Microstructure and composition of the trilobite exoskeleton

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Teigler, D.J. & Towe, K.M. 1975 07 15: Microstructure and composition of the trilobite exoskeleton. *Fossils and Strata.* No. 4, pp. 137–149, Pls. 1–9. Oslo. ISSN 0300–9491. ISBN 82-00-04963-9.

Representatives of the major orders of trilobites have been studied by polarized light and electron microscopy as well as electron probe, X-ray diffraction and amino acid analysis. Data were collected with respect to crystallography-mineralogy, layering-lamination, pore canals, and organic matrices. A C-axis preferred orientation of microcrystalline calcite is characteristic. Layering, lamination, pore canals, and a pseudopleochroism vary in appearance and distribution. Phosphate layers occur in two species. Decalcified cuticles reveal a structurally preserved, biochemically degraded meshwork of organic material. The trilobite exoskeletal microstructure compares more favorably with that of calcified ostracodes than with the typical, generalized arthropod cuticle.

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By comparison with other invertebrate groups that are common in the fossil record the microstructure and composition of the trilobite cuticle has received very little attention. With the exception of a recent study by Dalingwater (1973) there have been no extensive papers published on this subject to date. Cayeux (1916) figured a section of *Trinucleus goldfussi* showing a thin external fibrous layer and a "lamellar" inner layer. Størmer (1930) described a few well-preserved specimens in which evidence of layering and laminations could be seen. Rome (1936) also observed laminations in some specimens of *Phacops* and Kielen (1954) illustrated a specimen with a pigmented layer. Pore canals are also frequently mentioned and while the composition of the exoskeleton is generally considered calcitic there have been scattered but persistent reports of phosphate in the trilobites (von Zittel, 1887; Richter, 1933; Rhodes & Bloxam, 1971). Primarily because of these few observations the currently held textbook conception of the trilobite cuticle is that of a calcareous-phosphatic structure with pore canals and a layered and laminated microarchitecture which is in general directly comparable with that of the typical extant cuticle (Harrington, 1959). In extant arthropods the external skeleton or cuticle is secreted by the epidermal cells forming the inner part of the body wall and it is commonly composed of several layers consisting primarily of microfibrils of chitin associated with protein. It usually contains pore canals and is stiffened and hardened over most of the body surface by a process of tanning and in the case of many crustaceans by the deposition of calcium salts (Richards, 1951).

The present study is aimed at examining representative, well-preserved trilobite exoskeletons with a view towards comparison, both compositionally and microstructurally, with that of extant arthropods as well as among the trilobites themselves.

MATERIALS AND METHODS

The majority of the specimens examined in this study were obtained from the collections of the National Museum of Natural History and the United States Geological Survey housed at the Smithsonian Institution, Washington, D. C. Additional specimens were made available by Dr. Ewa Tomczykowa, Instytut Geologiczny, Warsaw, Poland. The taxonomic and locality data for all of the species studied are listed in Appendix I. The selection of material was limited

to well-preserved specimens that were neither museum types nor exceptional or one-of-a-kind specimens from rare localities. This left a minimum to work with and although an attempt was made to select a standard region of the cuticle for comparison, more often than not only fragments of cuticle were available for sectioning. Accordingly, the coverage is necessarily spotty geographically, stratigraphically, anatomically and taxonomically for the twenty species studied.

The trilobite material was embedded, where necessary, in epoxy resin and sectioned for polarized light microscopy according to methods similar to and modified from those developed by Nye *et al.* (1972). Mineralogical and chemical determinations were made on selected material by Debye-Scherrer powder X-ray diffraction methods and electron probe microanalysis. Transmission electron microscopy of the calcified cuticle was accomplished using either the single- or double-stage replica techniques on polished and etched surfaces as well as on untreated surfaces. The organic matrix from within selected exoskeletal fragments was isolated by means of Na-EDTA decalcification at pH 8. Aliquots were used for amino acid analysis by high-sensitivity ion-exchange chromatography or for subsequent epoxy embedding and ultramicrotomy.

RESULTS

The most characteristic and consistent feature of the trilobite exoskeleton is its mineralogy and crystallography. In agreement with Sorby (1879) and Cayeux (1916) all of our specimens which represent well-preserved, primary cuticle material (*i.e.*, not impressions or silicified material, etc.) are constructed of calcite crystals which have a C-axis preferred orientation more or less perpendicular to the skeletal surface. This is a statistical preferred orientation of the crystals as regards their crystallographic C-axis and is best observed on *thin* thin-sections in cross-polarized light where it manifests itself as a zone of darkness (optical extinction) through the cuticle wall perpendicular to its surface (Pl. 1). Such a high degree of crystallographic preferred orientation is not, except for the ostracodes, common among extant calcified arthropod cuticles (Dudich, 1931) where a more irregular, mosaic pattern is often seen. We have been able to detect two general types of optical extinction within the trilobites studied. The first is a homogeneous extinction (Pl. 1: 3, 5, 6) and the other is a patchy extinction (Pl. 2: 1, 2). In the former the zone of darkness between crossed polars is rather uniformly dark within the major layer. In the latter there appear to be irregular patches of uniform darkness separated by areas less dark. The two types grade into one another in many instances and all species have one or the other or both.

From a morphological point of view the electron microscope is capable of providing the best information on the calcite crystals. Here we have found that in some of the cuticles examined the calcite exhibits a crudely layered aspect parallel or subparallel to the surface (Pl. 2: 5, 6). The individual crystals are difficult to define with certainty but appear to be irregularly shaped and plate-like so that in plan view (Pl. 2: 1–4) a sutured, mosaic pattern can be discerned. This type of pattern was seen in *Phacops, Isotelus* and *Agnostus* and it compares with that observed in some ostracodes (Pl. 2:3) as well as in some salcareous foraminifera (Towe & Cifelli, 1967), both groups of which can also have a C-axis preferred orientation.

As expected in fossil material of Paleozoic age, the calcite composition is not magnesian. Whether or not there was any magnesium in solid solution in the original trilobite cuticle cannot be stated with any reliability from our data since magnesian calcites rapidly lose their magnesium with diagenesis. Some magnesium was likely to have been present as this is a common constituent of extant calcified arthropod cuticles (Vinogradov, 1953).

As stated in the introduction, it is a commonly held generalization regarding the trilobites that a certain proportion of calcium phosphate is present in the cuticle mixed with the calcium carbonate. Our evidence does not support this generalization although we did find at least two specimens with discrete outer layers composed of calcium phosphate. Our attention was drawn to these specimens (an *Ellipsocephalus* from Sweden and a *Calymene* from Poland) because of the presence of a thin, pink covering layer. In thin-section these layers behave quite differently as compared to the rest of the cuticle (Pl. 3). They are yellow-brown in color which in polarized light becomes a dark gray appearing almost isotropic due to the very low birefringence. The layers are uniform in thickness (~ 20 μm), clearly follow the surface of the calcite below, and are penetrated by pores, all of which argue in favor of a primary origin for the layer itself but not necessarily for its phosphatic composition. Samples of the outer layer in both species were carefully scraped from the surface and analyzed by powder X-ray diffraction. Plate 4: 6–8 shows that in both species the mineral is a member of the apatite group, comparing favorably with an apatite standard.

The distribution of the phosphatic material in *Calymene* was studied by means of electron probe analysis on a polished, transverse section. Traverses across the cuticle were made for calcium and phosphorus and these were compared with an apatite standards as well as with traverses across a specimen of *Isotelus* similarly prepared. The results are given in Plate 3: 1–3. It can be seen that the phosphorus content (dashed line) is essentially negligible throughout all of the cuticle in *Isotelus* as well as the bulk of the cuticle in *Calymene,* rising sharply only at the location of the outer, pink layer in the latter. We attempted to compare these data with those from extant arthropod material and although there have been several chemical analyses of various crustaceans reporting phosphorus (Clarke & Wheeler, 1922; Lowenstam, 1972; Vinogradov, 1953; Bøggild, 1930) we were unable to locate any specific information regarding its mineralogy and distribution within the cuticle itself. Accordingly, therefore, we undertook to examine a known phosphorus accumulating crustacean, the crab. Electron probe analysis (Pl. 4: 1, 2) of both Recent and fossil crabs (*Cancer* sp., Late Miocene) shows that phosphorus, while present throughout the cuticle, is in both instances more concentrated toward the outermost exocuticular portion. Powder X-ray diffraction analysis (Pl. 4: 3–8) shows that the phosphorus-rich outer portions in both the Recent and fossil crab contain calcite but only in the fossil material is the phosphate in the form of a crystalline apatite. It is amorphous to X-rays in the Recent crab. The point of comparison with the trilobites is that in both cases the phosphorus is concentrated toward the exterior of the cuticle. But in the trilobites the phosphate mineral is not admixed with calcite. If this apatite layer in the trilobites is indeed a primary layer there is the possibility that, like the crab, in the living condition the phosphorus accumulated in an amorphous condition.

Although the calcite mineralogy and crystallography of the trilobite cuticle is consistent throughout the group, few other microstructural features that we investigated were so uniformly present. The presence of an organic matrix preserved within the calcite exoskeleton is something we observed with regularity where it was feasible to do so and inasmuch as calcification in general is a process intimately related to an organic precursor this was an expected and predictable result for reasonably well-preserved specimens.

If pieces of trilobite cuticle freed from the rock matrix are placed in a solution of ethylenediaminetetracetic acid (EDTA) at pH > 7.0 and left undisturbed the calcitic material will be gently and completely dissolved, usually within 24 hours. The organic matrix (and pyrite) left after decalcification retains the shape of the original fragment (Pl. 5: 1, 2) but is exceptionally fragile and delicate, subject to breakage with the slightest agitation. Plate 5: 1 shows a fragment from *Phacops rana* including a portion of the eye. The organic matrix mimics the original fragment even to its presence within the original calcitic lenses of the eye. In the light microscope the organic matrix of the trilobites is brownish in color and generally amorphous in structure. In the electron microscope, however, a delicate meshwork is seen in ultrathin-sectioned material (Pl. 5: 4). There are openings and spaces that are in reasonable agreement with the size of the "crystals" as illustrated in Plate 2. In platinum-shadowed preparations (Pl. 5: 3) the network appears to be more granular in aspect. Bate and East (1972) have figured a similar organic matrix meshwork from within the ostracode carapace.

With the help and guidance of Dr. P. Edgar Hare (Geophysical Laboratory, Washington, D. C.) we attempted to obtain data on the amino acid composition of the isolated organic matrix by means of high-sensitivity ion-exchange chromatography. Utilizing extreme care to avoid contamination (Hare, 1964), we were unable to detect any meaningful amino acids at a sensitivity of 10^{-9} moles. The amino-sugar glucosamine which is the basic building-block of chitin (sensu stricto) was especially searched for but not found. It is apparent from these data that the organic matrix of the trilobites has been, like that from other older fossil material, diagenetically altered to a non-proteinaceous, carbonaceous material. The absence of glucosamine in particular does not mean that the original material was not chitinous but only that it is not now chitinous in a biochemical sense.

In the introduction we raised the point that the trilobite cuticle is generally regarded as being layered and laminated in a fashion similar to that of the typical extant arthropod. Our data, summarized in Table I, fail to support this generalization and we find that in this microstructural aspect there is wide variation.

Layering was distinguished by changes in either mineralogy, mineralogical microstructure, or pigmentation. Lamination, on the other hand, is a generally finer-scaled repetitive feature within a layer and usually related to organic substances within this layer. The crudely laminated aspect of the crystals making up the cuticle alluded to earlier (Pl. 1: 3, 4; Pl. 2. 5, 6) is not here considered as true lamination. Almost half of the trilobite species included in this study lack any evidence of layering or lamination. In the others no more than two layers could be distinguished and in each case the outermost layer was always the thinner. Three types of outer layers

could be discerned: (1) a prismatic layer, (2) a pigmented layer, and (3) a phosphatic layer. The prismatic layer is the most common and is so named because it is constructed of elongated blocks roughly perpendicular to the cuticle surface (Pl. 1). These prisms are larger than the crystals of the underlying microcrystalline layer. In most instances they form an outer layer up to 20 or 30 μm in thickness. The pigmented layer, also very thin, is a dark, brownish layer which is generally microcrystalline helping to distinguish it from the prismatic layer which is itself occasionally pigmented. The phosphatic layer has been described above. It is worth emphasizing here that because of the thinness of these layers in many instances the outer layer will be found to have been worn away from some portions of the calcite cuticle. Thus it is difficult to make any definite statements about the distribution of the outer layer on the exoskeleton as a whole. The taxonomic distribution of these various outer layers is given in Table I.

The layered and lamellar microstructure of many living arthropods, especially among the insects, has been studied intensively by a number of workers including Bouligand (1965), Neville (1965), and Locke (1964). In particular, Neville (1965) has shown that daily growth patterns are visible in the cuticle of a number of insects where non-lamellate day layers having fibrils oriented in a preferred direction alternate with lamellate night layers having helicoidally oriented microfibrils.

Based on the paleontological literature as well as on the generalized structure of living arthropod endocuticle we expected to find abundant lamination in the trilobites. Contrary to this expectation we found little evidence for lamination and nothing directly comparable with the laminations often described in Recent arthropods. With respect to the ostracodes Bate and East (1972) concluded that the structural matrix of the carapace is also quite different and unlike that present in insects and decapod Crustacea.

All of the laminations we observed in the trilobites were confined to the principal layer. That is, we never recognized, unequivocally, any lamination in the thin, outer layer. All of the laminations seen could be related to the presence of dark, brownish organic pigmentation within the principal layer. Such laminations were found in all six examined specimens of *Phacops rana* from New York and in two species of *Isotelus*, one from New York and one from Vermont. On the other hand, *Phacops logani* from Tennessee exhibited no lamellae whatever. If a fragment of laminated *Phacops rana* cuticle is etched briefly in EDTA the lamellae will stand out in relief to some extent (Pl. 6: 1). Such material was replicated for electron microscopy and the results (Pl. 6: 2) show that there is no essential difference in the texture of the calcitic material forming the lamellae from that of the rest of the cuticle. Only a slight furrowing (arrows) can be seen. Etched material of the non-laminated *Flexicalymene* showed a similar structure but without furrowing. These data support the contention that the lamination is due to variation in the organic concentration within the cuticle rather than in some basic variation in the nature of the calcite crystals.

Whether or not the laminations are controlled by taxonomy, ecology, diagenesis or a combination thereof remains to be determined. The evidence based on our data favors taxonomy as the major influence. This, we argue, is the likely case because: (1) All specimens of *Phacops rana* from the Moscow Formation (Windom Member) near Buffalo, New York show laminations as do those of *Phacops accipitrinus* from Gotland figured by Rome (1936). However, those of *Phacops logani* from the Linden Group in Tennessee do not. (2) Specimens of *Isotelus gigas* from the Trenton Limestone in New York display laminations but specimens of *Odontopleura trentonensis* from this same locality do not. (3) *Isotelus platymarginatus* from the Upper Chazy Valcour Limestone in Vermont also have laminae while the specimens of *Bumastus subglobosus* from there do not.

Accordingly, if laminations were a general feature of the Trilobita, being well-preserved only under unusual diagenetic conditions, we would expect all trilobites from one such locality to show them and this is not the case. The same line of reasoning should hold for the laminations if they were primarily controlled by environment. Until further work can be done, a genetic control appears to be the major factor in their distribution.

In thin-sections of certain trilobites (see Table 1) ordered accumulations of some of the brownish organic material within the cuticle exhibit a phenomenon known as pseudopleochroism. The brownish color of the organic substance varies in its intensity in plane-polarized light depending on the orientation of the thin-section with respect to the polarizers. The maximum intensity (deepest brown coloration) corresponds or coincides with the position of maximum extinction of the calcite in the cuticle (under crossed polars) when this direction (C-axis) is perpendicular to the vibration direction of the lower polar (Pl. 7: 1, 3). Conversely, the weakest brown coloration is 90° to this position when the calcite extinction (C-axis) is parallel to the vibration direction of the lower polar (Pl. 7: 2, 4). In optical parlance: E, weak; O, strong. This phenomenon has been described by Hudson (1962) in the unrecrystallized mollusk shells

from the Middle Jurassic Great Estuarine Series of Western Scotland. As noted by Hudson there may be a correspondence between microscopic organic inclusions and the absorption-pleochroism; such inclusions in this case having an index of refraction near the E-ray of calcite at 1.486. The biological significance of this phenomenon is unknown but it is of interest to note that the brownish organic material in the laminations of *Phacops rana* does not exhibit pseudopleochroism while the organic material within the eye lenses does (Pl. 7: 3). Neither the Recent nor fossil crab sections with brownish material exhibit the phenomenon. We conclude that two types of organic material may have been present – one which in association with the calcite of the cuticle gives rise either intrinsically or diagenetically to a pseudopleochroism and one which does not.

"Pore canals are widely but not universally found in arthropod cuticles" (Richards, 1951: 182). This statement concerning extant arthropods applies similarly to the trilobites (see Table 1) with the proviso that we are using the term "pore canal" to include all primary duct-like processes actually observed in the cuticles studied. That is, we do not here include secondary borings nor do we distinguish semantically between different types on the basis of presumed functional differences.

The pore canals observable in trilobite cuticles have a considerable size range as well as mode of preservation. Some pores are 75 μm or more in diameter while others are very narrow being 2–3 μm in diameter. The pore canals can be observed as true openings in the cuticle, or filled secondarily with calcite, pyrite or organic material. When the fine pores are filled with calcite they are best observed in thin section with cross-polarized light since the crystallographic orientation of the calcite fillings is commonly different from the C-axis preferred orientation of the cuticle itself. In most of the trilobite cuticles that have pore canals the canals are usually of one size range but we noted the presence of two distinct size populations (bimodal) in some species. *Calymene,* for example, has in addition to the narrow pore canals about 3–4 μm wide another set of pores 20–30 μm in diameter. *Odontocephalus* also has two sets of pore canals with the very large population easily visible and averaging 70–80 μm in diameter. Some of these varations are illustrated in Plate 8.

The pore canals of many living arthropods are twisted or helical in structure and the lamellar nature of the cuticle in sections produces a parabolic pattern. Bouligand (1965) has proposed a model demonstrating that this parabolic pattern could be due to an optical illusion created by sectioning obliquely through a stack of single sheets, each having its component fibers arranged in parallel. This stack, similar to plywood, is arranged such that each sheet is rotated slightly creating a twisted structure. As a result of this rotation the pore canals can also be apparently twisted or helicoidal (Neville, 1969). The parabolic pattern has been reported by Neville & Berg (1971) in the cuticle of a Jurassic fossil lobster (*Eryma stricklandii*) and twisted pore canals have been observed by Rolfe (1962) in ceratiocaridid Crustacea from the Middle Silurian. The parabolic pattern has been observed in Recent crab and crayfish cuticles and we have observed it in a fossil crab along with evidence of twisted pore canals (Pl. 9: 1, 2) where the pitch of the helical pores is in register with the light-dark laminations. Bate and East (1972) did not find evidence of parabolic structure in any calcified ostracodes and only indistinctly in uncalcified species. Harding (1964) figured one twisted pore canal in a calcified ostracodes cuticle but other photos figured by him of the same species do not appear to have twisted canals.

If twisted pore canals were observable in trilobites this would be evidence in support of the viewpoint that the trilobite cuticle is directly comparable with the generalized typical arthropod cuticle and would further indicate that a comparable lamellar structure must have been present originally, its absence now being therefore due to some diagenetic change. But in all of the trilobite material studied here only one vague instance provided any real evidence for helical pore canals (Pl. 8: 5). Observations of this material in the electron microscope (Pl. 8: 6) did not support the vague light microscopic image.

In addition to twisted or helical pore canals, the microstructure of some extant arthropod surface tubercles in polarized light is characteristic. As Bouligand (1965, 1971) has illustrated, such tubercles tend to exhibit a spiral pattern when sectioned tangentially. Our data on the crab cuticle confirm this general pattern (Pl. 9: 4, 5). However, the pattern for the tubercles in *Phacops rana* is completely different and, as with the helical pore canal data, lends no support to the comparison between the trilobite cuticle and that of the typical arthropod. As shown in Plate 9: 3 the cross-polarized light view of the tangentially sectioned tubercles is one of a dark cross or pseudo-optic axis figure. This figure is similar to that displayed by the corneal lens covering in phacopid eyes (Towe, 1973) produced by the radial distribution of the crystallographic C-axes in the cuticle. A similar pattern has been observed in the "eye spots" of some ostracode carapaces and we have noted its presence also on the nodes of *Agnostus pisiformis.* Whether or not these crystallographically oriented tubercles and nodes functioned as light

sensors similar to the phacopid eyes themselves or to the ostracode "eye spots" is not known, but the possibility exists. This could be especially true for *Agnostus* where the calcified cuticle is unusually thin and transparent.

While many of the trilobites have pore canals a number of the cuticle fragments studied show no evidence at all of any pore canals (Table 1). Most extant arthropods have some evidence of pore canals although certain areas of the cuticle may lack them (Richards, 1951). The trilobites, therefore, would have to be considered unique if a substantial number of their representatives lacked these structures. However, this finding may be biased in several ways and thus may be more apparent than real. Firstly, the fragments studied in some instances may represent portions of the cuticle that lacked pore canals while other portions of the exoskeleton not studied in fact contained these structures. Secondly, the pore canals could have been totally obliterated by diagenesis. The total obliteration of larger pore canals (> 10 μm) appears unlikely but in the case of narrow canals it is a possibility. As stated above, the pore canals are preserved in several ways, being filled with calcite, organic material or iron minerals. To obliterate pore canals completely without recrystallization or solution-reprecipitation of the cuticle, it is necessary to fill them with calcite in crystallographic optical continuity with the cuticle itself and therefore with the same C-axis preferred orientation. If the calcite filling is grown at any other orientation then it will be readily detected from the cuticle in cross-polarized light. For example, such is the case in our specimen of *Odontocephalus* (Pl. 8: 2). Pore canals filled with pyrite or organic material are seen best without polarized light but it can be noted that in cross-polarized light such canals when incompletely filled often disappear when traced forward (see

TABLE 1.

Specimen (No.)	Region sectioned	Outer layer	Laminations	Pore canals	Pseudo-pleochroism
Paradoxides (2)	Cephalon	p	+ i	N, i	+
Olenoides (2)	Pygidium	p	—	—	+
Ellipsocephalus (2)	Cephalon	A	—	N	—
Olenellus (2)	Cephalon	pr	—	—	—
Agnostus (6)	Whole	—	—	N	—
Isotelus (N.Y.) (4)	Pygidium and pleural fragment	p, pr	+	N	+
Isotelus (Vt.) (2)	Pleural fragment	p, pr	+	N	+
Cryptolithus (10)	Cephalon	—	+ i	—	—
Tretaspis (1)	Cephalon fragment	—	—	—	—
Bumastus (3)	Cephalon	pr, i	—	i	+ i
Proetus (2)	Cephalon	—	—	N, i	—
Odontopleura (2)	Whole	—	—	—	+ i
Diacalymene (2)	Pygidium	—	—	W	+
Calymene (1)	Cephalon	A	—	N, W	—
Flexicalymene (10)	Whole	pr	—	N–W	—
Acastopyge (1)	Cephalon	—	+ i	N	—
Odontocephalus (2)	Cephalon	—	—	N, W	—
Scutellum (1)	Pygidium	pr	—	—	—
Phacops (N.Y.) (11)	Whole	pr	+	N–W	E
Phacops (Tenn.) (3)	Pleural fragment	pr	—	N	E

+	present	p	pigmented	i	indistinct
—	absent	A	apatite	N	narrow (> 10 μm)
pr	prismatic	E	eye lenses only	W	wide

for example Pl. 8: 3). Thus in the absence of the opaque filling the canals might not be seen at all. The narrower the pores, the greater this possibility.

Størmer (1930) figured "minute tubulae or canaliculae" in the cuticles of some Scandinavian trinucleids where they were preserved as radiating lines of pyrite grains. This feature is conspicuous in *Tretaspis seticornis* but only in the sections containing pyrite. The other sections of *T. seticornis* figured from the same locality do not show pore canals nor do the other trinucleids figured by Størmer. Our trinucleid specimens (*T. clarkii* and *Cryptolithus tesselatus*) failed to show pore canals, although the "stay lines" also mentioned by Størmer are visible.

We conclude from these data that the diagenetic obliteration of narrow pore canals through calcite filling in optical continuity with the cuticle can be a common occurrence and that trilobites appearing to lack pore canals need to be carefully evaluated from this point of view. Therefore, in Table 1 we list those specimens in which we were unable to see evidence of narrow pore canals but this does not necessarily imply that these species were in fact lacking them in life. Only a careful evaluation of many thin-sections of the same species from different localities can clarify these data.

SUMMARY AND CONCLUSIONS

Twenty species of trilobites ranging from Cambrian through Devonian in age have been studied with regard to microstructure and composition. Only the mineralogy and crystallography of the principal layer is consistent throughout all of the specimens studied. All other features were found to vary. The data can be summarized as follows:

(1) MINERALOGY. – The highly calcified principal layer of all twenty species is composed of calcium carbonate in the form of calcite. Two species were found with an outer layer composed exclusively of apatite (calcium phosphate).

(2) CRYSTALLOGRAPHY. – The calcite of all twenty species is statistically oriented with the C-axis of the component crystals more or less perpendicular to the cuticle surface. The ultrastructural morphology of the component crystals is plate-like parallel or subparallel to the cuticle surface and the boundaries of the crystals are sutured and irregular, rarely with straight edges or primary crystal faces.

(3) LAYERING. – Twelve of twenty species have a principal layer as well as a thinner outer layer which can be prismatic in structure, pigmented, or composed of apatite. The remaining eight species have only the principal layer.

(4) LAMINATIONS. – Fine-scale lamellae related to the distribution of organic material occur within the principal layer in six out of twenty species. Parabolic structure was not observed.

(5) PSEUDOPLEOCHROISM. – This optical phenomenon related to the preservation of organic material in the calcitic cuticle could be observed in seven of twenty principal layers and only in the schizochroal eye lenses of two phacopids.

(6) PORE CANALS. – Fourteen out of twenty species show evidence of some type of pore canal structure preserved in the cuticle. Two of these have two types of pore canals with two distinct size ranges. None of the pore canals is distinctly twisted or helical.

(7) ORGANIC MATRIX. – Structurally well preserved organic material isolated from selected cuticles shows a fine reticulated meshwork ultrastructure but is devoid of original biochemical constituent amino acids.

From the data given in this report, and as summarized above, we conclude that trilobite exoskeletal microstructure and composition do not compare as favorably with that of a typical generalized arthropod cuticle as has been thought. That is to say, the three-fold division of the typical arthropod cuticle into an outer epicuticle, a middle exocuticle and an inner laminated endocuticle is not found in the majority of trilobites. This does not mean, however, that the

trilobites lacked the three-fold division in life since the epicuticle in extant arthropods is generally a thin, non-chitinous and uncalcified layer and is unlikely to have been preserved except under unusual circumstances. It is possible therefore that the outer thin layer observed in many trilobites represents the exocuticle and the inner or principal layer corresponds to the endocuticle; the epicuticle being lost with the other soft tissues in fossilization.

The fine endocuticular lamellae commonly observed in extant arthropods (especially insects) are generally lacking among the trilobites. These laminations are not universally found in arthropod endocuticle as the work of Bate & East (1972) and Harding (1964) shows in some calcified ostracode carapaces. The presence or absence of lamellae in trilobites may be due to genetic factors or to fossil preservation but the absence of parabolic structures (and helical pore canals) indicates that those lamellae that are found are not the same as those normally observed in the typical extant arthropod endocuticle.

Narrow pore canals, often observed in trilobite cuticles, are apparently subject to the vagaries of fossilization and we believe that their absence in a given specimen may be due to diagenetic obliteration. Only by checking several specimens from different localities can it be established whether or not a given species lacked small pore canals in life. The larger wide pore canals, less likely because of their size to be obliterated, may have functioned differently from the narrow ones and as with extant arthropods could have been ducts containing a sensory apparatus – an uncertain conclusion.

The structurally preserved but biochemically destroyed organic matrix isolatable from trilobite cuticle is in keeping with the pattern of preservation found in other older fossil material. The reticulate meshwork observed in the electron microscope compares favorably with a similar pattern seen in decalcified ostracode cuticle (Bate & East, 1972). The absence of biochemical evidence for chitin does not mean that the trilobite cuticle lacked this material but only that it is not now chitinous. It is highly probable that at least some chitin and protein was present in the living trilobite cuticle simply by analogy with extant arthropods.

The crystallography, mineralogy and high degree of calcification of the trilobites also compares favorably with that of many (but not all) ostracodes where the carapace too has a pronounced C-axis preferred orientation, is calcitic and is highly calcified (Sohn, 1958). The trilobite cuticle is not phosphatic. Although two species were found with distinct outer phosphatic layers showing evidence to indicate they could be primary, the distribution of the phosphate is completely separate from the carbonate and this is not typical for many extant calcareous-phosphatic arthropod cuticles. In spite of some evidence to support the primary nature of these outer phosphatic layers it is not certain and until this can be confirmed with other specimens the significance of this finding is not clear.

The trilobites studied range in age from Cambrian through Devonian and represent six orders. No clear pattern or correlation with either geologic age or with systematic position could be discerned from the data and it appears that most of the microstructural and compositional features in one species or another can be dated from the Cambrian, but the data are too limited to draw any firm conclusions in this respect.

Throughout this work we have emphasized the lack of correspondence between the microstructural and compositional details of the trilobite cuticle and that of the typical arthropod (insects and decapod Crustacea). At the same time, we have pointed out several similarities with the calcified ostracode carapace. It is our conclusion that no other group of arthropods living today compares in as many ways as favorably with the trilobites in this respect as do the calcified ostracodes. A phylogenetic relationship between these two groups of arthropods is not, on the basis of shell structure and composition, out of the realm of possibility.

ACKNOWLEDGEMENTS. – This study was made possible by a postdoctoral fellowship from the Smithsonian Institution through the Office of Academic Studies to Dian Teigler. Although there is not enough space to thank all the many members of the staff of the Smithsonian who have aided this project, the authors would like to particularly thank Mr. Donald Dean for his advice on thin-sectioning techniques, Mr. Charles Obermeyer for help in performing the electron probe analyses, Mr. Warren Blow, Mr. Fred Collier and Dr. Michael Taylor for assistance in locating and identifying specimens and Mr. Larry Isham for his aid in preparing some of the illustrations for this manuscript. We are also grateful to Dr. P. E. Hare for carrying out amino acid analyses of our samples. In addition, we would like to thank Dr. John Dalingwater, Dr. Halszka Osmólska and Dr. Ewa Tomczykowa who have aided us in discussing problems and by providing material for study. Dr. Richard A. Robison and Dr. I. Gregory Sohn reviewed drafts of the manuscript and made helpful suggestions for improvement.

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APPENDIX

Each specimen in this study has been assigned a USNM catalog number here listed under its name. A second number in parentheses is the older USNM number of the suite of specimens from which the individual was taken in those instances where such a number existed.

Specimen	*Locality*	*Age*
Paradoxides oelandicus 203467	Borgholm, Öland, Sweden	Middle Cambrian
Olenoides neolenus 203468	Antelope Springs, House Range, Utah	Middle Cambrian
Ellipsocephalus polytomus 203469 (23928)	Borgholm, Öland, Sweden	Middle Cambrian
Olenellus logani 203470 (137537)	Forteau Bay, Labrador, Canada	Middle Cambrian
Agnostus pisiformis 203471 (23905)	Tomta Närke, Sweden	Middle Cambrian
Cryptolithus tesselatus 203472 (72057)	Cold Brook, Poland, New York	Middle Ordovician
Isotelus gigas 203473-75	Grays Brook, Russia, New York	Middle Ordovician
Isotelus platymarginatus 203476	Isle la Motte, Vermont	Middle Ordovician
Tretaspis clarkei 203477	Whitehead, Mont Joli, Perce, Quebec	Late Ordovician
Bumastus subglobosus 203478 (72225)	Isle la Motte, Vermont	Middle Ordovician
Odontopleura trentonensis 203479	Grays Brook, Russia, New York	Middle Ordovician
Flexicalymene meeki 203480 (40926)	Waynesville, Ohio	Middle Ordovician
Proetus signatus 203481	Potok, Poland	Late Silurian
Diacalymene diademata 203482	Mielnik, Poland	Middle-Upper Silurian
Calymene cf. beyeri 203483	Radoszewo, Poland	Late Silurian
Acastopyge shergoldi 203484	Karwia, Poland	Late Silurian
Odontocephalus selenurus 203485 (79160)	Union Springs, New York	Early Devonian
Goldius palifer (Scutellum) 203486 (72519)	Koneprusy, Bohemia	Early Devonian

Specimen	*Locality*	*Age*
Phacops rana 203487-92	Buffalo, New York	Middle Devonian
Phacops logani 203493-94 (27849)	Big Sandy River, Benton County, Tennessee	Early Devonian
Unidentified phacopid 203495	Decatursville, Tennessee	Silurian
Cancer sp.	Virginia Beach, Virginia	Recent
Cancer sp.	Chuckatuck, Virginia	Late Miocene

EXPLANATIONS OF PLATES

Plate 1

Fig. 1. Isotelus gigas (203473) viewed in cross-polarized light to show a typical "patchy" extinction of the calcite cuticle. Note also the outer prismatic layer. Cross section of a portion of a cephalon. X325.

Fig. 2. Isotelus gigas (203475) cross section of a pleural fragment, also with patchy extinction. The step-like surfaces of the prismatic layer are cross sections of terrace lines (arrow). X175, cross-polarized light.

Fig. 3. Flexicalymene meeki (203480) pygidium viewed in cross-polarized light to show the zones of extinction. This is an example of the more homogeneous extinction of the microcrystalline calcite which is crudely layered. These are not true laminations. The prismatic layer has a somewhat different extinction pattern and larger crystals. X260.

Fig. 4. The same section as in Fig. 3 but viewed with ordinary light. The prismatic layer (PR) is visible as well as portions of some pyrite-filled pores (arrows). X260.

Fig. 5. Flexicalymene meeki (203480) a thoracic segment showing the extinction zones and the outer prismatic layer (left side of cuticle). X140.

Fig. 6. Phacops rana showing patchy and homogeneous extinction. X140.

Plate 2

Figs. 1–4. Electron micrographs of replicas of the surface of three trilobites and an ostracode showing the similar plate-like, sutured appearance of the surfaces. The sutured plates of the ostracode are smaller.

Fig. 1. Agnostus pisiformis (203471) X8,900 (unetched). Fig. 2. *Isotelus gigas* (203471) X4,400. Fig. 3. Unidentified marine ostracode X28,000 (unetched). Fig.4. *Phacops rana* (203489) X4,900. Figs. 5 & 6. Electron micrographs of carbon-platinum replicas of cross sections of different specimens of *Phacops rana* showing the typical arrangement of crystals in the cuticle. There is a rough shingle-like orientation parallel to the cuticle surface. X5,300.

Plate 3

Fig. 1. Electron probe scanning profile across a section of *Calymene* sp. (203482) showing the distribution of P (dashed) and Ca (solid).

Fig. 2. A similar electron probe scanning profile of *Isotelus gigas* (203473) for comparison.

*Fig. 3.*A back-scattered electron photograph of the same cross section of *Calymene* as in Fig. 1 showing the sharp distribution of phosphorus at the outer edge (C – cuticle, M – plastic embedding medium).

Fig. 4. Photomicrograph of a section of *Calymene* sp. (203482) showing the apatite layer on the exterior which corresponds to the region of high phosphorus concentration in Figs. 1 and 3. X240.

Fig. 5. Cross section of *Isotelus gigas* (203473) such as was scanned in Fig. 2. Although the section is laminated and has a pigmented prismatic outer layer (PR), no region contains phosphorus. The wide surface pits are characteristic of this species.

Plate 4

Figs. 1 and 2. Electron probe scanning profiles showing the distribution of P (Kα) and Ca (Kα) in a cross section of fossil (Fig. 1) and Recent crabs (Fig. 2). The graph of Fig. 1 begins at approximately the middle of the cuticle and extends to the exterior while that of Fig. 2 scans the entire cuticle thickness. In both cases the amount of P tends to increase in the outer third of the cuticle. The dip in Ca in Fig. 2 represents a less calcified area rich in organic material.

Fig. 3–8. Powder X-ray diffraction patterns. Fig. 3. Sample from the upper surface of a Recent crab (same specimen used in Fig. 2), calcite. Fig. 4. Calcite standard. Fig. 5. Upper surface of a fossil crab; mixture of calcite and apatite. Fig. 6. Apatite standard. Figs. 7 and 8. Samples of the pink outer layer of *Ellipsocephalus polytomus* (203469) and *Calymene* (203482) respectively. Both are apatite.

Plate 5

Fig. 1. A fragment of a schizochroal eye from *Phacops rana.*

Fig. 2. The same fragment as in Fig. 1 after complete decalcification in EDTA showing the residual organic material and some pyrite.

Fig. 3. Electron micrograph of organic material such as in Fig. 2, which was air-dried and shadowed with platinum-carbon. X32,000.

*Fig. 4.*Electron micrograph of phacopid organic matrix which has been embedded and sectioned showing the delicate meshwork. X9,400.

Plate 6

Fig. 1. A section of *Phacops rana* (203489) cuticle lightly etched and coated with platinum-carbon, viewed in reflected light. Note the lamina which have etched differentially. X260.

Fig. 2. Electron micrograph of the replica in Fig. 1 showing the slight furrowing (arrows) indicating the direction of the laminae. X6,000.

Figs. 3–6. The various appearance of laminae within the same specimen and in different specimens from the same locality. Fig. 3. *Phacops rana* (203491). X120. Fig. 4. *Phacops rana* (203489). X260. Fig. 5. *Phacops rana* (203489). X230. Fig. 6. *Phacops rana* (203489). X100.

Plate 7

Figs. 1 and 2. A section of *Isotelus gigas* (203472) to show the effect of the pseudopleochroic material when viewed at right angles in plane polarized light. The vibration direction is east-west. X185.

Figs. 3 and 4. A calcite lens of a phacopid eye which also contains pseudopleochroic material. There is however no pseudopleochroism in any other region of this specimen (unidentified, 203495). X185.

Plate 8

Fig. 1. Section of *Odontocephalus selenurus* (203485) showing two of the many very large pore canals present in the pygidium. X185.

Fig. 2. Another area in the same section of *Odontocephalus* viewed in cross polarized light to bring out the narrow pores also present in the cuticle (an oblique view of a large pore can be seen at the left). X260.

Fig. 3. Flexicalymene meeki (203480) showing some of the many pore canals discontinuously filled with pyrite. X145.

Fig. 4. Calymene sp. (203482), one of the large pores present in the cuticle. Compare with the small pore from the same specimen, Plate 3, Fig. 4 (arrow). X200.

Fig. 5. Photomicrograph of a possibly twisted pore from a phacopid cuticle (203495). X720.

Fig. 6. Electron micrograph of a replica of a pore from the same section as in Fig. 5, indicating that the pore is not helicoidal. X3,900.

Plate 9

Fig. 1. A large twisted pore in the cuticle of a fossil crab. X1,000.

Fig. 2. An oblique section of fossil crab cuticle showing the parabolic pattern indicative of twisted layers. X570.

Fig. 3. A tangential section in polarized light of tubercles on the cephalon of *Phacops rana* indicating a radial C-axis orientation of the calcite crystals in each. X140.

Fig. 4. A tangential section of a tubercle of a fossil crab showing the spiral pattern characteristic of this type of cuticle architecture. X350.

Fig. 5. The same section as in Fig. 4 viewed with polarized light. Contrast this to the trilobite tubercle pattern seen in Fig. 3. X350.

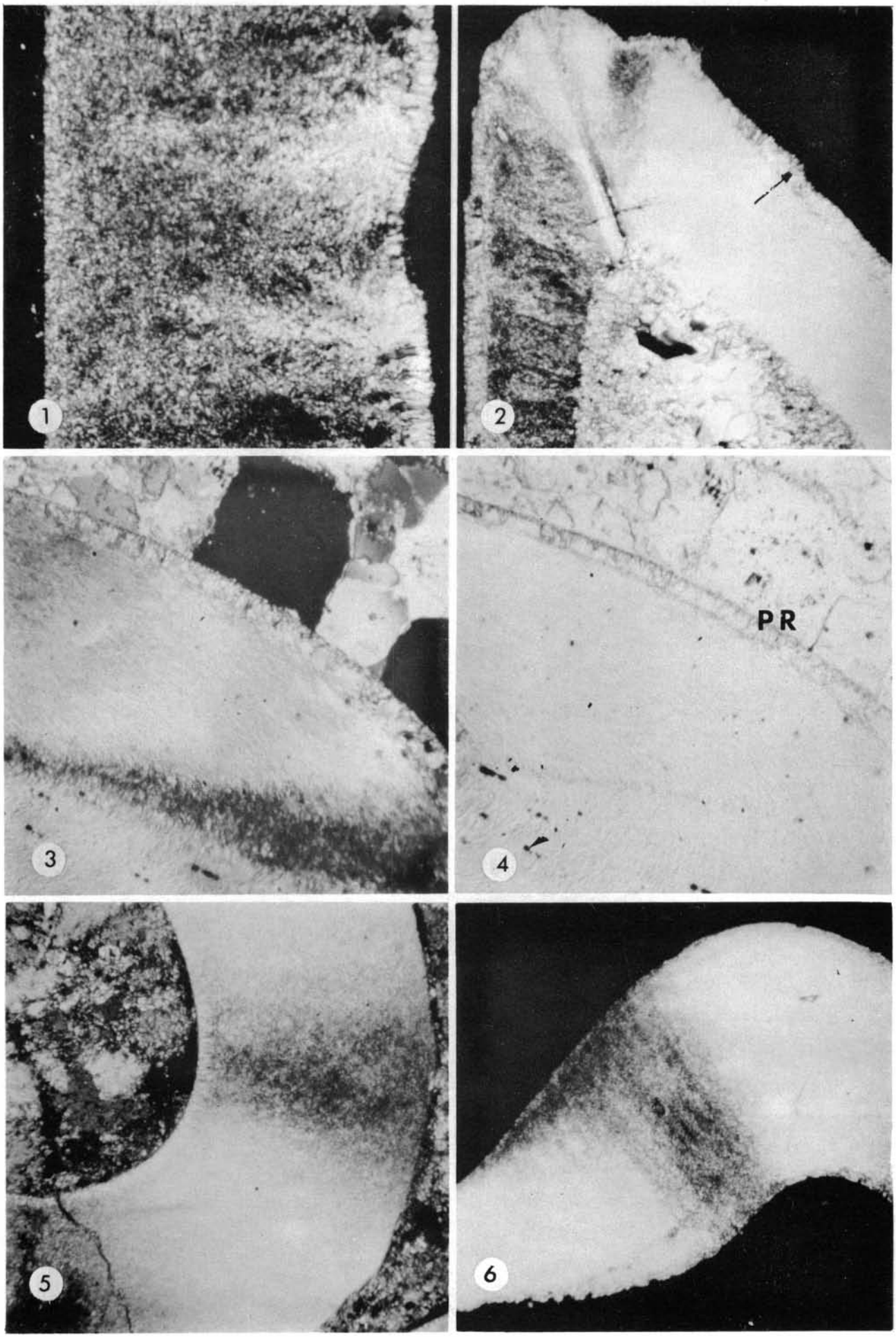
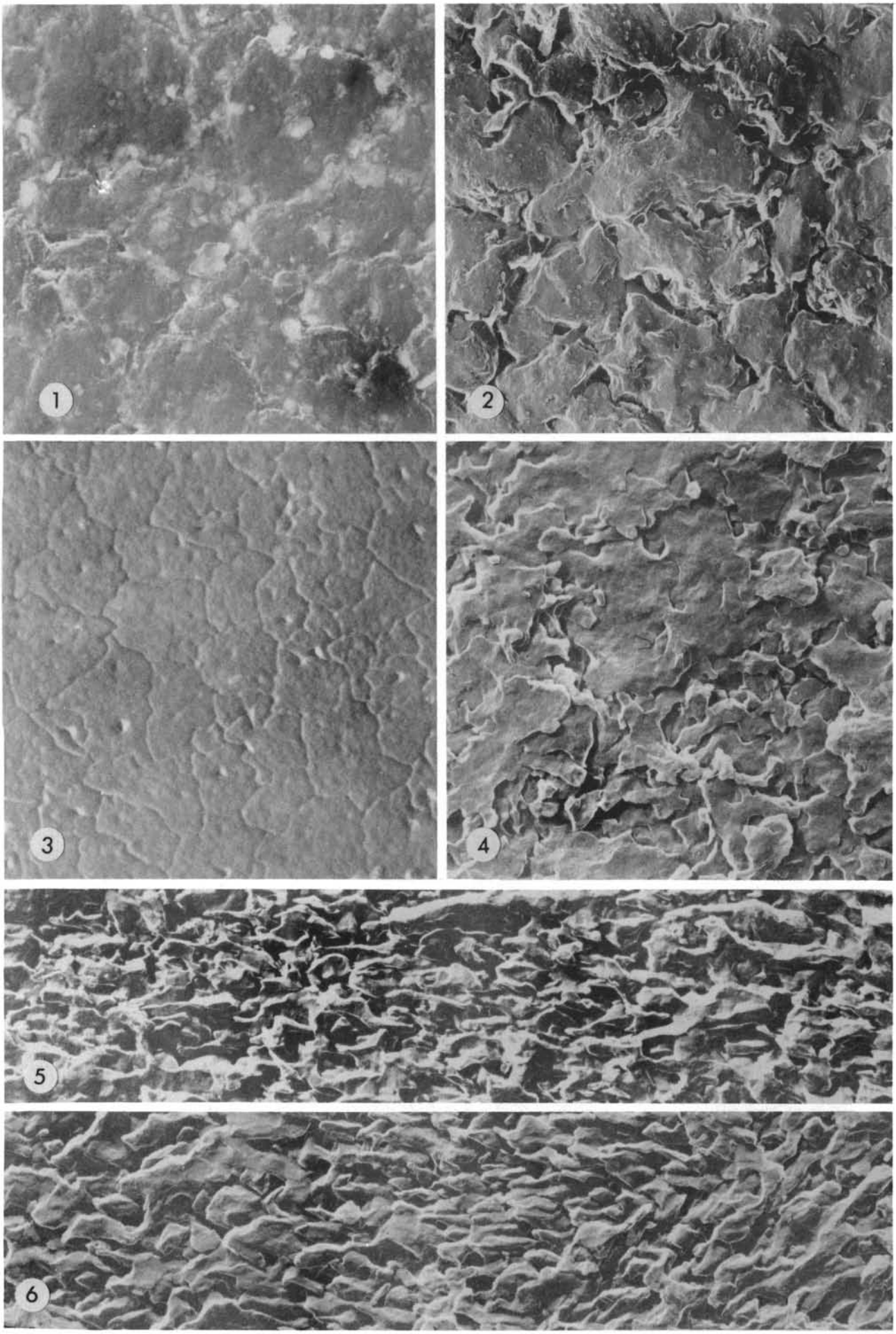


Plate 1.



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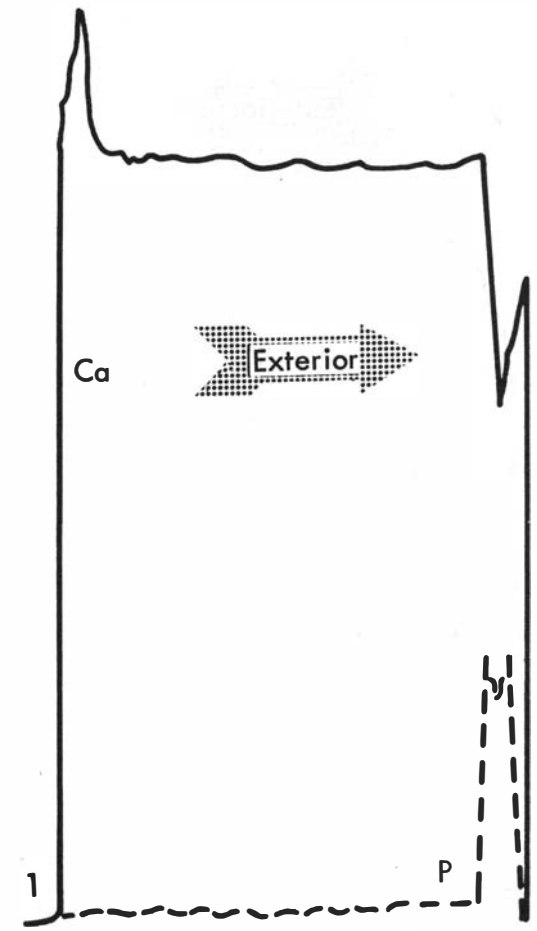

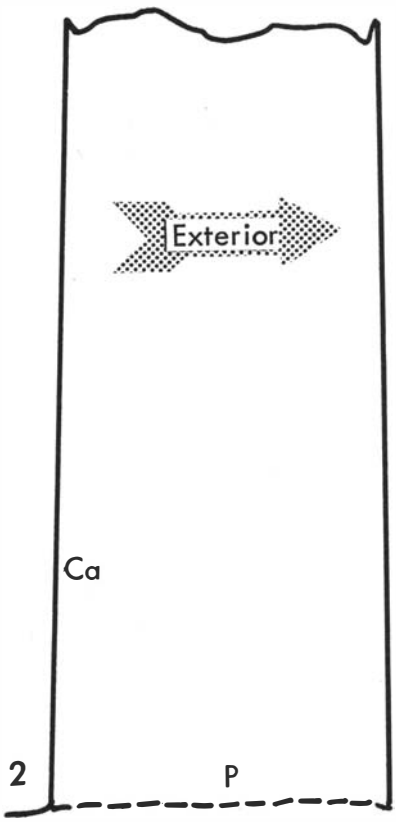

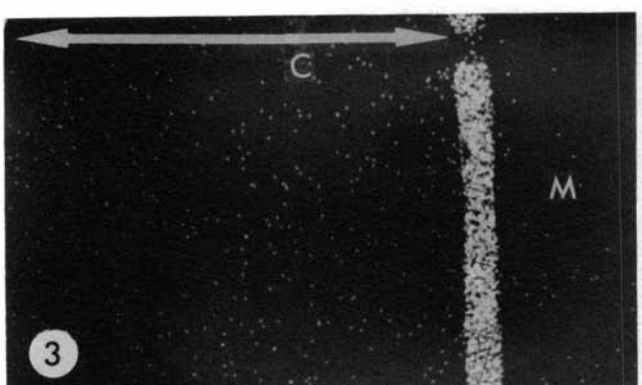

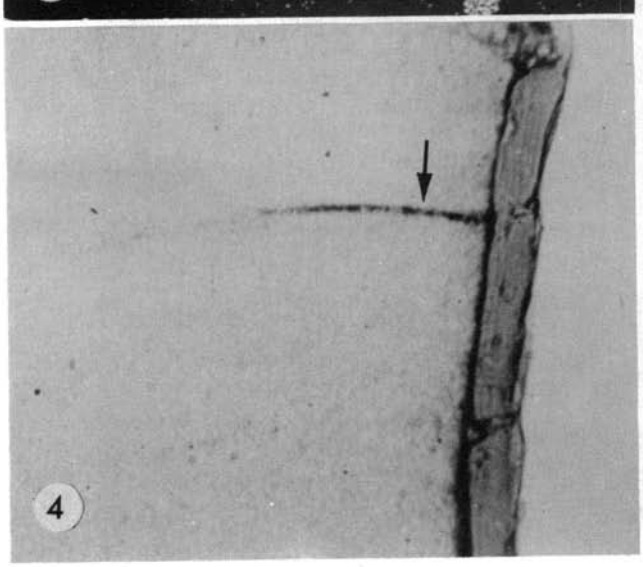

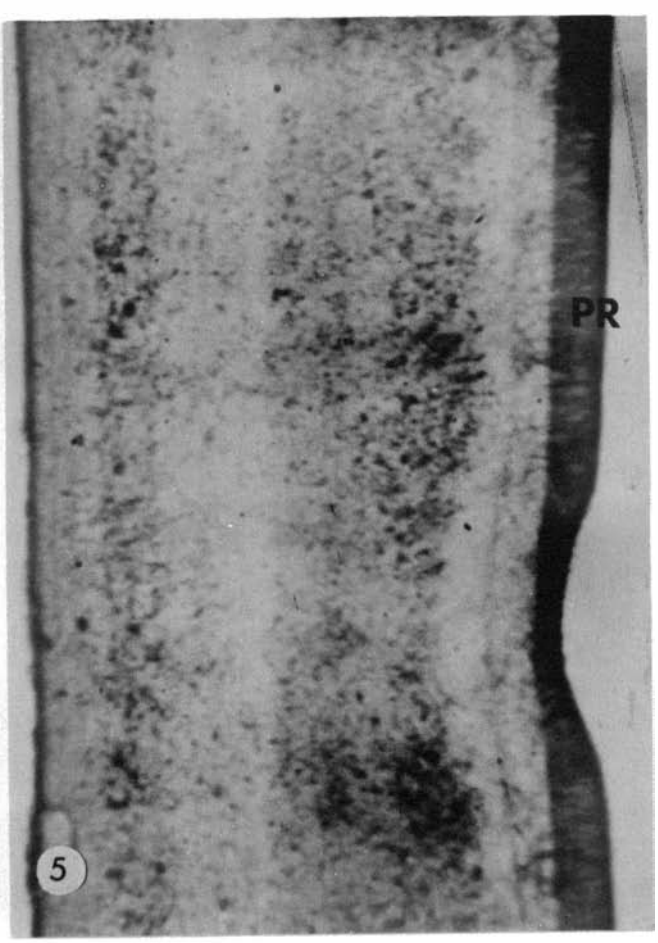

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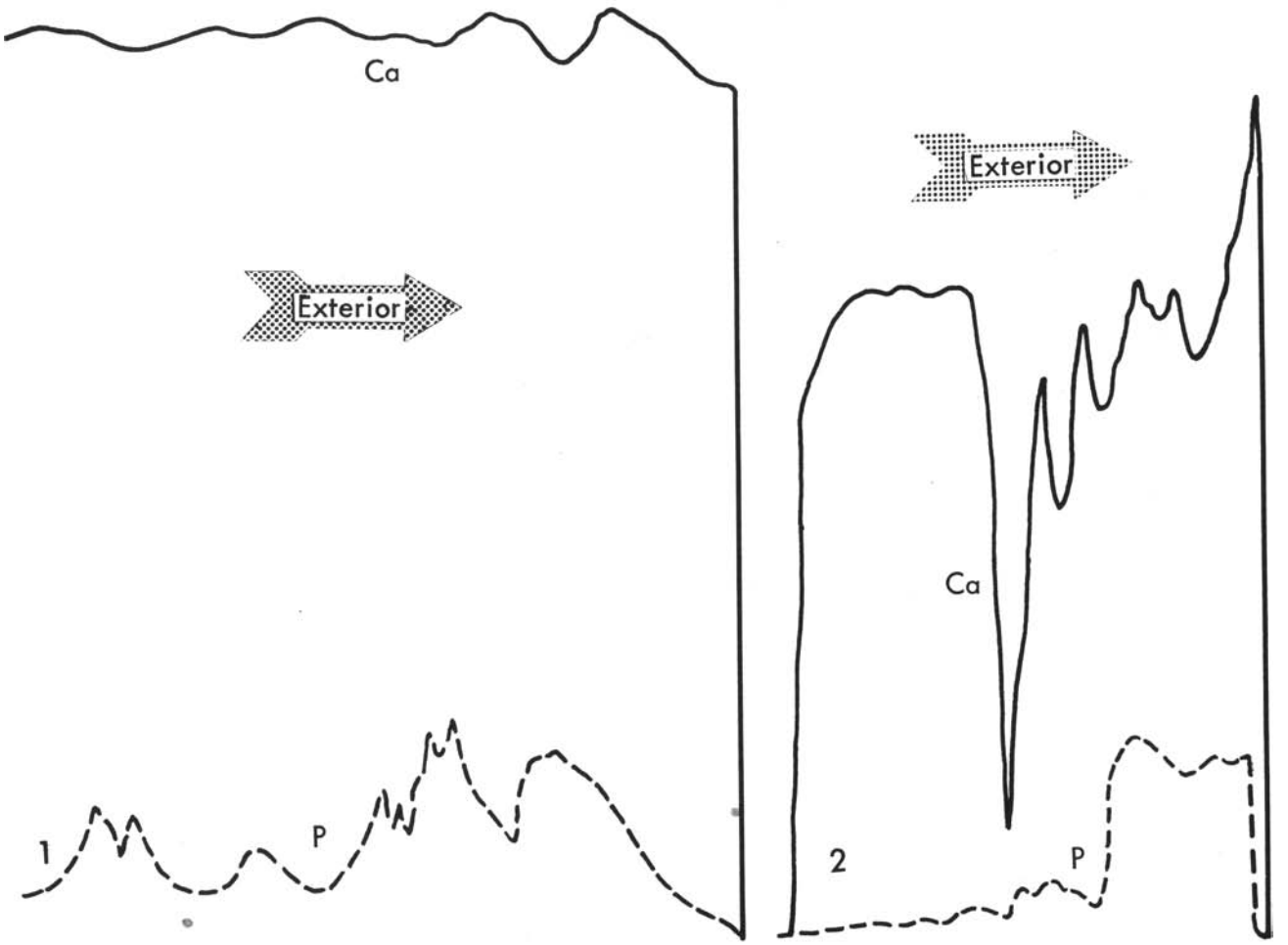

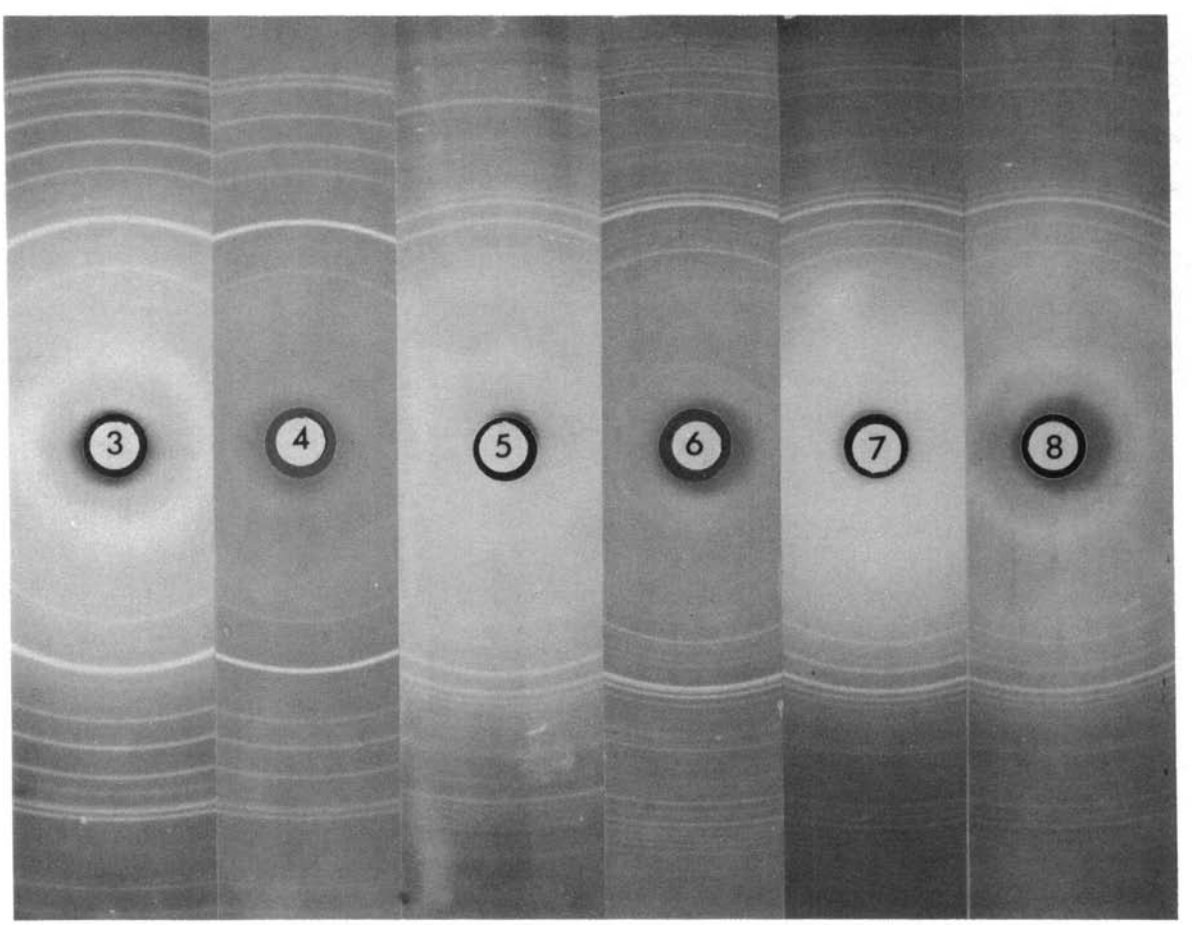

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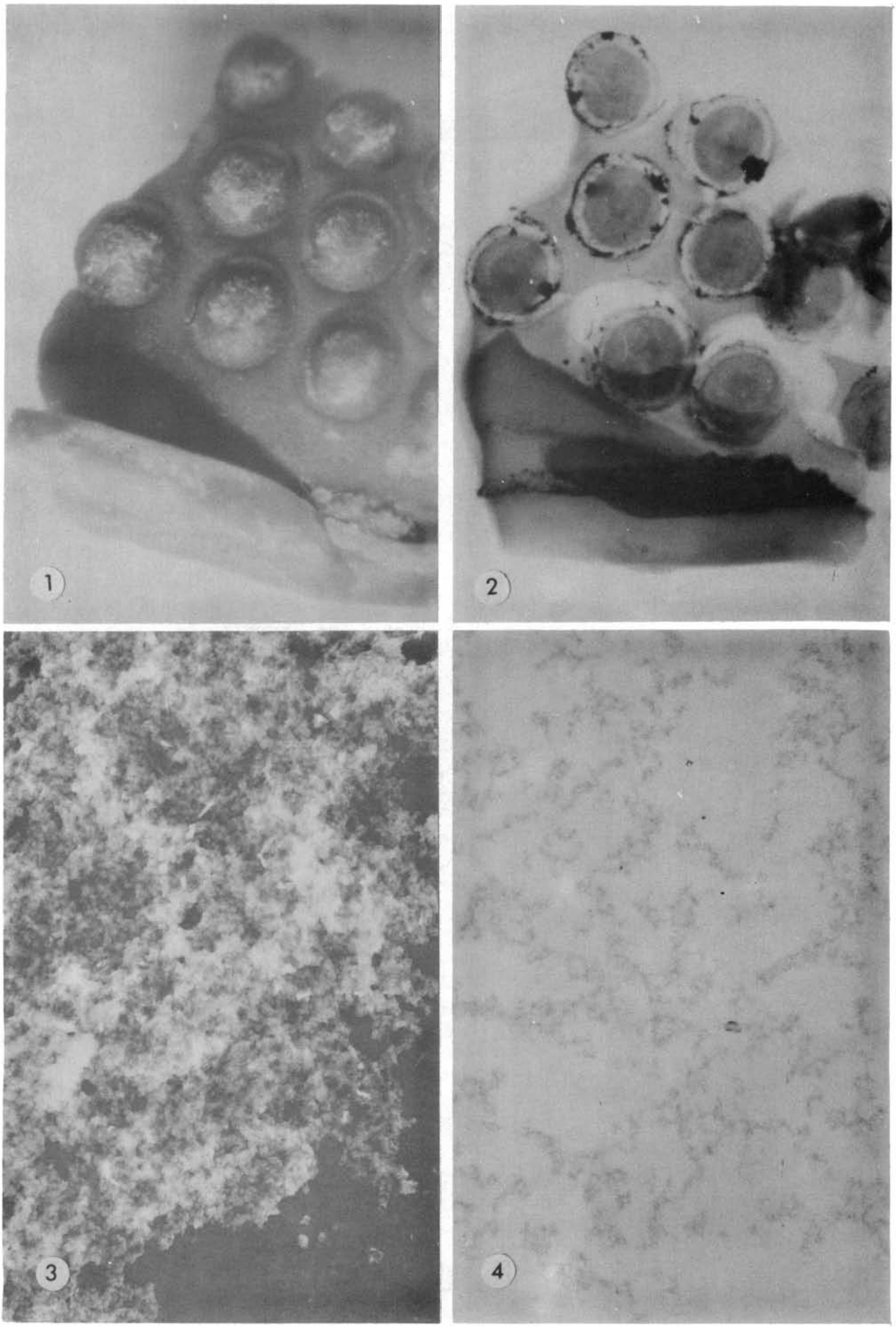

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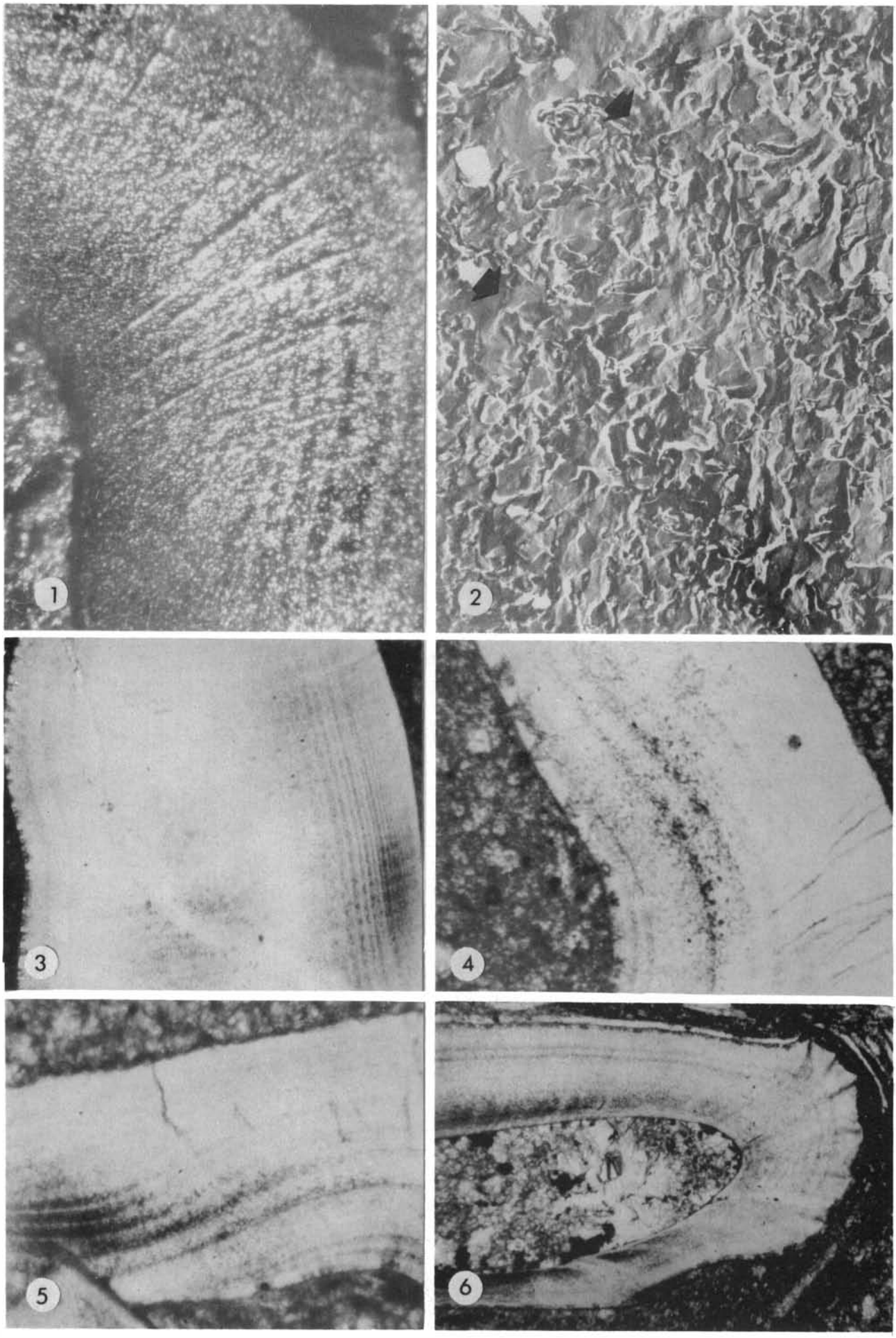

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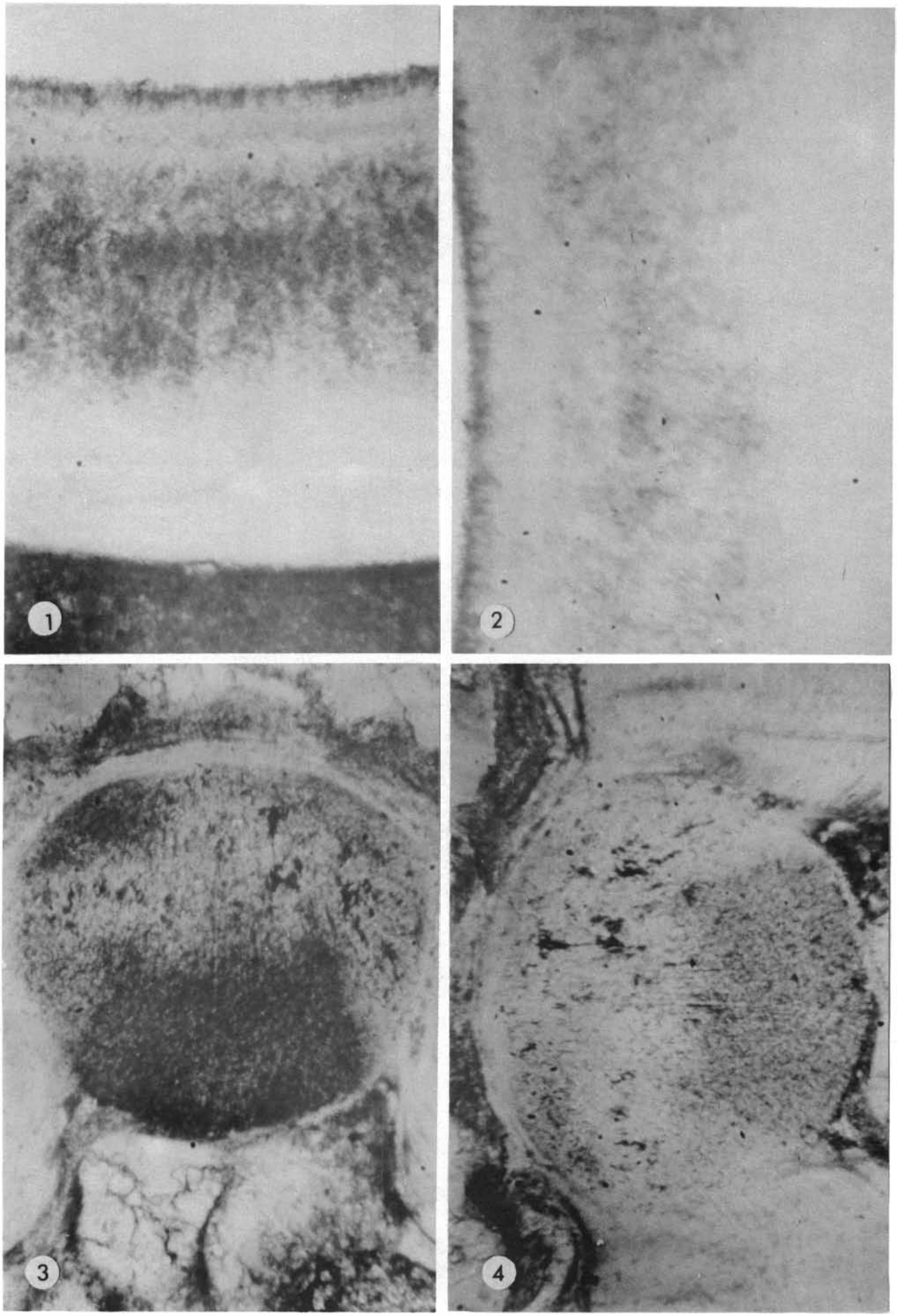

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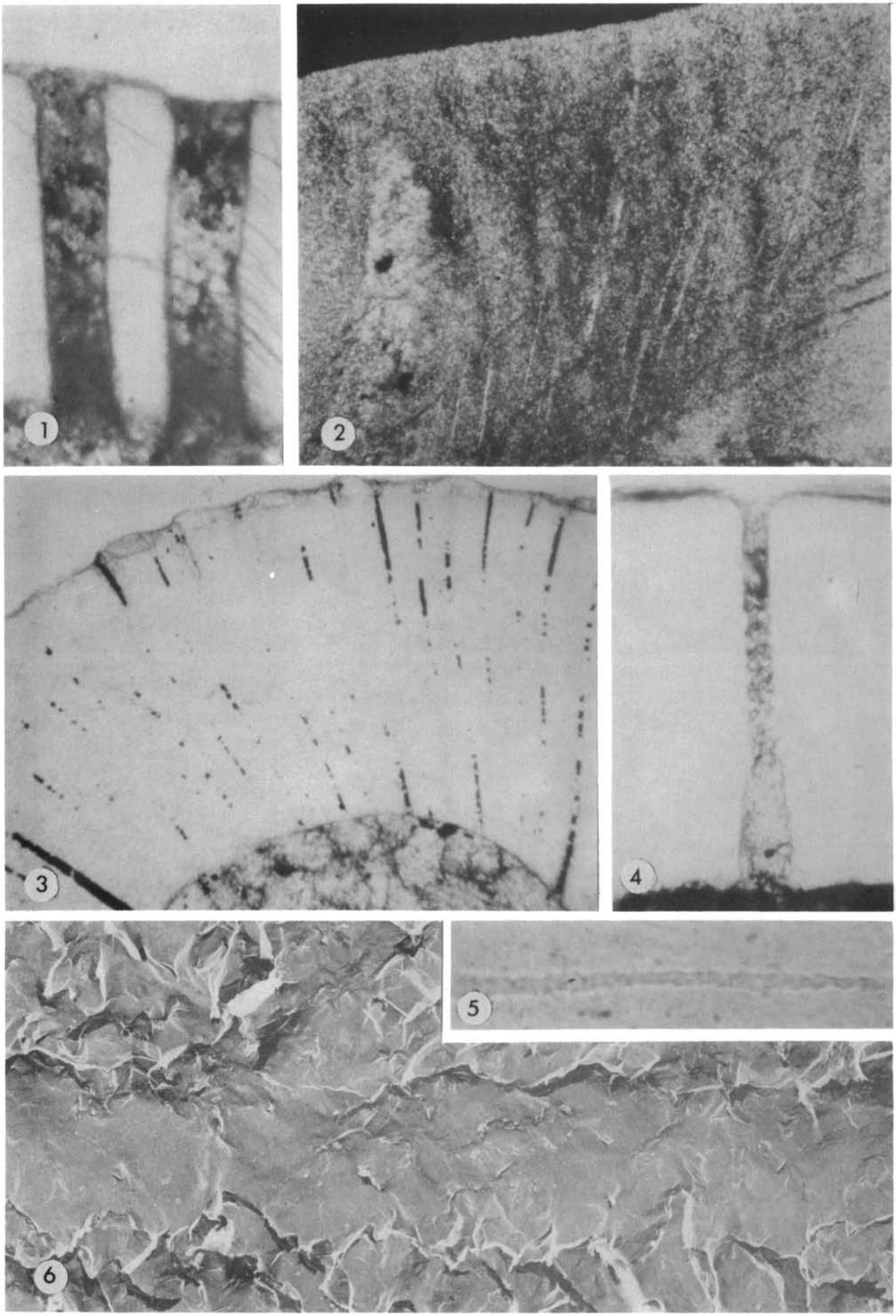

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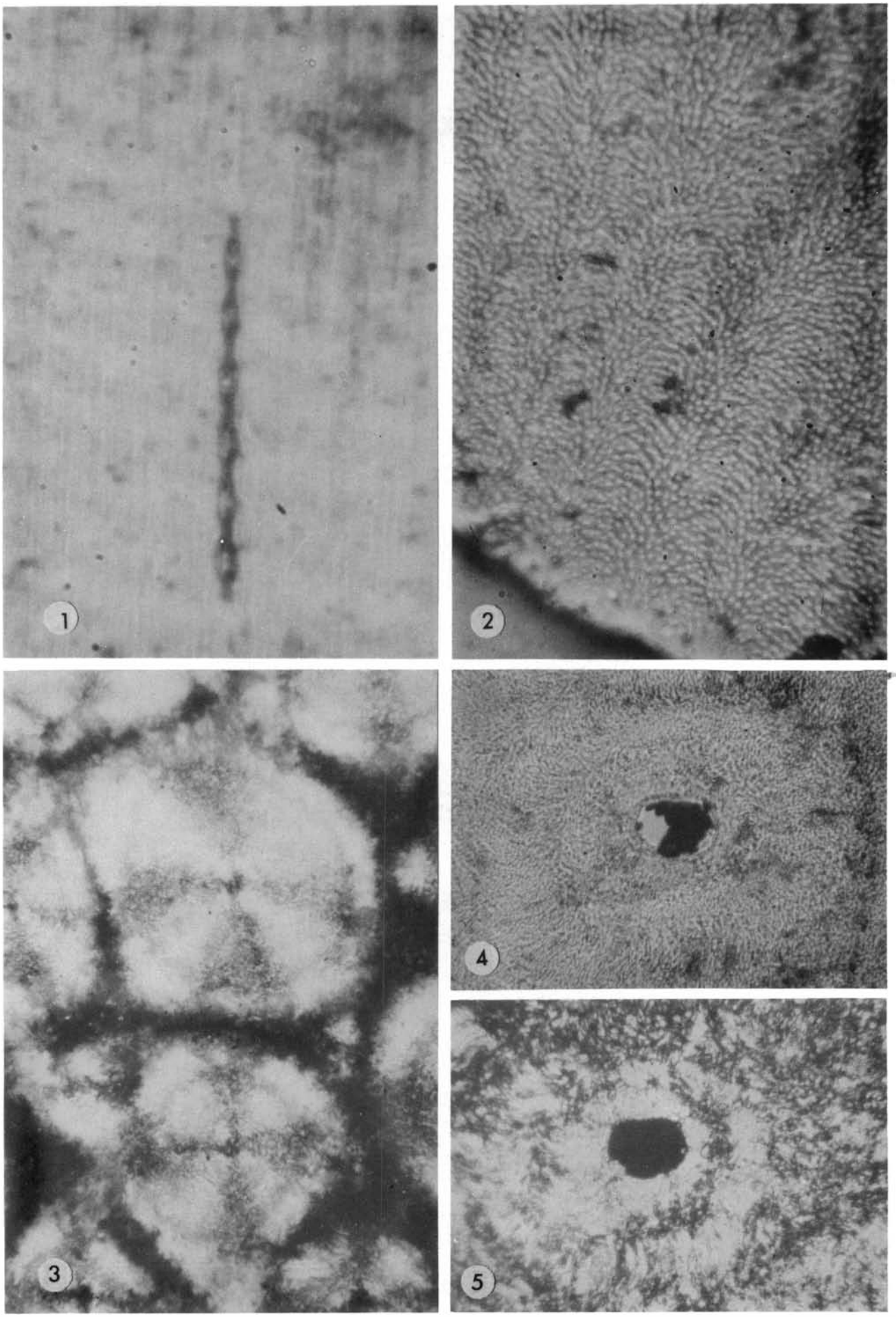

Plate 9