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Vaterite, a Constituent of the Eggshells of the Nonparasitic Cuckoos, *Guira guira* and *Crotophagi ani*

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Summary. The crystalline spherules occurring in patches on the outer surface of the eggshells of *Guira guira* and the bloom on the entire outer surface of those of *Crotophagi ani* were shown by X-ray diffraction analysis to be vaterite.

Key words: Vaterite — Calcium — Eggshells — Cuckoos.

Introduction

Of the three polymorphs of calcium carbonate, calcite, aragonite, and vaterite, the first mentioned is the principal inorganic constituent of avian eggshells [1], and the second is the major component of the calcified shell of reptile eggs [2]. Both polymorphs occur in fossils [3]. The unstable [4] polymorph, vaterite, occurs rarely in sediments [5] or biological materials [6-9]. It has been found, for example, in the reproductive system and egg capsules of the ampullarid snail [9-11] and in the eggshells of the Brown pelican [12]. The latter study did not associate vaterite with a particular part of the shell, but subsequent studies [13] of members of the Pelecaniformes revealed that this polymorph was the major component of a stratum of spherules which overlay the true calcitic shell of the eggs of the Cormorant (*Phalacrocorax carbo*), Shag (*Ph. aristotelis*) and Gannet (*Sula bassana*). Indeed, the fine structure of this outer stratum was sufficiently distinctive for these shells to be assigned to a separate category in an arbitrary classification of eggshells [14]. Vaterite was found also [13] in the mineral deposit on the outer surface of "shell-less" hens' eggs and on a domestic duck egg which had a thin layer of albumen and shell membranes around the outside of a normal shell. From analyses of minerals other than Ca^{2+} in the eggshells of a range of birds, it was deduced [13] that phosphate, a poison of calcite formation [15], was perhaps the cause of the abrupt change in the crystal form in which $CaCO_3$ is laid down on the eggshells of members of the Pelecaniformes. As the size and life style of these sea birds preclude studies of the physiology of their shell gland or the ionic environment of its lumen, the pertinence of the phosphate hypothesis in nature cannot be gauged. Moreover, the conditions leading to the aberrant "shell-less" egg in domestic hens would provide only a part of the story. It is known [16] that the composition of the fluid in the shell gland of domestic hens changes during the period that an eggshell is forming. Thus, for example, the Mg^{2+} concentration increases, a change which is reflected in the eggshell. The concentration of this element rises progressively from a low level in a narrow band immediately behind the inner surface to a peak at the outer surface of the shell [17, 18]. As this feature is not common to the eggshells of all birds [19], there may be important interspecific differences not only in the ionic environment in the shell gland, and changes thereof during shell formation, but also in the means whereby calcite formation continues even though an inhibitor, such as Mg^{2+}, is present.

The appearance of the eggshells of the nonparasitic cuckoos, *Guira guira* and *Crotophagi ani*, has always intrigued egg collectors and curators who have to factor large collections of birds' eggs. The shells of *G. guira* merit the vernacular description, Wedgewood pottery, the blue surface of the shell is overlayed with irregularly shaped white patches. A delicate white bloom covers the shells of *C. ani;* the bloom contains many fine scratches through which the blue color of the underlying shell can be seen. These unusual features attracted our attention because of our interest in the fine struc-

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Table 1. Diffraction patterns of crystals from eggshells compared with synthetic vaterite

Crotophagi ani		Blue-eyed Shag		*Guira guira*		Synthetic vaterite[a] Turnbull (4)		Synthetic vaterite Meyer (36)	
d-spacing A	I/I_1	d-spacing A	I/I_1	d-spacing A	I/I_1	d-spacing A	I/I_1	d-spacing A	I/I_1
4.24	6	—	—	4.24	11	4.23	24	4.23	21
3.57	55	3.57	73	3.56	61	3.57	59	3.57	57
3.29	82	3.30	100	3.28	100	3.29	100	3.30	100
2.73	100	2.73	69	2.72	85	2.73	97	2.73	94
—	—	—	—	—	—	—	—	2.32	6
—	—	—	—	—	—	—	—	2.22	6
—	—	—	—	2.109	7	2.113	7	2.117	15
2.063	47	2.061	58	2.059	52	2.061	66	2.065	62
1.855	12	—	—	1.850	20	1.851	19	1.858	26
1.824	32	1.825	52	1.821	42	1.818	52	1.823	72
1.646	11	—	—	1.647	14	1.643	28	1.647	26

[a] Specimens prepared in the laboratory

ture of avian eggshells in general [13, 14], and our studies have shown that the appearance of the eggshells can be accounted for in terms of an abrupt change from the true (calcitic) shell to an outer cover formed from vaterite. Not only has this study identified birds other than Pelecaniformes that have eggshells made from two of the polymorphs of calcium carbonate but, as both species of nonparasitic cuckoos can be kept in captivity, it has indicated the possibilities for systematic studies of the conditions in vivo which cause this unique switch in crystal form. Moreover, such studies would provide also an opportunity for studying the chemistry of vaterite formation in a warm-blooded animal with the emphasis on a normal rather than on the abnormal situation which results in this polymorph being deposited in gallstones [20, 21], kidney stones [22, 23] and calculi of the bladder [24], etc. It would seem, also, that studies of vaterite formation in warm-blooded animals would provide information which would complement that which to date has been derived from studies of invertebrates [9].

Materials and Methods

Fragments of the eggshells of *Guira guira* and *Crotophagi ani* were obtained by courtesy of Dr. C.J.O. Harrison of the British Museum, London, and those of the blue-eyed shag (*Phalacrocorax atriceps*) were collected (December 1975) in the Antarctic by members of the British Antarctic Survey, Cambridge, England.

SEM Observations

Samples of powders were sprinkled on the surface of double-stick tape mounted on aluminum stubs and coated under vacuum with Au. Pieces of eggshell were glued (DAG 915; Acheson Colloids Ltd., Princes Rock, Plymouth, England) to aluminum stubs and coated under vacuum with Au. A Steroscan S4 (Cambridge Scientific Instruments, Cambridge, England) operating at an accelerating voltage of 10 or 20 kV was used.

X-ray Diffraction

The sample was packed in a glass capillary and examined by the Debye-Scherrer method using copper K_α radiation, at 40 kV and 20 mA. Samples of vaterite prepared by the method of Turnbull [4] were examined in the SEM and analyzed for purity in an X-ray diffractogram.

Results

To the naked eye there was a marked difference between the outer surface of the eggshells of *G. guira* and *C. ani*. The blue surface of the shells of *G. guira* was overlayed with irregularly shaped white patches. A faint white bloom coated the shells of *C. ani*. As boiling with NaOH (5% w/v) did not change the appearance of the eggshells, at least to the unaided eye, it was concluded that the white material was inorganic. This was confirmed by SEM studies and X-ray diffraction analysis of powder scraped from untreated shells. The X-ray diffraction patterns obtained with the Debye-Scherrer camera were essentially identical with those of vaterite prepared in the laboratory (Table 1). X-ray diffractograms of this preparation showed that it contained only a trace of calcite; crystals of the latter were not seen during SEM examination.

The fine structure of the outer surface of the cuckoo eggshells was masked by a film which was removed in boiling NaOH (5% w/v). The origin of the film is not known; it may have been acquired in

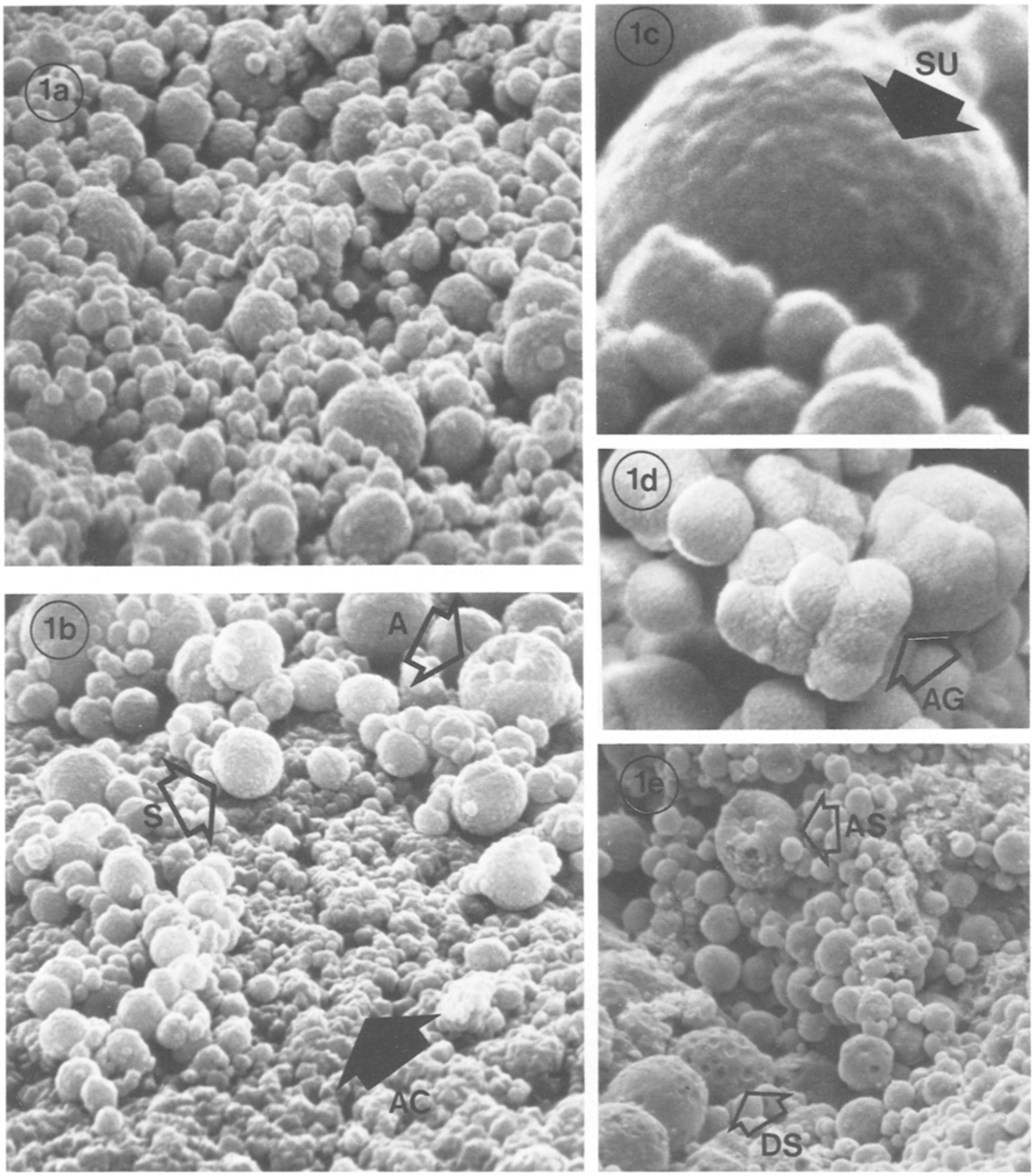

Fig. 1. Electron micrographs of vaterite spherules. **a** A general view of the spherules of a white patch on the surface of the eggshell of *Guira guira*; × 5200. **b** Spherules, both simple (S) and aggregates (A) at the edge of a white patch on the eggshell of *G. guira*. The angular crystals (AC) at the surface of the true shell are evident; × 5300. **c** A spherule from the bloom on the eggshell of *Guira guira* showing spherical units (SU) at the surface; × 21,000. **d** Aggregate spherules (AG) in vaterite prepared by the method of Turnbull [4]; × 5000. **e** Dimpled spherule (DS) and aggregate spherules (AS) in the cover on the eggshell of the blue-eyed shag; × 2400

the nest or during the many years the eggs were stored in a museum. When the film was removed, it was evident that the outer surface of the eggshell of *C. ani* was covered with spheres. Spheres occurred only in the white patches (Fig. 1a) on the outer surface of the eggshells of *G. guira;* the areas between these patches had a rough surface due to the angular form of the surface crystals (Fig. 1b). X-ray diffraction of the material scraped from these areas gave the pattern characteristic of calcite.

As there was no difference in the appearance of the spherules observed in situ on shells cleaned with boiling NaOH and those removed by scraping with a sharp scalpel, it was concluded that the removal of the film had not changed the fine structure of the stratum of spheres overlying the calcitic shell. It is evident from Fig. 1 a and b that there was a wide range in the size of the spherules and that two or more spherules had fused during formation. A much higher incidence of fused spherules was evident (Fig. 1d) in the vaterite prepared in the laboratory by the method of Turnbull [4]. Indeed, the conglomerates of spherules in this preparation were similar to those found in vaterite recovered from snails [8]. The large dimpled spherules which were a notable feature in the cover on the eggshells of cormorant, shag, and gannet [13] and blue-eyed shag (Fig. 1e) were not seen in the material on the cuckoo eggshells. The large spherules of the latter had a composite appearance when studied at high magnification (Fig. 1c), and in this respect the topology of their surface resembled that of the vaterite prepared in the laboratory. Further evidence that the spherules were polycrystalline came from examination of a spherule which had been fractured when scraped from the eggshell of a cuckoo. It was seen to be formed from fused spheres. In this respect, the spherules on cuckoo eggshells resemble those taken from the reproductive system of ampullarid snails [10].

It was evident from SEM examination of radial sections of cuckoo eggshells that the deposition of calcite in the outer crystalline layer of the shell did not end abruptly; the outer surface was formed from angular crystals of calcite (Fig. 1b; Fig. 2a, c) which gave a rough border between the calcite shell and the stratum of spherules. Not only did the spherules rest on a rough surface, they were present also in the outer part of the pore canal (Fig. 2b, d). In the latter situation there was a suggestion that spherules may well have been forming at the same time as the calcite. Thus some spherules appear to be embedded in the wall of the pore canal (Fig. 2d).

Discussion

The present study of the eggshells of two species of nonparasitic cuckoos, *Guira guira* and *Crotophagi ani*, has shown that a switch from the deposition of calcite to that of vaterite during formation of an eggshell occurs in birds other than members of the Pelecaniformes [13]. It adds, moreover, to the rather restricted list of biological materials in which vaterite is laid down naturally [6–9] rather than as a response to damage, as in mollusc shells [11], or in pathological conditions such as gallstones [20, 21], kidney and related calculi [22–24], and aberrant otoliths of cod [25]. This study raises two questions: Why is vaterite formed? How is vaterite formed?

To account for vaterite on the eggshells of gannet and related species, Tullett et al. [13] invoked an argument about the need for some sea birds (e.g., the cormorant) to avoid large increases in the amount of storage calcium in the bones during the breeding season so that there was not a drain on the energy required for flight. Likewise, the structural strength of the skeleton of the gannet, birds which plunge from upwards of 50 feet when catching their prey in the sea, was put forward as a possible reason for these birds having vaterite in their eggshells—the specially adapted bones being unable to accommodate sufficient reserves of Ca^{2+} for shell formation. In both examples, it was deduced [13] from analyses of the phosphate content of the shell that release of this substance during the breakdown of skeletal rather than medullary bone would lead to levels in the lumen inhibitory to calcite formation. These arguments as to the cause of vaterite formation would seem to be inapplicable to the eggs of the nonparasitic cuckoos and, if there is a *raison d'être* for the occurrence of vaterite, some selective pressure outwith the basic physiology of the parent has to be sought. Although it is not yet possible to identify such a pressure, the nesting habits of the nonparasitic cuckoos might be expected to pose problems to the egg. Thus the naturalists' description of nests containing upwards of 30 eggs intermingled with vegetation might be construed as evidence that the shell requires protection from mechanical damage due to collisions with other eggs and from microbial colonization of its outer surface. It has been demonstrated [26] that an eggshell covered with vaterite spherules is remarkably resilient to damage from repeated assault with a ball bearing, but the resilience is lost when the cover is removed. Although the author did not speculate as to the nature of the defense offered by the cover, it would seem reasonable to assume that the vaterite spherules collapsed under impact thereby protecting the underlying calcite. It has been noted [27] also that microorganisms digest the organic cuticle when they grow on the surface of the hens' eggshell. As digestion frees the pores of capping material, the contents of the eggs are rendered liable to microbial infection. It may be therefore that selection has favored a shell with an inorganic cover on which microbial colonization, leading to a weakening of the physical defenses of the egg, cannot occur. The surmise [13] that the poisoning of calcite formation by phosphate leads to an abrupt switch from calcite to vaterite formation on the eggshells of members of the Pelecaniformes does not take into account all the factors which can influence the crystal form of calcium carbonate. Now that two species of birds offering the prospect of laboratory studies are available, future studies can be put into a wider perspective.

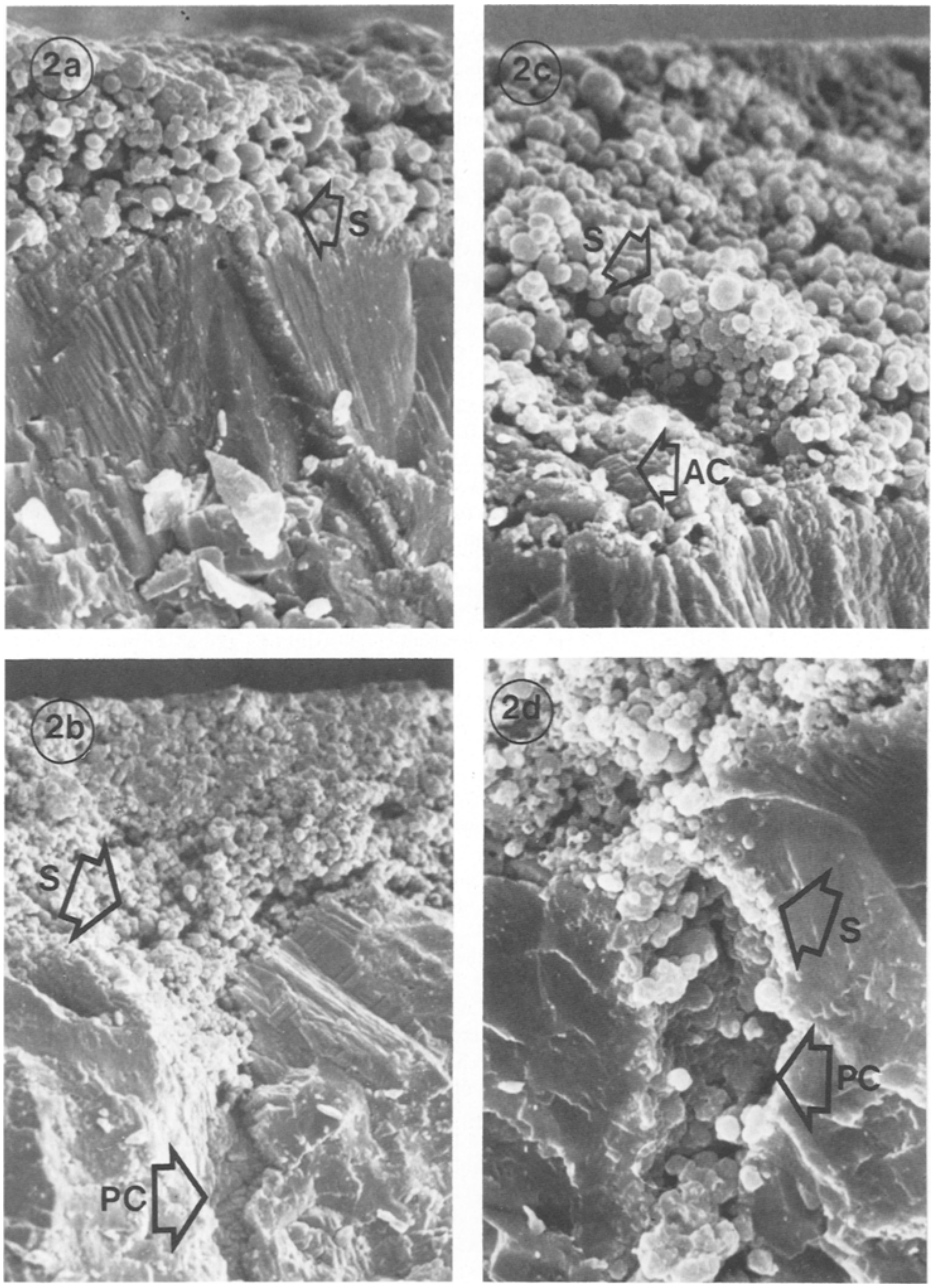

Fig. 2. Electron micrographs of the outer edge of radial sections of the eggshell of *Guira guira* and *Crotophagi ani*. **a** Spherules (S) on the outer surface of the eggshell of *C. ani*; × 2000. **b** A stratum of spherules (S) overlying the outer orifice of a pore canal (PC) in the eggshell of *C. ani*; × 1150. **c** Spherules (S) overlying angular crystals (AC) on the outer surface of the eggshell of *G. guira*; × 2400. **d** Spherules (S) embedded in the wall of the pore canal (PC) in the eggshell of *G. guira*; × 2000

Although vaterite can now be regarded as a naturally albeit rarely occurring form of calcium carbonate in biological materials, little is known about the conditions leading to its deposition or, for that matter, to the maintenance of this metastable polymorph [28] once it is formed. Unlike calcite and aragonite, the vaterite crystal is disordered [29]. It has been suggested [30] that its hexagonal symmetry with a pseudo cell [31] would cause stacking problems along the c-axis. There is ample evidence of its instability when in contact with water [4, 32]. The properties of vaterite have been determined on material obtained by either rapid precipitation [4] or slow ionic diffusion [30]. With rapid separation from the mother liquor and drying, the former method gives mainly vaterite whereas ionic diffusion gives a mixture of all three polymorphs. It has been demonstrated repeatedly that cations such as Ba^{+} and Mg^{2+} tend to increase the proportion of vaterite [33, 34]. When examining factors that might determine which of the polymorphs would form in sea water, Simkiss [35] noted that Mg^{2+} favored the formation of aragonite and a low pco_2 of vaterite during the relatively slow precipitation of crystals in synthetic sea water. In biological systems the influence of the ionic environment, pco_2, temperature, etc. may well be muted or accentuated by nucleation sites provided by organic material. Indeed, this has been shown by Kitano [34], and in the review by Watabe [8] of crystal growth of calcium compounds and biological systems, emphasis was given to the important role of a fibrous organic matrix in the crystallization process in general. Along with collaborators, this worker demonstrated [9] a matrix formed of proteins and acid mucopolysaccharides in the vaterite-rich spherules in *Pomacea palludosa*. Thus future studies of the conditions leading to vaterite formation in the lumen of the oviduct of *G. guira* and *C. ani* would have to give at the outset equal weighting to the ionic environment of the uterine secretion and its content of fibers having the potential to provide nucleation sites.

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