Judging from the studies of the role of glycoprotein spheres on the eggshells of domestic hens (Board 1980; Board & Halls 1973), R_1 can be tentatively identified as the resistance that prevents flooding of the pore canals. Such an interpretation would be in accord with that of Lack (1968). This resistance would also prevent the outer orifices of the pore canals from becoming blocked with extraneous materials. As the deposition of mud, debris, crystals etc. could be expected to increase the resistance that R_1 offered to the flux of respiratory gases, the question arises: how is this resistance prevented from attaining a value that would impede the embryo's exchange

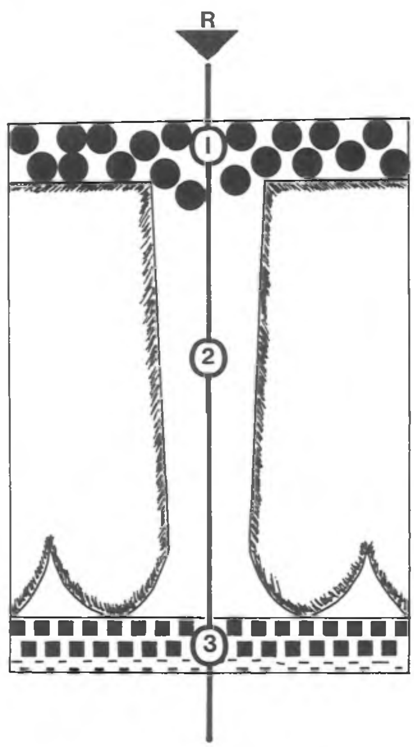


Figure 5. The eggshell of Greater Flamingo considered as a resistance network. Three major resistances, acting in series, are recognized: R_1, the layer of spheres on the outer surface of the shell; R_2, the pore canal, and R_3, the shell membranes.

of respiratory gases with the nest atmosphere? Several possible answers suggest themselves. For example, the deposition of debris on the surface of R_1 might increase the resistance locally such that the relatively direct diffusion pathway depicted in Figure 5 could not function; the pore canals would exchange with the void spaces in the sphere layer and these would exchange across the entire outer surface of the powdery layer. Alternatively the fissures present in the outer surface of this layer may be kept open as a consequence of egg turning, a possibility suggested by studies of the incubated eggs of Grebes (Board *et al.* 1981). A third possibility would be that through turning, the outer surface of the powdery layer is progressively worn down so that R_1 never offers a serious impediment to the flow of respiratory gases yet its thickness never diminishes to such an extent that the waterproofing of the eggshell is impaired.

Field studies

There would thus appear to be *a priori* reasons for assuming that eggshells are adapted to the nest environment and also to features resulting from the parents' own adaptations. Preening oils, for example, could presumably cause resistance R_1 (Figure 5) to increase progressively during incubation. Moreover attrition between eggs during turning can modify the shell's surface. Thus the cuticle on the shells of the Helmeted Guinea-fowl *Numidia meleagris* is worn away during incubation but the counter-sunk orifice of the pore canals protects the plug of cuticular material (Board & Perrott 1981). As this plug becomes stained during brooding by the parent, it would appear to be operating as a filter such that extraneous materials do not block the pore canals or contaminate the shell membranes. These observations suggest that there is a need to shift the emphasis in field studies concerned with the breeding biology of birds. To date much attention has been given to those features of bird behaviour that ensures heating, turning and protection of the eggs (White & Kinney 1974; Drent 1975; Howey *et al.* 1977). The present discussion suggests that behaviour alone cannot assure the well-being of the developing embryo and that shell adaptations compensate for those factors that the parents cannot control or, on occasions, may even accentuate.

One possible approach in field studies would be to modify eggshells—i.e. remove the layer of spheres on the eggshells of waterfowl—and monitor weight loss with them. Changes in the fine structure of the shell's surface during incubation would be another approach and the non-destructive technique introduced by Board (1981*a*) could make a useful contribution to such studies. The swapping of eggs could be another approach but probably the most useful method, as suggested by Board (1981*b*), would be the use of dummy eggs to measure changes in the conductance and the content of extraneous material of the 'shell'.

Practical implications

It would seem reasonable to assume that those practices adopted by the poultry industry would be appropriate should there be a need to wash eggs. Wash water should be maintained at a temperature greater than that of the egg (Board 1980). The presence of phosphates in the covers on the eggshells of flamingoes would presumably inactivate certain of the quarternary ammonium compounds that are used as disinfectants. Thus there would appear to be a need for studies of disinfectants so that ones appropriate for the treatment of waterfowl eggs can be identified.

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Summary

Eggs laid in 'wet places' need to be adapted so that the pore canals in the calcareous shell are not flooded with water or occluded with mud, nest debris, preening oils or salts. The shell of the Greater Flamingo *Phoenicopterus ruber roseus* was taken as an example. Its surface layer of spheres rich in calcium, phosphorus and sulphur is probably the adaptation that fits the egg to the nest environment. Field studies could establish the extent of the contribution of the outer layer of spheres to the well-being of the embryo.

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