A Comparative Study of the Ultrastructure and Mineralogy of Calcified Land Snail Eggs (Pulmonata: Stylommatophora) [1]

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ABSTRACT This study indicates that eggs containing calcium carbonate crystals occur in at least 36 of the 65 known families of the land snails (class Gastropoda: order Stylommatophora). Eggs from 22 of these families were available for examination. The x-ray diffraction data, available for the first time for 21 of these families, shows that these egg shells are all made of calcite only, or of a combination of calcite with smaller amounts of aragonite. All of the snail (body) shells examined were made of aragonite only. This is the first ultrastructural investigation of these egg shells, and it indicates that the eggs exhibit enough structural diversity to allow identification of parental animals to genus, and often to species level solely on the basis of egg shell ultrastructure.

All of the calcified eggs may be divided into two groups: (1) partly calcified, with discrete crystals of $CaCo_3$ dispersed in the jelly layer, and (2) heavily calcified, with a hard, brittle egg shell made of fused crystals of $CaCO_3$ much like an avian egg. Both types of calcified eggs occur in oviparous as well as in ovoviviparous snails. Because of the wide distribution of calcified eggs in the Stylommatophora, and because of the occurrence of heavily calcified eggs in ancient families such as Partulidae, Endodontidae, and Zonitidae, the calcified egg is viewed as a primitive land snail trait associated with terrestrial adaptation. The function of the calcified egg shell, in addition to mechanical support of egg contents, is to supply the developing embryo with enough calcium to form the embryonic shell by the time of hatching.

The land gastropods are probably the single largest group of molluscs; Boss ('71) has critically estimated the order Stylommatophora to number 15,000 out of the total of 46,810 species of molluscs. This is a very diverse group, indicated by the fact that Taylor and Sohl ('62) list 65 families and 2,184 genera or subgenera in this order. That a number of these snails deposit eggs which have calcium carbonate crystals in the outer layer has been known since the work of Swammerdam in 1737 and Spallanzini in 1803 (Turpin, 1832).

The structure and mineralogy of land snail [3] eggs has received little attention, although the eggs of the freshwater pulmonates (all uncalcified) are the subject of an entire monograph (Bondesen, '50). In 1917, Standen gave a list of some snails which form calcified eggs, but this work is rather limited. Kelly ('01) examined two land snail eggs and found that they were made of calcite, with some calcium phosphate. Prenant ('27) also examined several eggs and found them to be calcite; Alaphilippe and Michon ('63) noted that the egg of *Calachatina* contains calcite. Bayne ('66, '68a)

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[3] In this paper, the terms "land snail" and "land pulmonate" are used synonymously with "Stylommatophora."

examined histochemical and biochemical properties of land snail eggs, some of which are of the calcified type.

The purpose of this paper is to document the ultrastructural and mineralogical characters of calcified eggs from the whole order of land snails for the first time.

MATERIALS AND METHODS

Whenever possible, eggs were obtained in a fresh state, immediately following deposition by snails cultured in the laboratory. In some cases, only dried museum specimens were available. In a few cases, as for *Strophocheilus oblongus*, dried out museum specimens were compared with freshly deposited material to assess possible changes in ultrastructure or mineralogy — no changes were seen.

For scanning electron microscopy, eggs were usually air-dried or freeze-dried. Often, the material was treated with 5% sodium hypochlorite to remove adherent organic material, debris, and the outer jelly layer where applicable. Then, the eggs were coated with gold metal and examined in a JEOL JSM U-3 model scanning electron microscope at 15 kv or 25 kv.

For powder x-ray diffraction, the egg shells were first mechanically separated from the outer jelly layer when present, and from the inner albumen fluid, and then treated with 5% sodium hypochlorite to remove organic substances. The shell was then gently crushed to a powder and packed into lithium borate glass tubes of 0.5 mm diameter. Operating conditions for the diffraction were 35 kv, 15 mA, for three to six hours, using Cu Kα radiation with a Ni filter; the Debye-Scherrer camera was loaded with Kodak Medical X-ray film in the Straumanis method (Cullity, '56). Line positions were measured and the material was identified by the Hanawalt method, using ASTM cards (op. cit.). The diffraction patterns were also compared with known calcite and aragonite preparations. Several mixtures of calcite and aragonite were made in different proportions, and it was found that a minimum of about 4% aragonite in calcite was needed to just barely identify the major (3.4Å) line of aragonite. In a few cases, the shells of some snails were examined with a diffractometer. However, this method was not used extensively since it is not as sensitive as the Debye-Scherrer method and also requires considerably larger amounts of sample.

For electron diffraction, several pieces of an egg of *Strophocheilus oblongus* were embedded in Spurr resin (Spurr, '69) and sectioned with a diamond knife. Selected area electron diffraction of the inner layer was obtained using a JEOL 100B microscope, operating at 80 kv, mag 3, camera length 2, using gold metal standard (See JEOL Instruction Manual for 100B).

The sources of the eggs are given in table 1. For 13 families of snails, freshly deposited eggs were available; these are all of the ones collected by the author (labeled "A" in table 1), and also *Strophocheilus oblongus, Rumina decollata, Agriolimax reticulatus, Varohadra yeppoonensis,* and *Cepaea nemoralis.* A more detailed account of some of the materials and methods can be found in Tompa ('74a).

RESULTS

Mineralogy

The list of specimens which have been examined by x-ray diffraction is given in table 1. As can be seen, eggs of 30 genera representing 22 families of the order Stylommatophora have been examined; (body) [4] shells from 12 of these families were also investigated.

All of these eggs gave diffraction patterns of either purely calcite or of calcite with traces of aragonite. In all cases, while the calcite patterns were extremely strong, showing about 20 of the major lines (fig. 1C), the aragonite was represented only by its 3.4 Å line, or occasionally also by its 3.3 Å, 2.7 Å and 1.98 Å lines, barely visible (fig. 1D). Aragonite was detected in freshly deposited eggs laid by snails kept in the laboratory as well as in dried out museum specimens whose handling was not known,

[4] The term "body shell" is used to denote the shell of the animal, to avoid confusion with the "egg shell."

so that the presence of aragonite is not considered to be an artifact. Moreover, calcite is generally known to be so stable that it does not change into aragonite under most conditions. Interestingly, both partly calcified and heavily calcified egg types[5] showed aragonite traces (table 1).

From the appearance of the crystals when viewed with the scanning electron microscope, it appears that only calcite is found in the outer layer of the egg shell in most cases (figs. 9, 14, 16, 17, 28). The locus of aragonite is not easily determined in most of the eggs, but must be in the middle and/or inner layers. In the egg of *Strophocheilus oblongus,* however, the innermost layer appears to have aragonitic looking ultrastructure (fig. 23; see also Tompa, '75). Selected area electron diffraction of this inner layer confirmed the presence of aragonite mixed with calcite.

The body shells of snails from 12 families were also analyzed by powder diffraction using the Debye-Scherrer camera method (table 1). In every case, the shells have only the aragonite pattern (fig. 1A). The protoconch (embryonic shell) of an *Anguispira alternata* embryo was also examined and found to be made of aragonite (fig. 1B).

Egg shell ultrastructure

The structure of heavily calcified eggs is seen in plates 2 to 7. Among the most interesting is the egg shell of *Anguispira alternata,* seen in figures 6 to 9. The outermost layer of the egg is a gelatinous material which swells readily in water (fig. 6) and presumably functions in keeping the egg from drying out. This jelly layer can be removed either chemically (by sodium hypochlorite) or mechanically. Then it can be seen that the egg shell outer surface is composed of large calcite crystals (figs. 7,8). If an egg is fractured, a view of its edge reveals that there are actually three layers which form the egg shell: an outer layer of large crystal facets, a large middle layer, and a thin inner layer of small crystals (fig. 9). The middle layer commands particular attention because it is composed of layers of crystals alternating regularly with layers of organic material, as can also be seen in *Zonitoides nitidus* (fig. 13). The innermost layer of the *Anguispira* egg is made of very small, thin crystals. The details of egg shell formation and the induction of abnormal calcification are given by Tompa ('76b).

The ultrastructural features of the heavily calcified eggs are surprisingly varied among the species examined. Often, the shells of the eggs achieve a complexity of organization which parallels and even surpasses that of the avian egg (figs. 22-24, 26, 27). For example, pores are evident in several species of *Euglandina* (fig. 27), in *Acavus superba* (fig. 24), and in *Strophocheilus oblongus* (fig. 22). In other cases the functional equivalents of pores are presumed to exist (because of the rapid desiccation of these eggs when exposed to air convection) between the columns or stacks of crystals, but these are not morphologically clear (e.g., figs. 8, 9).

Most heavily calcified eggs have an outer hygroscopic jelly layer which prevents desiccation. However, although the giant egg of *Strophocheilus* does not have such a layer, it is actually very resistant to desiccation. Whether this egg has a very thin outer cuticle is not yet clear.

Partly calcified eggs, such as those of *Triodopsis multilineata* are often laid in a collapsed state (fig. 2) and imbibe water from the moist soil until they become spherical. They then become so turgid that they bounce several times when dropped onto a hard surface. The ultrastructure of these eggs is quite interesting, but difficult to deal with technically (almost impossible to section). The crystals are embedded in the jelly layer, but can be removed by dissolving the supporting jelly with sodium hypochlorite (fig. 3). When examined, they show innumerable fine pores (fig. 5) from penetration by organic material. Nevertheless, such crystals give a sharp x-ray diffraction pattern when examined with the powder method, suggesting that each

[5] See DISCUSSION for the definition of partly calcified and heavily calcified eggs.

TABLE 1

List of stylommatophoran land snails which form calcified eggs; those without a reference are from the present study

	Egg shell		Body shell			
	calc	arag	calc	arag	Source	Reference
Order Stylommatophora						
Suborder Orthurethra						
Fam. Partulidae						
Partula sp.	+	tr	–	+	JC	
Samoana sp.	+	tr			JC	
Fam. Cionellidae						
[a] *Cionella lubrica*	+	–	–	+	A	
Fam. Valloniidae						
Vallonia costata	+	tr	–	+	A	
V. pulchella	+	tr	–	+	A	
Fam. Achatinellidae						
Auriculella turritella	+	tr			U	
Fam. Amastridae						
Leptachatina cersalis	+	tr			U	
Fam. Pyramidulidae						(Standen, '17)
Suborder Mesurethra						
Fam. Filholiiddae						(Cox, '60)
Fam. Dorcasiidae						(Standen, '17)
Fam. Strophocheilidae						
Caryodes dufresii					U	
Strophocheilus oblongus	+	tr	–	+	U,MC,B,	
Suborder Sigmurethra						
Fam. Subulinidae						
Euonyma lymnaeiformis					U	
E. platyacme					U	
Homorus pileatus					U	
Opeas gracilis					U	
O. tugelense					U	
Prosopeas achatinacean					U	
P. paioense					U	
P. roepstorfi					U	
Pseudoglessula cyanostoma					U	
Rumina decollata	+	–	–	+	JB	
Subulina octona					U	
S. striatella					U	
Tortaxis pilsbryi					U	
Fam. Achatinidae						
Achatina fulica	+	–			U,YK	
A. zebra					U	
Cochlitoma simplex					U	
Limicolaria saturata					U	
Fam. Streptaxidae						(Berry, '63,'65a)
Fam. Acavidae						
Acavus superba	+	–	–	+	U	
A. waltoni					U	
Fam. Haplotrematidae						
Haplotrema concavum	+	–			A	
Fam. Rhytididae						
Paryphanta busbyi	+	–	–	+	U	
Fam. Bulimulidae						
Bulimulus apodometes	+	tr	–	+	U	
Fam. Orthalicidae						
Liguus incisus					U	
Oxystylus floridanus	+	–			U	
Fam. Urocoptidae						
Coelocentrum gigas	+	tr			U	

TABLE 1 *(continued)*

List of stylommatophoran land snails which form calcified eggs; those without a reference are from the present study

	Egg shell calc	Egg shell arag	Body shell calc	Body shell arag	Source	Reference
Fam. Endodontidae						
Anguispira alternata	+	–	–	+	A	
A. kochi	+	–	–	+	A	
Discus cronkhitei					A	
D. patula	+	–			A	
* *Helicodiscus notius*					A	
* *H. parallelus*					A	
Fam. Arionidae						(Quick, '60)
Fam. Philomycidae						
* *Philomycus carolinianus*					A	
Fam. Zonitidae						
Mesomphix subplanus	+	tr			U	
Retinella sp.	+	–			A	
Ventridens ligera	+	tr			A	
Zonitoides arboreus					A	
Z. nitidus	+	–	–	+	A	
Fam. Parmacellidae						(Standen, '17)
Fam. Milacidae *						(Focardi, and Quattrini, '72)
Fam. Limacidae						
* *Agriolimax reticulatus*	+	–			HW	
Fam. Helicarionidae						
* *Helicarion cuvierii*	+	–			BS	
Fam. Ariophantidae *						(Ghose, '60)
Fam. Urocyclidae						(Standen, '17)
Fam. Testacellidae						(Stokes, '58)
Fam. Polygyridae						
* *Allogona profunda*	+	tr			A	
* *Mesodon inflectus*					A	
* *M. zaletus*	+	–			A	
* *Polygyra septemvolva*	+	–			A	
* *P. texasiana*					HH	
* *Stenotrema fraternum*					A	
* *S. hirsutum*					A	
* *S. leai*					A	
* *Triodopsis albolabris*					A	
* *T. fraudulenta*					A	
* *T. multilineata*					A	
Fam. Oleacinidae						
Euglandina guttata					U	
E. rosea					A	
E. texasiana	+	tr	–	+	U	
Fam. Sagdidae						(Standen, '17)
Fam. Camaenidae						
* *Chloritis victoriae*					BS	
* *Varohadra yeppoonensis*	+	–	–	+	JB	
Fam. Bradybaenidae						(Standen, '17)
Fam. Helicidae						
Cepea nemoralis	+	tr			HV	
* *Helix aspersa*					A	
* *H. pomatia*	+	–			A	
Fam. Thysanophoridae						(Standen, '17)

Sources are given in ACKNOWLEDGMENTS.
(*) indicates a partly calcified egg; (tr) indicates trace composition;
(+) indicates major mineral component; (–) indicates none found.

"crystal" is itself a composite of minute particles.

It should be noted that all land snails thus far examined deposit individual eggs (i.e., each ovum is surrounded by its own egg shell or distinct jelly layer) and not egg masses or capsules, such as occurs in the freshwater pulmonates, where the aggregate eggs are surrounded by a common jelly envelope (Bondesen, '50) and in most prosobranchs.

Occurrence and size range

On the basis of this study and compilations from the literature, it seems that at least 36 of the 65 families of land snails have species with calcified eggs (table 1), representing three out of the four suborders. The most noticeable absence of calcified eggs is in the suborder Heterurethra. Thus far, only members of the successful family Succineidae, *Succinea* spp., *Omalonyx* sp., and *Oxyloma* spp., have been examined in this suborder; specimens from the other two families represented by five (mostly rare) genera were not available.

Especially interesting is the nature of slug eggs, since slugs, with a reduced or completely absent shell, have much less calcium available in their body for egg shell formation. The Testacellidae, an extremely unusual, specialized group, all deposit heavily calcified eggs. The only other slug which is presently known to form heavily calcified eggs is *Arion ater*, of the family Arionidae; most of the other members of this family form uncalcified eggs and a few have partly calcified eggs. Among the Limacidae, *Agriolimax agrestis* and *A. reticulatus* lay eggs which are speckled with calcium carbonate crystals (Quick, '60), but most species' eggs are not calcified. *Milax gagates* of the Milacidae is partly calcified (Focardi and Quattrini, '72), but there is very little information available for the rest of this group. Among the Philomycidae, the egg of *Philomycus carolinianus* is partly calcified. In the slug family Parmacellidae, the genus *Cryptella* is reported to have calcareous particles in the egg (Standen, '17).

The range of sizes for heavily calcified eggs spans two orders of magnitude, from 0.5 mm to 50 mm in maximum diameter. The largest land snail egg belongs to *Strophocheilus (Borus) popelairianus* of Colombia and Ecuador (Bequaert, '48); this egg is up to 51 mm long and 28-35 mm wide. Other giant eggs include to 20 × 30 mm eggs of *Strophocheilus oblongus*, and the 16 × 22 mm egg of *Acavus superbus*, which are approximately 0.30 and 0.25 mm in thickness respectively. The smallest eggs examined in this study are the heavily calcified eggs of the genus *Vallonia*. *Vallonia pulchella* eggs have been measured at 0.66 × 0.62 mm, with a thickness of only 10 μm (Tompa, '76a).

It appears that the largest eggs of land snails are all heavily calcified while the uncalcified and partly calcified eggs are usually between 1 and 8 mm maximum diameter (personal observations). One of the functions of the shell, therefore, is to support the large volume of egg albumen.

Taxonomic investigation of egg shell ultrastructure

In some cases, eggs of several species of a given genus were available for investigation, making it possible to examine species level characters. In the genus *Euglandina*, the eggs of the three species examined, *E. guttata*, *E. rosea*, and *E. texasiana* are distinctly different and identifiable on the basis of egg shell ultrastructure. For lack of space, only *E. rosea* and *E. guttata* are illustrated (figs. 26, 27); details can be found in Tompa ('74a). In the family Endodontidae, the eggs of *Anguispira alternata* (figs. 6-9) are also readily distinguishable from those of *A. kochi* (figs. 10, 11). In this same family, the eggs of *Discus patula* (not shown) could be differentiated from *D. cronkhitei* (Tompa, '74a). In the Achatinidae, the eggs of *Achatina fulica* (figs. 18, 19) and *A. zebra* (not shown) could be distinguished on the basis of size; ultrastructural comparisons were not conducted in this family.

Exceptions to this species level specificity were found. For example, in the genus *Vallonia*, the eggs of *V. costata* (figs. 14, 15) were found to be identical to the eggs of *V. pulchella* (not shown) on the ultrastructural level and by every other criterion; however, these two species are so closely related to each other that the species designation may not be valid. A similar situation was found in the genus *Zonitoides*, where the eggs of *Z. nitidus* (figs. 12, 13) were found to be indistinguishable from those of the closely related *Z. arboreus* (not shown). Nevertheless, eggs of these two genera were taxonomically distinct at the genus level. More detail is given in Tompa ('74a).

In most cases, eggs could be identified best on the basis of a combination of size, shape and ultrastructural appearance, and by these criteria, eggs from fossil deposits can be identified (Tompa, '76a). When only egg shell fragments are available, the scanning electron microscope is the single most valuable diagnostic tool.

An attempt was made to classify partly calcified eggs (figs. 2-5, 25) by dissolving the crystals from the jelly matrix and measuring crystal sizes and types, but this yielded no useful results; for these eggs, the distribution and range of sizes of crystals is too variable and non-specific. Nevertheless, many of these eggs can be identified using a list of possible sources (if available) on the basis of egg shape, size of each egg, presence or absence of crystals, and number of eggs deposited in a clutch. It must be emphasized that only freshly deposited eggs, i.e., eggs without noticeable embryos inside, should be used since the embryos dissolve the $CaCO_3$ crystals later in development rendering the egg totally uncalcified (Tompa, '74a, '75).

For many eggs, only five to ten specimens were available, while for others almost unlimited numbers were obtainable. On the basis of several years of observations and data collection involving numerous species, it seems apparent that most land snails of a given species produce eggs of constant size and shape, with consistently identifiable ultrastructural features. The major variable, depending on the size of the animal, its health, nutritional state and the time of the season is the number of eggs deposited in a given clutch. Therefore, all of the *Anguispira alternata* eggs laid by several hundred adults of varying age and size were essentially identical in size, shape and ultrastructure. The size range of ten *Vallonia pulchella* eggs, taken at random from ten different snails was $0.66 \pm 0.1 \times 0.62 \pm 0.09 \times 0.53 \pm 0.02$ mm (Tompa, '76a). The most dramatic exception to the above generalization is the case of *Strophocheilus oblongus*, where the data (unpublished) indicate that the size and weight of the eggs vary directly with the size of the parent animal. The clutch size is from one to eight eggs, and even the shape of the egg is variable between snails, ranging from almost completely spherical to very oblong.

All of the heavily calcified eggs examined were of a white color when in a clean state, with the exception of the eggs of *Ventridens ligera* and of some achatinids. In *Ventridens*, each clutch of eggs is of a uniform coloration, but the colors of different clutches range from light pink to dark brown. This coloration is in the outer jelly layer, not within the egg shell, so that mechanical scraping or light treatment with alkali removes the coloration and reveals the white colored egg shell beneath. In the Achatinidae, several snails, such as *Achatina fulica* deposit eggs of a yellow-green color. The nature or significance of this coloration is not known. No pigments were found in the egg albumen of any land snails.

DISCUSSION

All birds form calcified eggs and these consist mostly of calcite, with trace amounts of aragonite in the mammillary cores (Erben, '70). Within the reptiles, turtles have aragonitic egg shells, crocodiles have calcitic egg shells, and dinosaurs had egg shells made of calcite, with aragonitic knobs as in birds (op. cit.). The eggs of monotreme mammals contain a very thin layer of calcium carbonate (Brown, '75). Vaterite, another form of $CaCO_3$, has been recorded from pelican eggs (Gould, '72).

Besides molluscs, only arthropods appear to form calcified egg shells or egg cases among the invertebrates. The oothecae of cockroaches contain crystals of calcium oxalate, and those of mantids have calcium citrate (Brown, '75), while in the stick insect egg case there are crystals of calcium carbonate and calcium oxalate (Moscona, '48).

Among the mollusca, calcified deposits occur not only in land pulmonate snail eggs, but in the "egg case" of the cephalopod *Argonauta* (Morton, '67), in eggs of the land archeogastropod family Helicinidae (Berry, '65b), in the land mesogastropods of the family Cyclophoridae (Berry, '64), and among several genera of the aquatic mesogastropods of the family Ampullariidae — also known as Piliidae (Michelson, '61). No detail is known about the structure or mineralogy of the eggs from the first families mentioned, but it is now clear that in some of the Ampullariidae, the eggs are made of vaterite, a rather uncommon and less stable form of $CaCO_3$ (Hall and Taylor, '71; Watabe et al, '73). Interestingly, these aquatic prosobranchs, such as *Pomacea* come out of the water and lay their eggs on land (Andrews, '64). The presence or absence of calcified eggs of the Ampullariidae has been used to define generic limits (Michelson, '61).

Very little is known about any aspect of calcified land (pulmonate) snail eggs; even their occurrence is scarcely documented. Why there are no calcified eggs formed by freshwater pulmonates has not been seriously investigated (Bondesen, '50). Only a few lists of the occurrence of calcified eggs in pulmonates are available: Pilsbry (1894) used the size of eggs and the presence of heavily calcified egg shells in an attempt to classify the helicids; Standen ('17) gave the most extensive list of calcified eggs with comments on their places of deposition, but performed no mineralogical or structural investigations; Connolly ('15) included observations on the occurrence of eggs, their size and of ovoviviparous trends in his discussion of primitive traits in certain stylommatophoran families; Pelseneer ('35) brought together much useful information on gastropod eggs and their periods of incubation; a most valuable source of general information on slug eggs is the monograph of Quick ('60) on British slugs. On the basis of these works, and on new material presented in this paper, it is suggested that those families which can be considered primitive, such as the Partulidae, Endodontidae and Zonitidae (Morton, '67), among others, have heavily calcified eggs, and it is possible that heavily calcified eggs represent an ancient, basic stylommatophoran trait. Of all the suborders of the land pulmonates, only the Heterurethra lack this kind of egg. This supports the view that the Heterurethra are a unique group within the Stylommatophora (Cather and Tompa, '72).

Based on the degree of egg shell calcification, there are three types of land snail eggs (Tompa, '74b):

(1) Uncalcified egg — there are no deposits of $CaCO_3$ in the egg, e.g., *Succinea putris.*

(2) Partly calcified egg — with discrete, individual crystals of $CaCO_3$ in the outer egg layers; these particles are not fused, e.g., *Helix pomatia.*

(3) Heavily calcified egg — with a hard, brittle shell of fused $CaCO_3$, similar to bird eggs, e.g., *Strophocheilus oblongus.*

Standen ('17) states that heavily calcified eggs are laid in a soft state, which swell and then harden, so that they cannot again be passed through the aperture of the shell. This has not been witnessed in any cases during the present investigation and seems unlikely on the basis of work done on *Anguispira alternata* (Tompa, '75) and *Strophocheilus oblongus* (unpublished).

Most of the families of snails examined had a single kind of egg, only, in terms of the degree of calcification. The exceptions thus far found are: Helicidae have both partly and heavily calcified eggs (*Helix* vs. *Cepaea*); Endodontidae have both partly and heavily calcified eggs (*Helicodiscus* vs. *Anguispira*); Limacidae have uncalcified and partly calcified eggs (*Limax* vs. *Agriolimax reticulatus*); and the Arionidae have uncalcified, partly calcified, and heavily calcified eggs (*Arion fasciatus* vs. *Arion intermedius* vs. *Arion ater*). No doubt many more such examples will be found.

Calcified eggs are formed not only by oviparous snails, but also by ovoviviparous animals and those with delayed oviparity (eggs are retained in the terminal part of the oviduct for some time after their formation and are released in an advanced state of embryonic development). For example, *Helicodiscus parallelus* lays a single, partly calcified egg (fig. 25) which it keeps within its uterus for about 40 days before releasing it (Gugler, '72; personal observations) and *Achatina fulica* and *Limicolaria martensiana* hold their heavily calcified eggs for varying lengths of time before depositing them (Owiny, '74). An example of a heavily calcified egg in a truly ovoviviparous snail is *Partula*, in which the eggs hatch inside the oviduct (unless the snail is disturbed by handling and aborts the eggs). Presumably, the young eat the egg shell and are then released; this may also occur with *Limicolaria* if the eggs are retained until hatching (Owiny, '74).

There is too little data available to allow an analysis of the relationship between the degree of calcification of the egg and its site of deposit (and moisture content). One study indicates, however, that a heavily calcified shell does not improve water retention by the egg (Bayne, '68b). A brief account of the types of sites utilized for egg deposition is worth mentioning, nevertheless, to show the wide degree of adaptive radiation. Most land snails deposit their eggs into pockets of moist soil, beneath leaf litter, or in rotten decaying wood. There are tropical snails, however, which place their calcified eggs in various arboreal sites. *Pseudachatina downesii* reportedly deposits its eggs into the axils of the branches of the trees in which they live (Standen, '17); *Placostylus miltocheilus* lays its eggs in detritus which collects in the leaf axils of certain plants (Peake, '68); *Cryptaegis pilsbryi* leaves its eggs on the surfaces of leaves (op. cit.); *Cochlostyla* places its eggs between folded leaves (Sarasin and Sarasin, 1868); and according to Laidlow and Solem ('61) *Amphidromus* lays its eggs in nests made of folded leaves. Subterranean tunnels are used by *Testacella haliotidae* and others of this genus to deposit their eggs a meter or more below the surface (Standen, '17). In the genus *Libera* from the Society Islands, the snails have the peculiar habit of ovipositing into the wide umbilicus of their shell which is constricted so as to form a pouch; in some members of this genus, the eggs are retained in this position, until hatching, by a temporary shelly plate which partly covers the umbilical opening (op. cit.).

Obviously, the egg shell must be strong enough to withstand the chemical, physical and biological forces which challenge it during the incubation period, and yet it must be permeable enough to allow gaseous and water exchange across it to keep the embryo alive. While the most common duration of development is about one to two months, it varies from as little as two weeks in *Zonitoides nitidus* to over two years in *Testacella* (Stokes, '58). There is even a great deal of variability in the time of hatching for eggs of the same clutch, at the same temperature. For example, in *Anguispira alternata* the range of hatching times will vary from 35 days to over 70 days at 20°C.

Temperature and climatic conditions may also greatly affect incubation time. Quick ('60) notes that in England, *Agriolimax agrestis* eggs are laid in autumn and hatch in three to four weeks, with the young maturing in the following summer and dying in that fall. In Finland, with a more severe climate, the entire life cycle of this same species is different since the eggs are laid and the parents die in late August or September, and the eggs hatch only next June. Thus, the embryonic life lasts less than a month in England but more than eight months in Finland (op. cit.).

In birds, the egg shell is necessary for providing calcium to the developing embryo during incubation, and this may also be true for some reptilian calcified egg shells (see Simkiss, '67 for a review). The function of calcified egg cases in insects is not certain, although in the stick insect the calcium may be utilized by the embryo (Moscona, '48). Recently, it has been demonstrated that the developing land snail embryo absorbs calcium from the heavily calcified egg shell in order to form its first whorls of the body shell, called the protoconch (Tompa, '75). This resorption also occurs in partly calcified eggs. Whether uncalcified snail eggs have a higher concentration of calcium in the albumen fluid, or whether the embryo has a less calcified body shell at the time of hatching is not known and is well worth investigating. This same question of the source of calcium in uncalcified egg shells, and the embryonic calcium content by the time of hatching, comes up in the various reptilian groups where both calcified and uncalcified eggs occur (Simkiss, '67); the maximum tolerable calcium concentration in the albumen fluid of the egg is not known for any of these animals. With land snails, not only does the embryo absorb much of the egg shell calcium during incubation, but the neonate consumes the egg shell remnant immediately after hatching, thus making full use of all the calcium deposited in the egg (Tompa, '74a).

Table 1 documents the widespread occurrence of calcified eggs within the order of land pulmonates. More than half of the families of this 15,000 member group are listed, with many more certainly waiting to be discovered. The SEM's presented demonstrate that there is taxonomic value in their character, which deserves further work. Because all three types of eggs — uncalcified, partly calcified, and heavily calcified — occur in the land snails, a study of the physiological differences in the reproductive systems of these three kinds of snails may be of great biological interest. For this reason, biochemical analysis of the structural proteins of egg shells has been recently undertaken. Because of the rapidity with which some of these egg shells are formed, current investigation in this laboratory centers also on characterizing the calcium transport system involved, and comparing it with that of the avian shell gland.

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PLATE 1

EXPLANATION OF FIGURES

1 X-ray diffraction patterns from (body) shells and egg shells of representative specimens from table 1. A. Diffraction pattern of the (body) shell of an adult *Anguispira alternata*, giving only the aragonite pattern. B. Diffraction pattern of the larval shell (unhatched snail), 40 days old, of *Anguispira alternata*, again giving the aragonite pattern. C. Diffraction pattern of the egg shell of *Anguispira alternata*, giving a pure calcite pattern. D. Diffraction pattern of the egg shell of *Partula*, showing a calcite composition mixed with a lesser amount of aragonite. Three of the five aragonite lines which are visible are labeled (arrows). In most cases of detectable mixed composition in egg shells (table 1), only the 3.4 Å line could be seen; this is the strongest aragonite pattern seen of any egg shell.

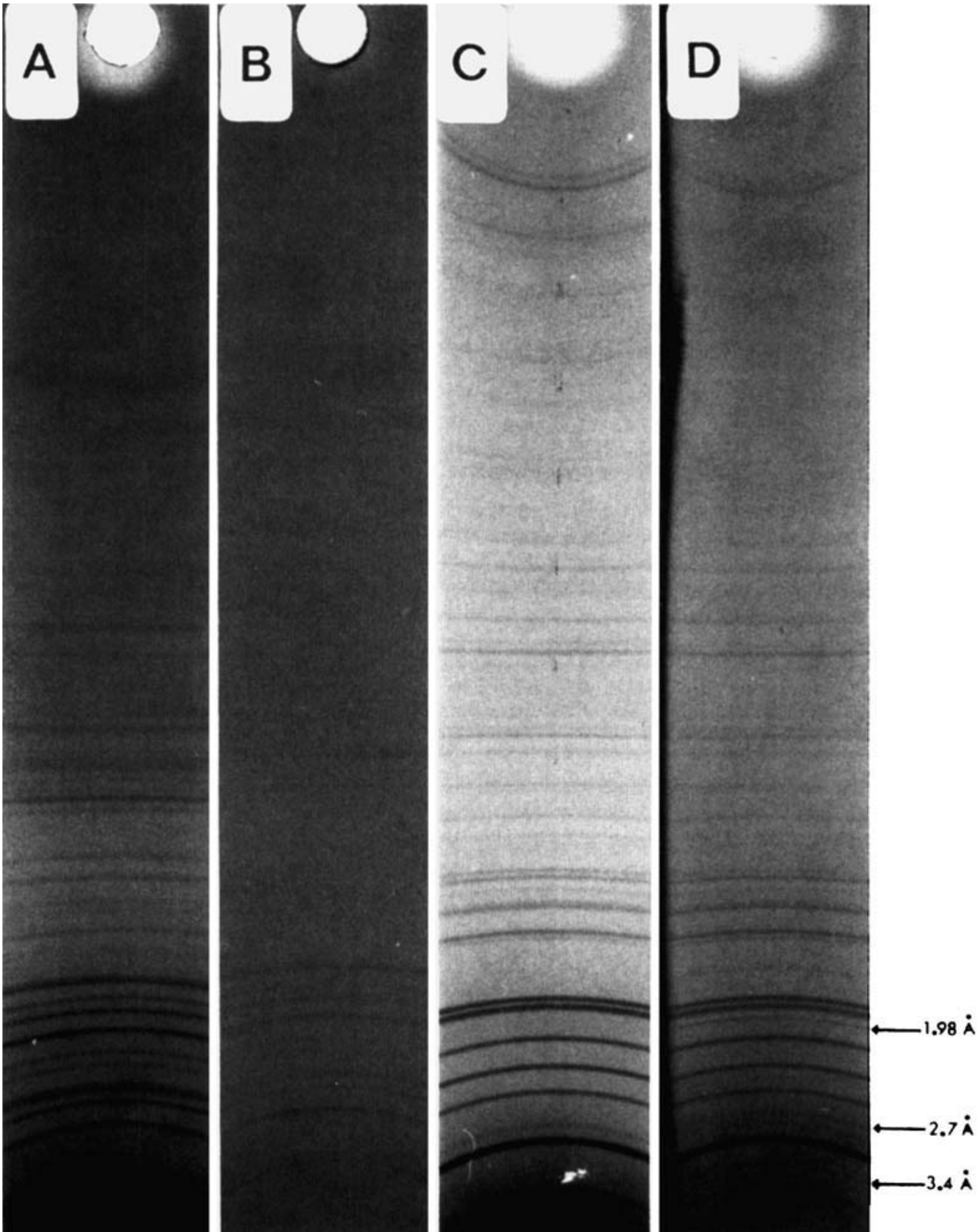

A
B
C
D
1.98 Å
2.7 Å
3.4 Å

PLATE 2

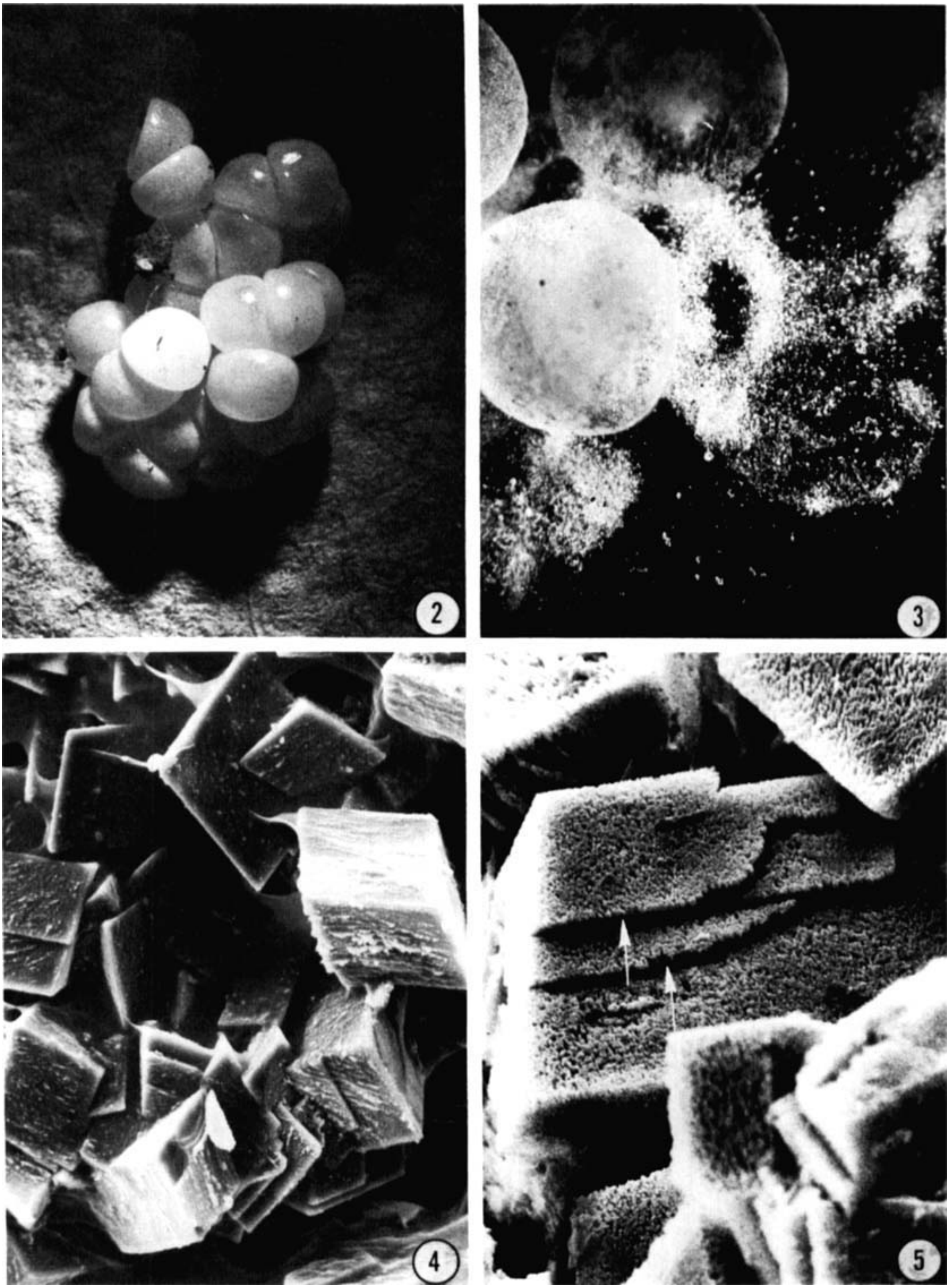
EXPLANATION OF FIGURES

2 Freshly deposited eggs of *Triodopsis multilineata.* Most of these partly calcified eggs are in a collapsed state, but will swell in moist soil and become completely round. × 3.

3 The partly calcified eggs of *Stenotrema leai,* treated with sodium hypochlorite to dissolve the jelly layer and release the crystals. The perivitelline membrane, which holds the albumen and ovum is obviously still intact. × 17.

4 Crystals from the partly calcified egg of *Helix pomatia;* alcohol fixed specimen, treated with sodium hypochlorite. × 500.

5 Crystals taken from figure 3, and examined in a scanning electron microscope. Note that the crystal appears to have been penetrated by an organic matrix, leaving innumerable small holes, and that there are growth steps on the surface (arrows). × 3,000.


2
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4
5

PLATE 3

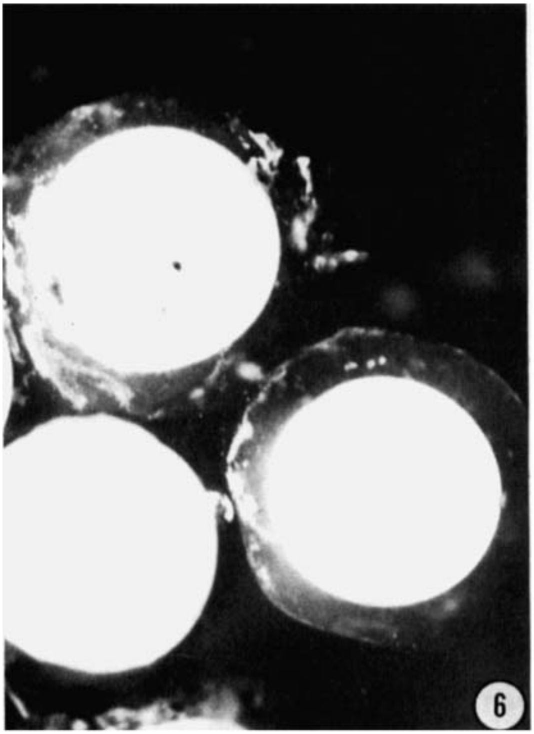
EXPLANATION OF FIGURES

6 Heavily calcified eggs of *Anguispira alternata,* placed into distilled water to reveal the (swollen) jelly layer outside of the egg shell. × 12.

7 *A. alternata* egg, with the jelly layer removed mechanically, revealing the solid, crystalline egg shell. (from Tompa, '76b) × 100.

8 Surface view of an *A. alternata* egg shell, jelly removed mechanically, revealing large calcite crystals on the egg shell outer surface. (from Tompa, '76b) × 1,000.

9 Cross section of specimen from figure 8, revealing three layers of crystals: outer layer of large crystal facets (0), middle layer (M) made of alternating crystals and organic material (arrow), and innermost layer (I) made of small crystals. The perivitelline membrane (not visible) is attached to this inner area. Freeze-dried egg, fractured. × 2,000.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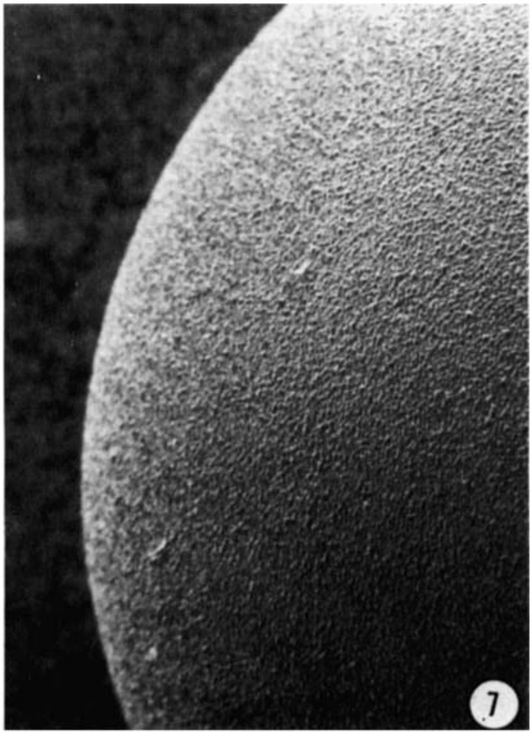
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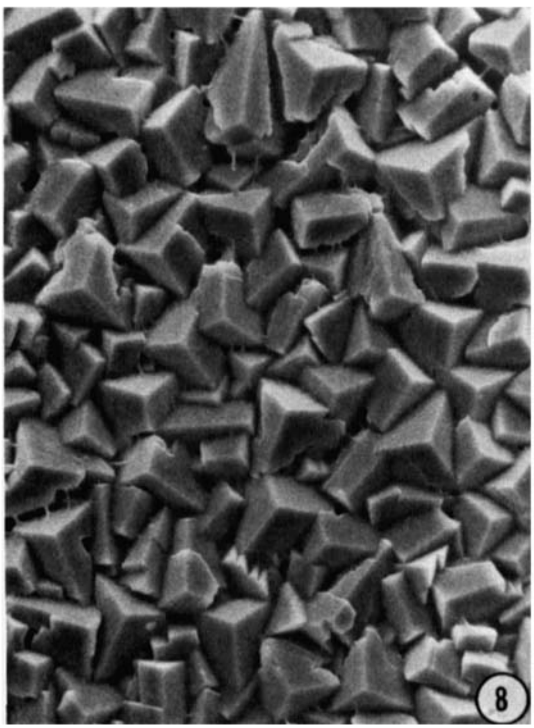
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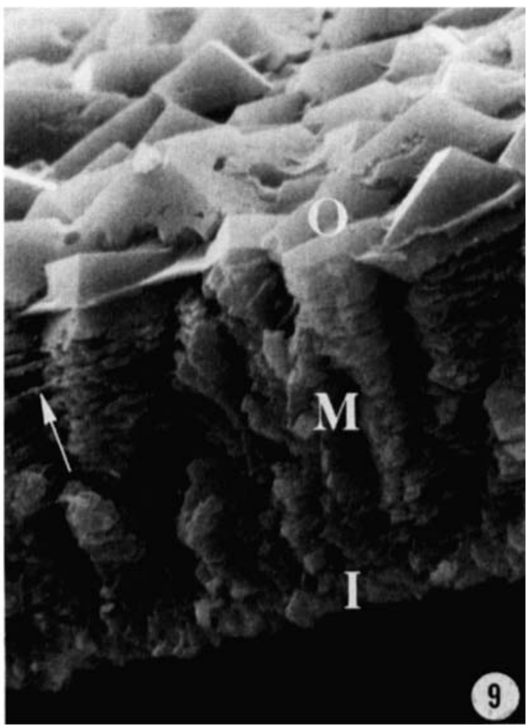

O
M
I
9

PLATE 4

EXPLANATION OF FIGURES

10 Outer surface of the egg shell of *Anguispira kochi*, treated with sodium hypochlorite. Note the strikingly different morphology from another species of this genus, in figure 8. × 1,000.

11 View of fractured egg of *A. kochi*, showing the cross section and inner surface (I). Note striking difference between this and figure 9. × 1,700.

12 Egg shell of *Zonitoides nitidus*, outer surface, treated with sodium hypochlorite to remove jelly layer. × 800.

13 Air-dried egg of *Z. nitidus*, fractured to reveal cross-section. Note the alternating layers of organic (arrow) and inorganic material, as in figure 9 of *Anguispira alternata*. Outer jelly layer (J) is visible. × 3,000.

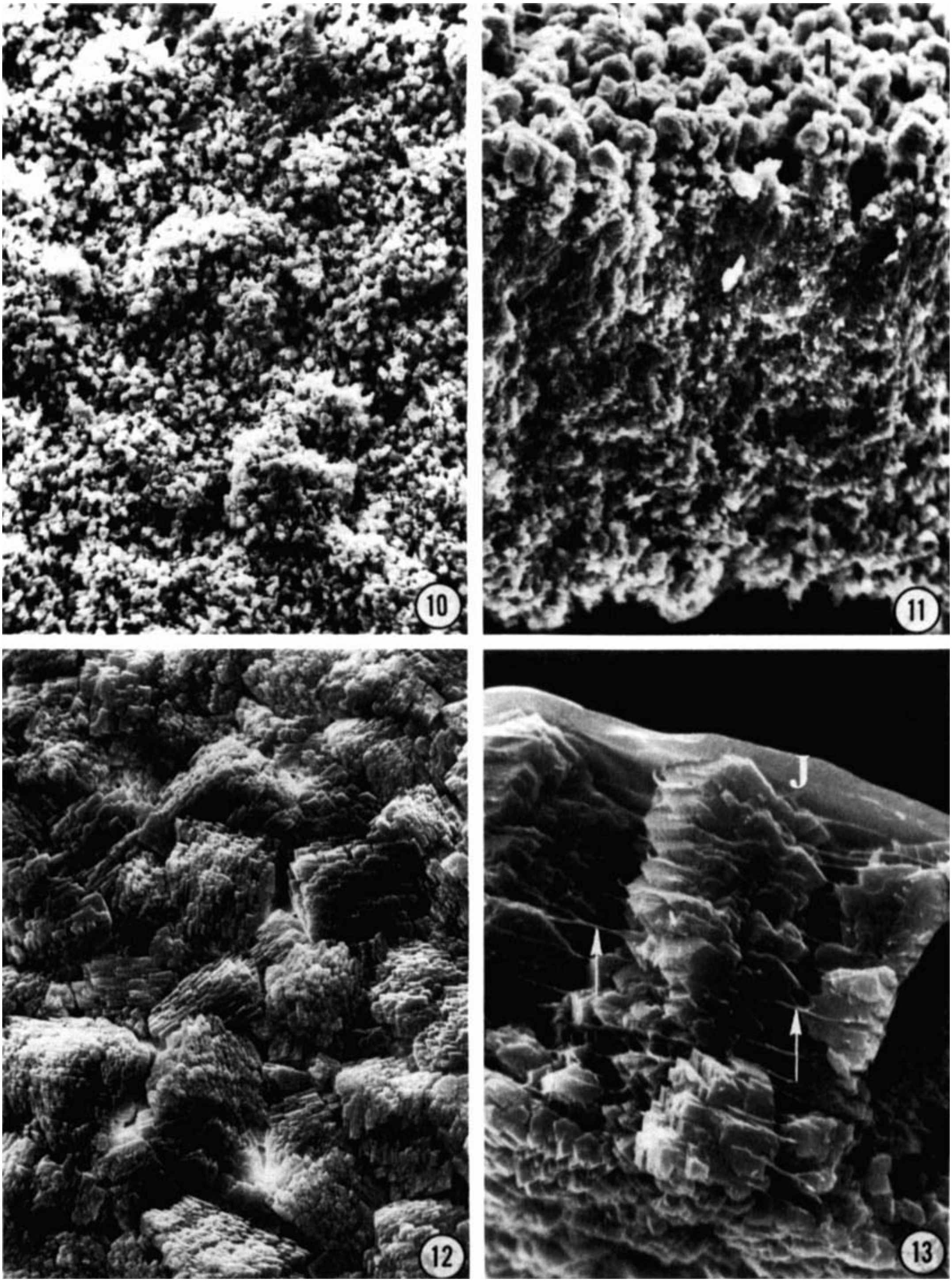

10
11
12
J
13

PLATE 5

EXPLANATION OF FIGURES

14 Untreated, air-dried egg of *Vallonia costata,* fractured to reveal cross section. The large crystals are restricted to the outer layer (0). This is the smallest egg examined in this study, measuring 0.63 mm in maximum diameter. × 1,000.

15 Surface view of the same egg, arrow indicates twinned calcite crystal. × 5,000.

16 Outer surface of the egg shell of *Cepaea nemoralis,* treated with sodium hypochlorite, showing largest crystals on the outside (O), smaller crystals in the middle (M), and inner surface revealed by the fracture. × 700.

17 Fractured egg shell of *Haplotrema concavum,* lightly treated with sodium hypochlorite. Three general layers are visible; an outer layer (O) consisting of large crystals, a middle layer (M) of very small particles, and the very thin layer made of rounded crystals, just barely visible in this photograph (arrow). × 1,400.

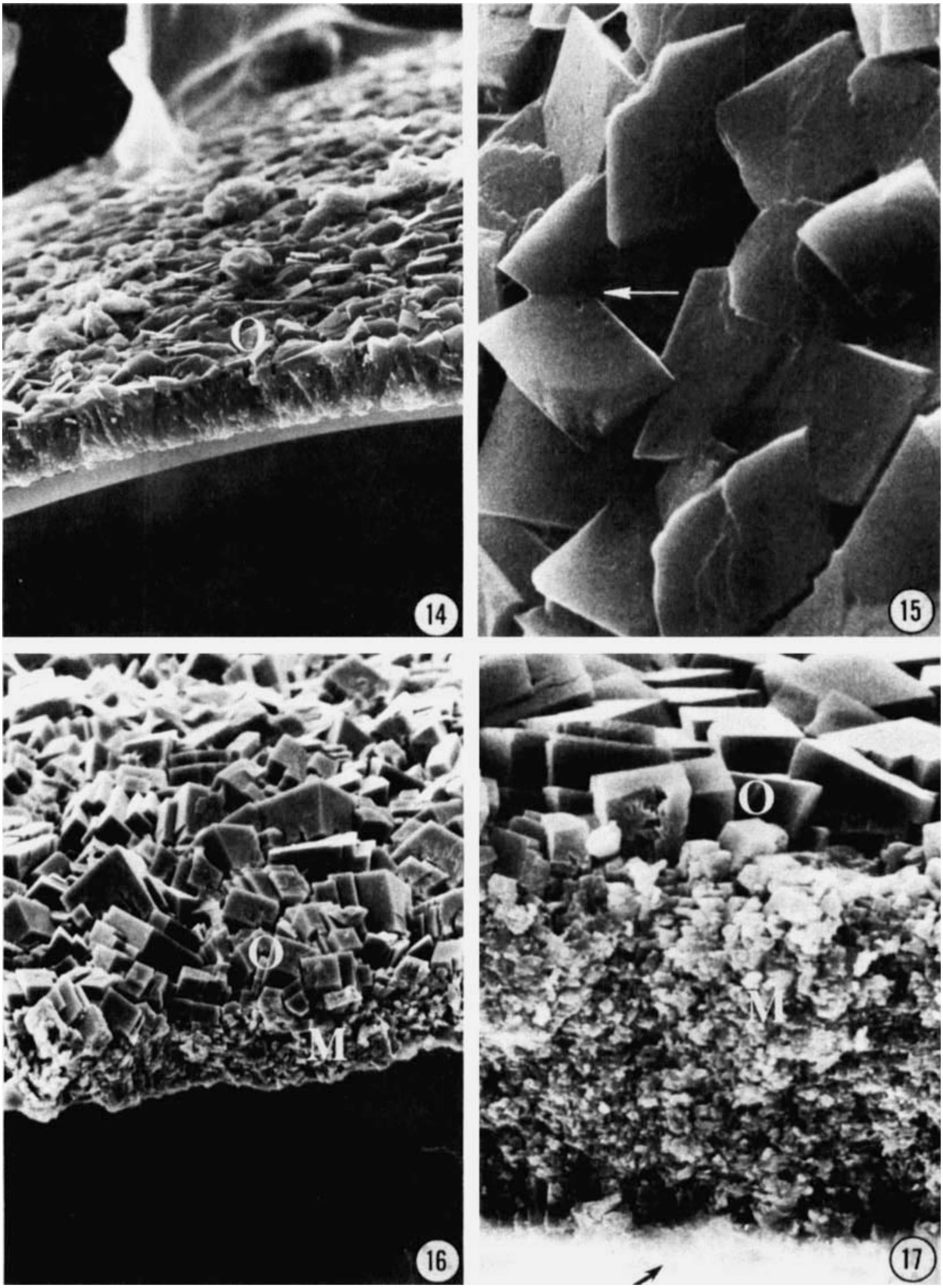

O
14
15
O
M
16
O
M
17

PLATE 6

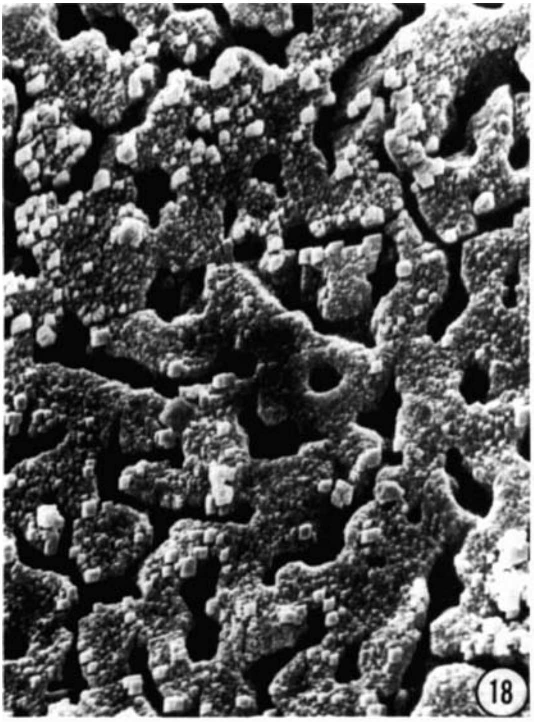
EXPLANATION OF FIGURES

18 Outer surface of the egg shell of *Achatina fulica*, treated with sodium hypochlorite. The unusual, reticulated surface reveals many very small crystals in the areas of crystal growth. Note that this is an extremely smooth, featureless surface if viewed at the same magnification as most of the other eggs illustrated. × 8,000.

19 Fractured egg shell of *A. fulica* treated with sodium hypochlorite. The entire egg shell is made up of reticulated network of organic material (dissolved) and inorganic matter. The outer layer is also unusual in that it appears to be made of a finer network of crystals than the inner layer (I). × 1,000.

20 Untreated egg shell of *Liguus fasciatus*, outer surface, showing numerous dome-shaped features. Each dome reveals spine-like projections when viewed at higher magnification (not shown; Tompa, '74a) making this one of the most unusual patterns seen. × 200.

21 Cross section of *L. fasciatus* egg, showing the columnar nature of the egg shell subunits. It appears that pores (arrowheads) connect the inner layer (I) with the outer surface. × 200.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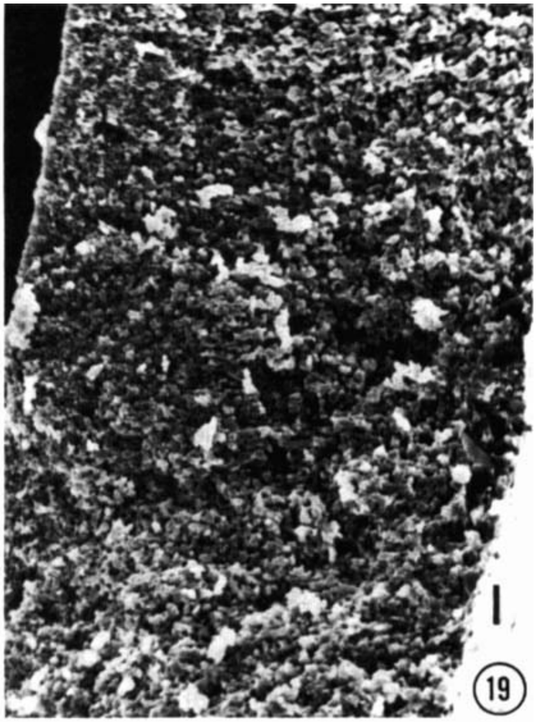
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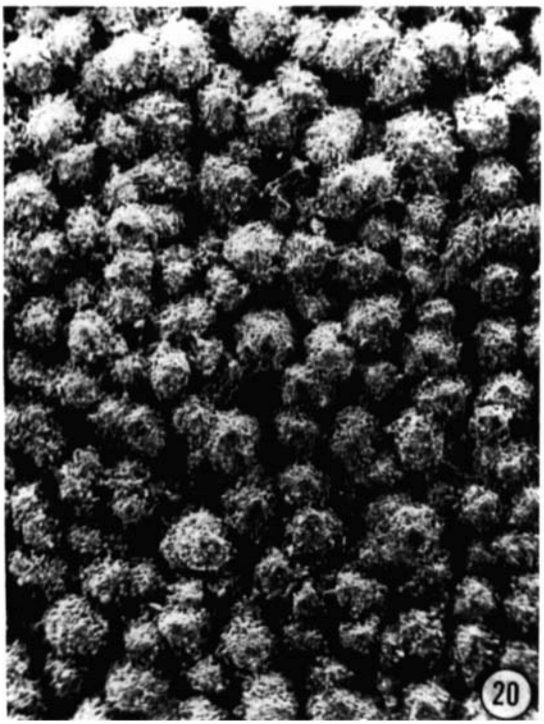
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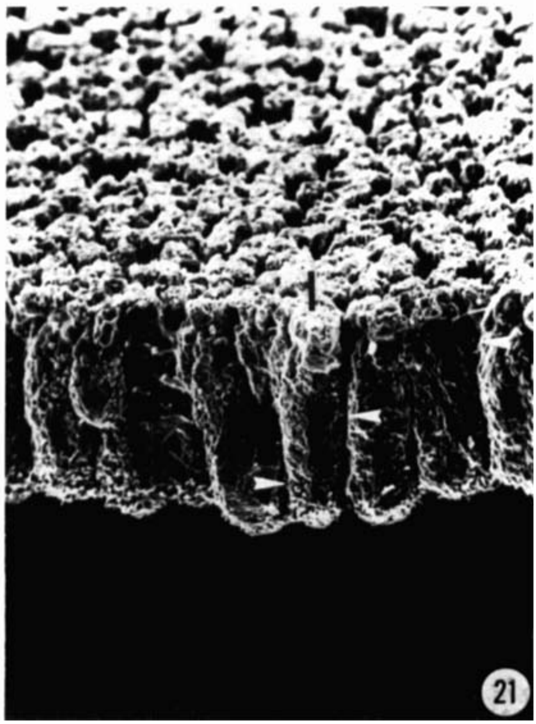

21

PLATE 7

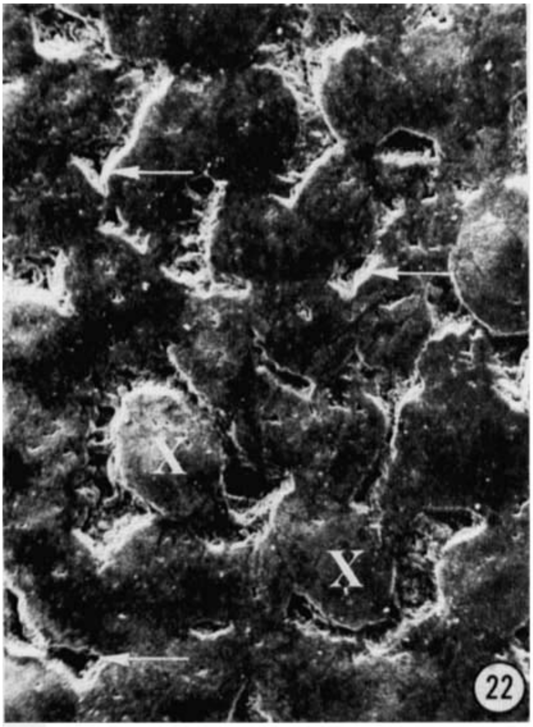
EXPLANATION OF FIGURES

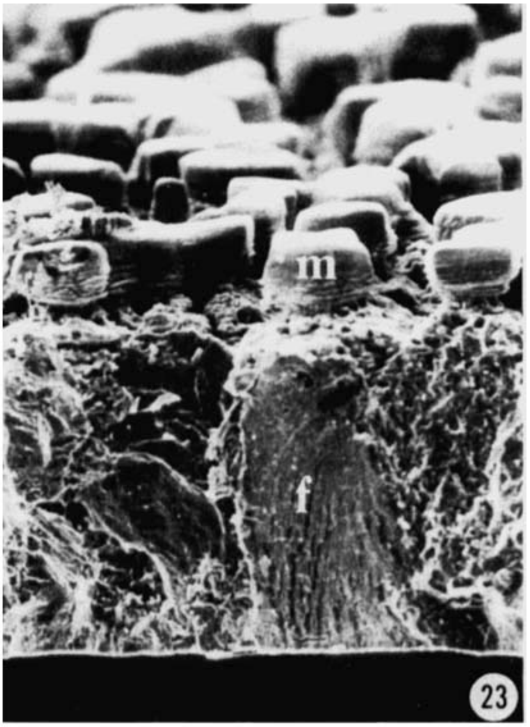
22 Outer surface of the egg shell of *Strophocheilus oblongus,* treated with sodium hypochlorite. This layer appears to be made of flat, circular areas fused with each other to various extents; several individual units are visible (X). Some areas suggest the presence of pores (arrows). × 150.

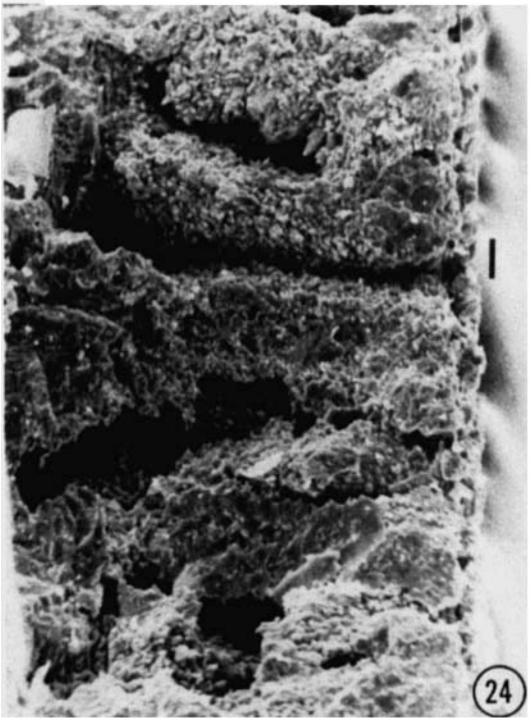
23 Fractured egg shell of S. *oblongus,* treated with sodium hypochlorite. The inner layer consists of mammillary knobs (m) which have a layered appearance. The fracture pattern at (f) suggests that the subunit of this egg shell consists of a long column, which has a mammillary knob (m) on the inside, and a flattened, circular top on the outside (X in fig. 22), with pores between the intersections of several units. This genus of snails makes the largest gastropod egg; this species has the second largest egg. × 300.

24 Egg shell of *Acavus superba,* treated with sodium hypochlorite, revealing the columnar nature of the egg shell subunits. Inner side is (I). This egg is 2.2 cm × 1.6 cm in diameter. × 300.

25 An air-dried egg of *Helicodiscus parallelus,* fractured to reveal the individual calcite crystals which have collapsed upon each other as the supporting jelly (arrow) dried out. The inner layer seen is only made of dried out albumen (A) from the egg content, not from calcium carbonate. × 2,000.


X
X
22


m
f
23


I
24

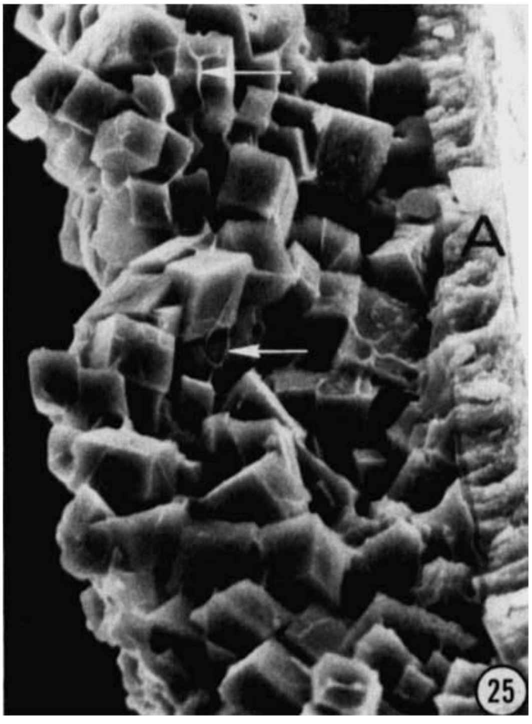

A
25

PLATE 8

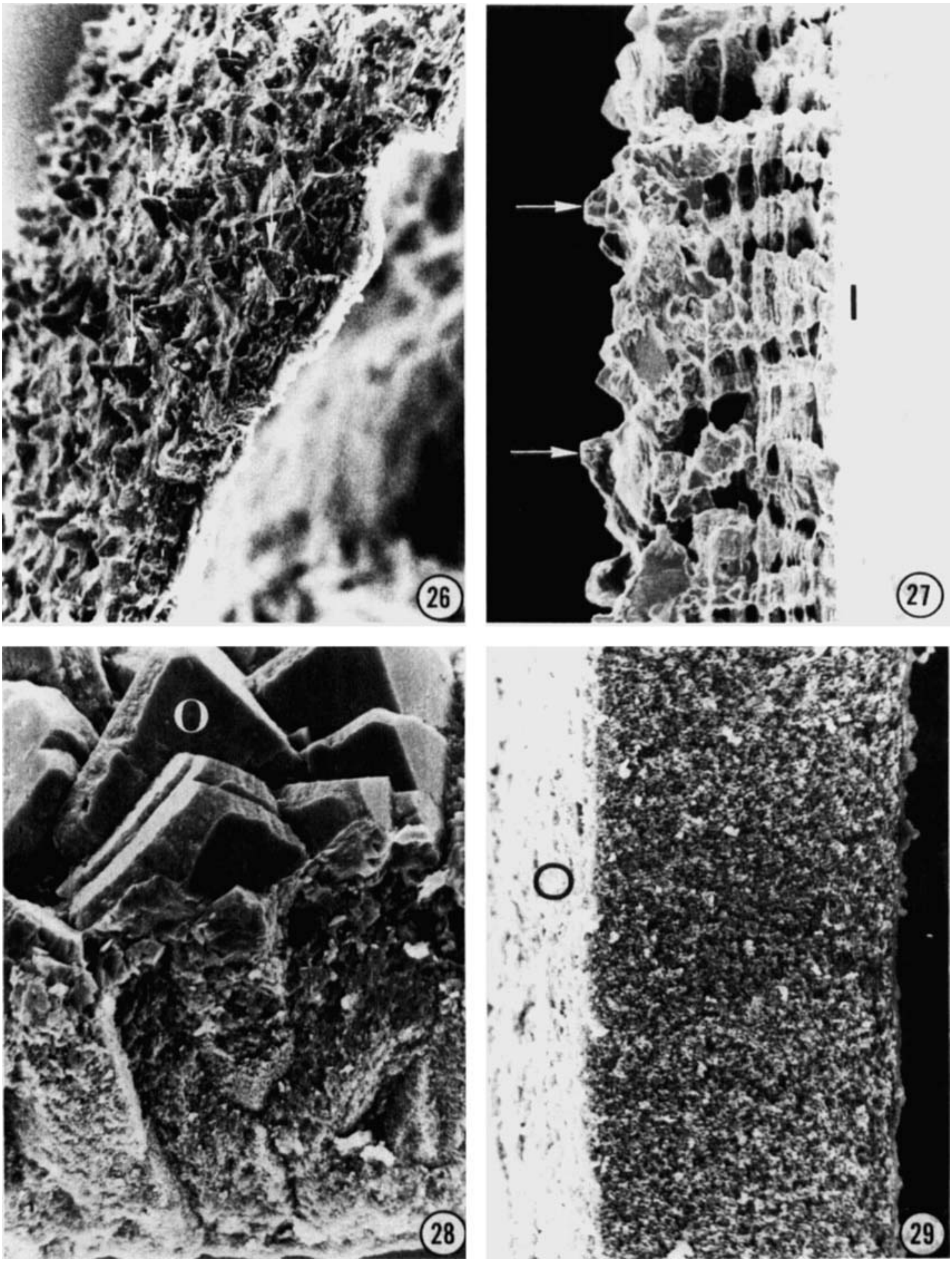
EXPLANATION OF FIGURES

26 The presence of spike-like crystals (arrows) seems to be characteristic of the genus *Euglandina.* This is the egg shell of *Euglandina rosea,* untreated. × 200.

27 Cross-sectional view of the egg of *E. guttata* showing the outer pike-like crystals (arrow), and displaying an extremely complicated shell structure, including air spaces of pores running from inside to outside, and numerous stratified layers near the inner surface (I). × 300.

28 Egg shell of *Rumina decollata,* treated with sodium hypochlorite, fractured to reveal cross section. Outer layer (O) is made of huge crystals. × 1,000.

29 Egg shell of *Limicolaria saturata,* treated with sodium hypochlorite, fractured to reveal cross section. The appearance is like that of *Achatina,* in that it also possesses a reticulated appearance. Outer surface is (O). × 600.


26
27
O
28
O
29