CUTICULAR ULTRASTRUCTURE OF THE TRILOBITE *ELLIPSOCEPHALUS POLYTOMUS* FROM THE MIDDLE CAMBRIAN OF ÖLAND, SWEDEN

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ABSTRACT. Hand specimens and polished sections of the cuticle of the trilobite *Ellipsocephalus polytomus* Linnarsson from the Middle Cambrian of Öland, Sweden have been examined in incident light and, after etching, with the scanning electron microscope. A thin (25–50 μm) outer layer comprises about twenty lamina units; the structure of these units is interpreted as representing the original inorganic material of the cuticle, and therefore also reflecting the structure of the original organic template. X-ray microanalysis strongly suggests that this outer layer is now composed of calcium phosphate. Cavities, 15 μm in diameter, in the outer layer connect to 3 μm diameter canals which extend across the principal layer of the cuticle: these resemble the gland ducts of a Recent millipede. Pore canal pathways may be represented by elongate openings on the undersurface of the outer layer, and structures resembling the interprismatic septa of Recent decapod crustaceans are seen in angled slices. Other primary microstructures identified are relict organic material and fibres which may have bound together the major layers of the cuticle. Horizontal tubules on the undersurface of the outer layer are possibly infilled borings of cyanobacteria.

Major subdivisions of *Ellipsocephalus* cuticle in life are proposed as: a very thin outermost epicuticle, an outer laminated layer, and a principal layer, the original structure of which is represented only by disc-like extensions on the perpendicular canals which pass across it.

TRILOBITE cuticular microstructure has been extensively investigated over the past twenty years (see Dalingwater 1973; Teigler and Towe 1975; Dalingwater and Miller 1977, Størmer 1980; Wilmot and Fallick 1989; Wilmot 1990*a*), yet our knowledge of the overall structure of the cuticle is far from complete, and the only detailed information on ultrastructure of lamina units has been provided by Mutvei (1981) from a *Flexicalymene* species from the upper Ordovician of Iowa.

In this paper we describe ultrastructural detail from an outer cuticular layer of the Middle Cambrian trilobite *Ellipsocephalus polytomus* Linnarsson, 1877, from Enerum, Öland, Sweden, which is superior to anything previously reported from any trilobite cuticle. We analyze our observations in relation to Recent arthropod material, assess the implications for views on the overall structure of the trilobite cuticle and outline areas for further investigation.

MATERIALS AND METHODS

The collections of the Naturhistoriska Riksmuseet in Stockholm contain specimens of the Middle Cambrian trilobite genus *Ellipsocephalus* preserved in different lithologies: shales, limestones and even conglomerates. However, the best-preserved material seems to be in the Middle Cambrian glauconitic limestones from Enerum and Borgholm on the Baltic island of Öland. Specimens of *Ellipsocephalus polytomus* from these localities are almost exclusively cranidia, although the collections also include a few complete dorsal exoskeletons. A series of pieces of glauconitic limestone containing cranidia of *Ellipsocephalus polytomus* and *Paradoxides* sp. fragments collected by Westergård from 'a boulder at Enerum, Öland in 1930' were selected for study.

The surface of the cuticle of *Ellipsocephalus* was examined and photographed in incident light.

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Slices of the limestone, approximately 2 mm thick, were cut away from blocks of material with a thin high-speed diamond wheel, after one face had been flattened on rotating wheels covered with carborundum-impregnated papers and lubricated with water, and polished to a mirror finish using ultra-fine diamond pastes on felt buffing wheels. Material prepared in this way was examined under a stereo binocular microscope in incident light. Further slices prepared in a similar fashion were etched in a supersaturated aqueous solution of ethylenediaminotetracetic acid (disodium salt) for up to three hours, with the etching process observed from time to time under a stereo binocular microscope in incident light. Etched slices were carefully washed in de-ionised water, air dried, gold sputter-coated and examined with a Cambridge S360 scanning electron microscope (SEM) under optimum conditions for high resolution (short working distance, high accelerating voltage, small aperture size, small spot size). In all, eleven etched preparations were made for SEM examination; each preparation contained at least two and as many as five sections of *Ellipsocephalus* cuticle, as well as those of *Paradoxides* sp. Most of the slices were deliberately cut in such a way that the cuticle was sectioned more or less perpendicular to the cuticle surface, though a few angled slices were accidentally produced and a few deliberately achieved. In addition, an accidental but fortuitous break of cuticle along a low angle from the horizontal was made; part and counterpart of this break were examined unetched with the SEM.

Two further preparations were carbon coated and analysed with the LINK system of X-ray microanalysis attached to a Cambridge S360 SEM.

All preparations are stored with their parent specimens (Ar 46218*a–f*) in the Sektionen för Paleozoologi, Naturhistoriska Riksmuseet, Stockholm (RM).

DESCRIPTION OF THE CUTICLE

Terminology

We follow Dennell's (1973) terminology for horizontal laminations of the cuticle: each *lamina unit* is considered to consist of a narrower *lamina* and a wider *inter-lamina*. This contrasts with the view of Bouligand (1965) who considered the lamination of arthropod cuticles as an artefact resulting from the sectioning of horizontal sheets of fibres with fibre orientation changing from one sheet to the next. (For a more detailed discussion see Dalingwater and Mutvei 1990).

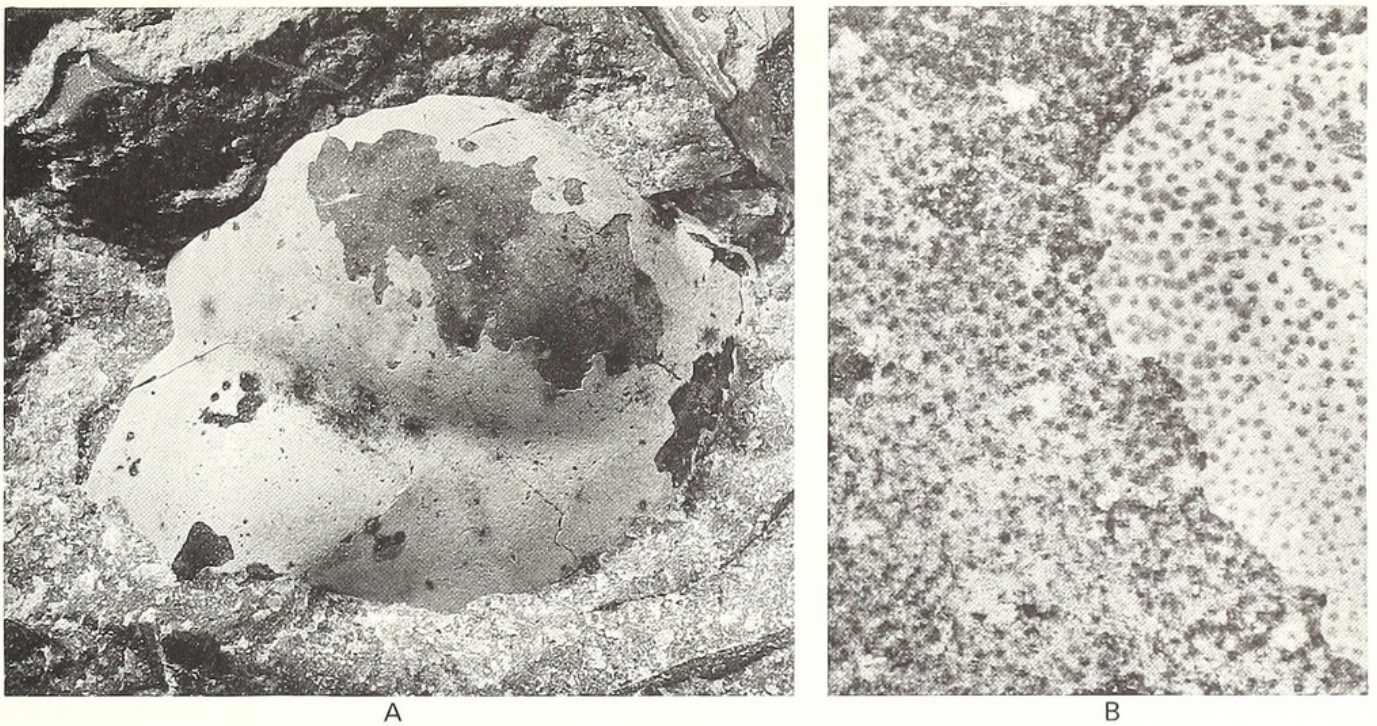
Hand specimens

A consistent feature of *Ellipsocephalus* specimens from Öland is a thin outer layer which has a faint pinkish tinge: this was commented on by Teigler and Towe (1975, pp. 138–139) who also established that a similar thin outer layer in a Silurian calymenid from Poland was composed of calcium phosphate, probably in the form of apatite.

Specimens of *Paradoxides* sp., in the same beds on Öland do not have a thin layer of this nature. The layer does not completely cover all parts of every *Ellipsocephalus* specimen: it is often worn away from the prominence of the glabella (Text-fig 1A) and, in a few examples, seems to be absent, possibly removed on the counterpart. In the latter situation, the brown exposed 'surface' of the cuticle has the shiny appearance characteristic of other well-preserved trilobites. It is possible to find two *Ellipsocephalus* cranidia side by side on the same bedding plane, one with a pinkish outer layer, the other apparently without. However, when examined under a microscope, at least traces of the outer layer can be found on all specimens. In the very rare 'complete' specimens of *Ellipsocephalus*, all parts of the dorsal exoskeleton are seen to be covered by the outer layer.

Hand specimens and polished slices viewed in incident light

When the surface of the outer layer is viewed in incident light at low magnifications, almost its entire area appears to be patterned with small circular punctations, about 15 μm in diameter (Text-fig. 1B). The spacing of these punctations is somewhat irregular: in places they are almost contiguous, contrasting with small clear patches, but on average they are 15 μm apart. In areas where the outer



TEXT-FIG. 1. *Ellipsocephalus polytomus* Linnarsson from Enerum, Öland, Sweden. Specimen RM Ar 46218*c*. A, cranidium, × 5. B, detail of surface punctations, × 100.

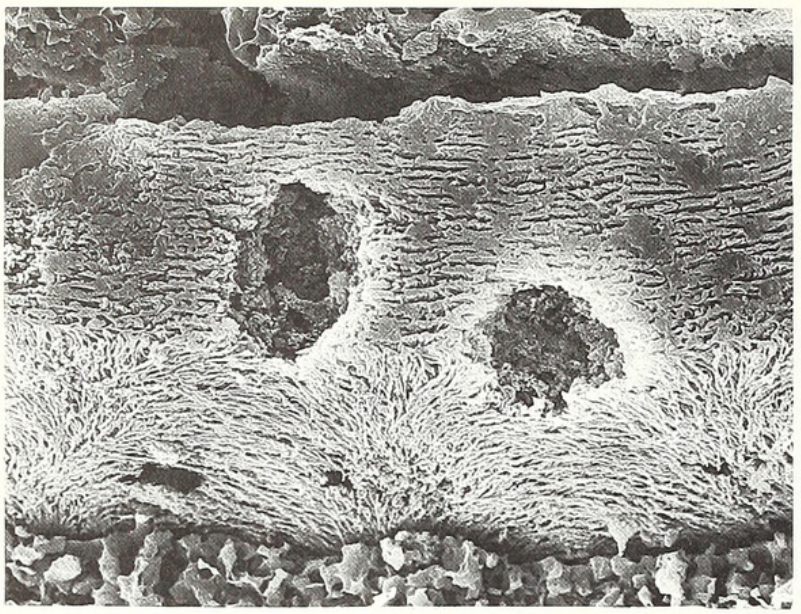
layer has been worn away or removed on the counterpart, the punctations can still clearly be seen, and also some light circular areas about 40 μm in diameter each perforated by a minute (*c.* 1 μm) opening. These light circles are about 200 μm apart: a similar spacing to that of the patches devoid of punctations on the outer layer surface.

In polished slices, sections of *Ellipsocephalus* cuticle can easily be identified by their shape and by the possession of a thin whitish outer layer, which at higher magnifications is seen to contain darker spherulitic structures around 15 μm in diameter. The region of cuticle below the outer layer is dark brown and penetrated by numerous fine perpendicular canals which stand out as they are paler than the ground material of the inner layer.

SEM preparations

General structure of the cuticle. The great predominance of cranidia on the surface of the hand specimens led us to assume that the great majority, if not all, of the *Ellipsocephalus* material was of sections of that part of the cephalon. As in material examined with the light microscope, the shape of many of the sections reinforced the validity of this conclusion.

The etching process left a thin outer layer, 25–50 μm thick, standing clear and unaltered from the rest of the cuticle, up to 200 μm thick, which was etched inwards. Not only could the outer layer be viewed in perpendicular section, but its inner undersurface could also be examined, for example in preparation RM Ar 46218*b*-E4 (Text-fig. 2). In that particular preparation, the perpendicular face clearly shows that the outer layer comprises about twenty lamina units, each just over 1 μm thick. The majority of preparations show a similar aspect to that of E4, but a few are different, possibly the result of: (i) slight differences in preparation technique, including direction of sectioning and quality of polishing; (ii) original differences in the cuticles, possibly including those related to the size of the animal; (iii) localized diagenetic differences. In preparation RM Ar 46218*e*-E9 (Pl. 1, fig. 1), the outer layer is somewhat thicker (nearly 50 μm thick) than in most other preparations and the lamination is very clearly defined. There are about thirty-five lamina units, each nearly 1·5 μm thick except for the outer five units which are thinner. In areas of a few preparations, for



TEXT-FIG. 2. Scanning electron micrograph of etched perpendicular section of *Ellipsocephalus polytomus* Linnarsson cuticle outer layer, also with a view of undersurface of that layer. Preparation RM Ar 46218*b*-E4, ×800.

example RM Ar 46218*b*-E1 (Pl. 1, fig. 2), the lamination is less clear and transforms laterally into a zone of semi-prismatic calcite crystallites, and in one preparation, RM Ar 46218*b*-Ei (Pl. 1, fig. 3), the lamination is penetrated by calcite crystallites. Preparations RM Ar 46218*b*-E1 and -Ei are both perpendicular sections (deduced from the perpendicular pathways of their canals) and so this transformation or penetration is a real phenomenon and not an artefact produced by angled sectioning.

In many sections round or elliptical cavities, up to 15 μm in diameter and up to 20 μm high, extend from the lower edge of the outer layer to near the surface of the cuticle. However, they never reach beyond the uppermost fine lamina units, nor was any connection between these cavities and the cuticle surface observed in any of the sections examined.

Another feature in many sections is an outermost non-laminate region of cuticle, up to 2 μm thick and with a dense homogenous appearance. This can be seen most clearly in Plate 1, figure 1 and Plate 3, figure 4.

The main region of the cuticle (for convenience termed the principal layer) consists of fine crystallites, presumably of calcite, sometimes with their long axes arranged roughly perpendicular to the cuticle surface. This region shows little detail apart from this feature, but is penetrated by perpendicular canals, approximately 3 μm in diameter (Pl. 1, fig. 4). These canals have disc-like lateral extensions about 0·5 μm thick and on average the same distance apart (Pl. 1, fig. 5). Preparations in which the principal layer is etched deeply inwards illustrate how numerous and ubiquitous these canals are (Pl. 1, fig. 6).

Lamina unit ultrastructure. At higher magnifications, considerable ultrastructural detail can be resolved. At first, a bewildering array of apparently different structures was observed. But eventually, by always taking micrographs at a standard series of screen magnifications, it became clear that at least some of the apparent variation was the result of viewing essentially similar

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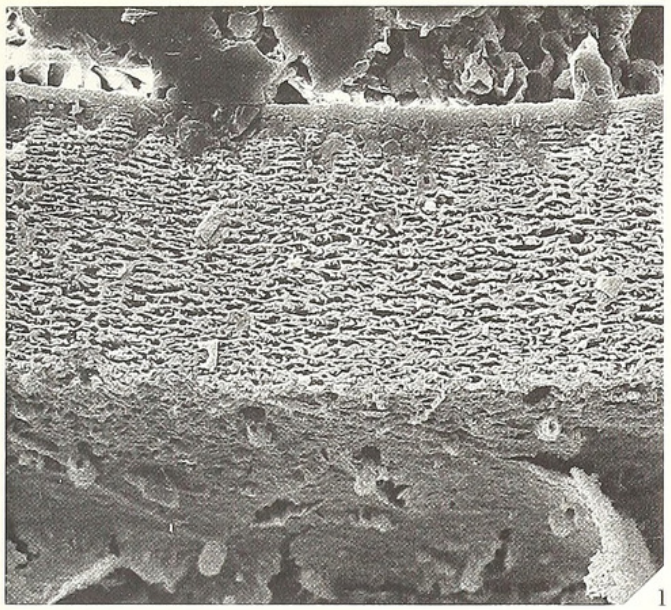
EXPLANATION OF PLATE 1

Figs 1–6. *Ellipsocephalus polytomus* Linnarsson, Middle Cambrian, Enerum, Öland, Sweden. Scanning electron micrographs of etched sections of cranidial cuticle. 1–3, outer laminated layer in preparations RM Ar 46218*e*-E9, *b*-E1, *b*-Ei, respectively, all ×650. 4–6, perpendicular canals in the principal layer in preparations RM Ar 46218*b*-E5, ×300, *b*-E5, ×8000, *e*-E9 ×250, respectively.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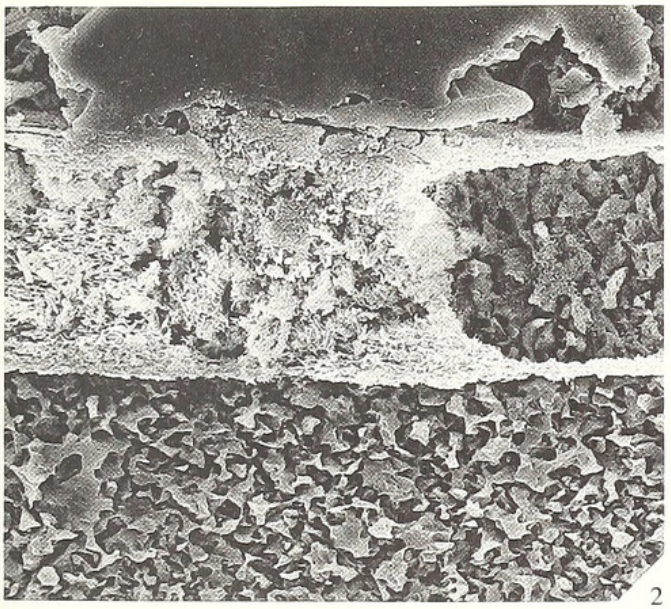
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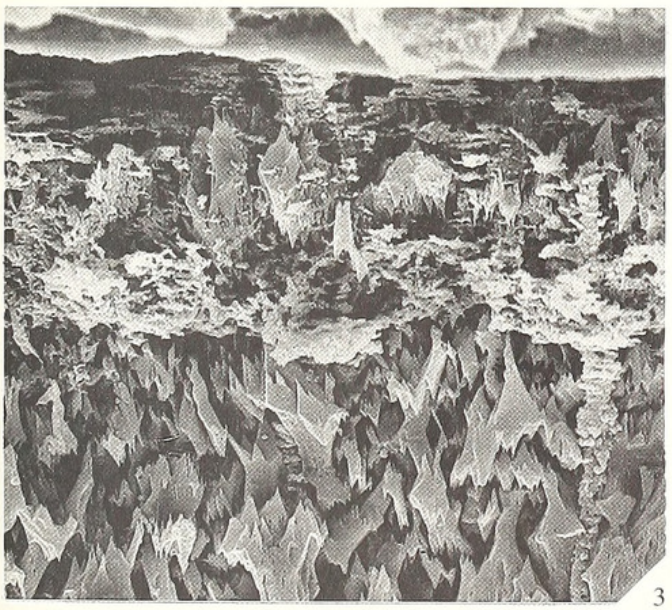
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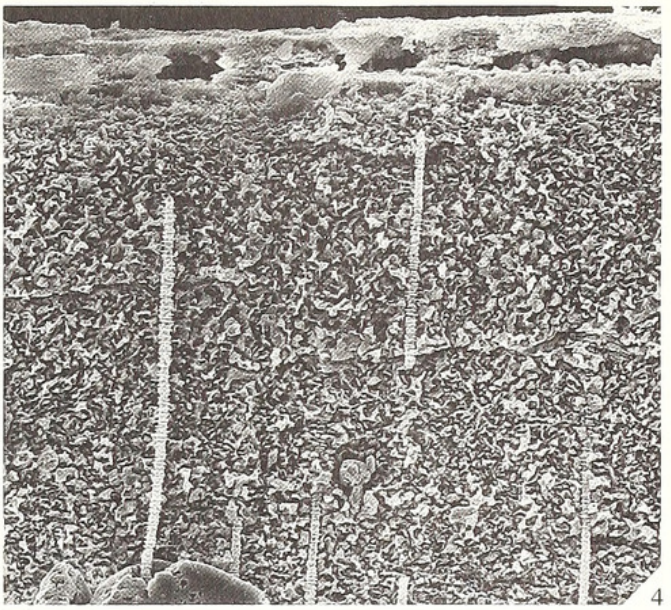
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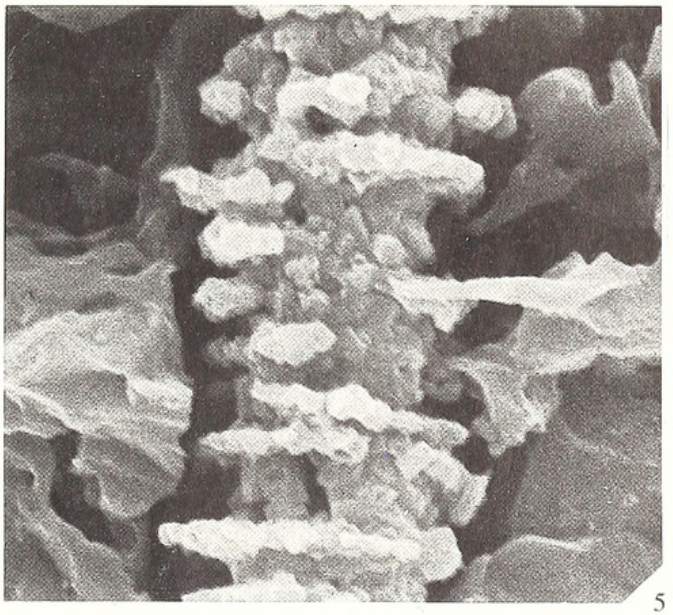
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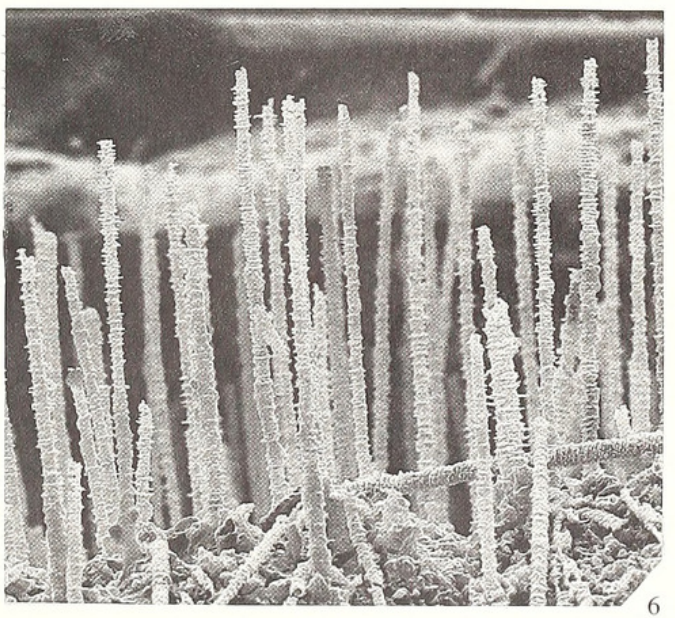
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structures at arbitrary magnifications, with slight differences in preparation method, angle of slicing and angle of viewing also contributing to variability. Plate 2, figure 1 shows lamina units with their sectional edges flattened by the polishing procedure, whereas those in Plate 2, figure 2 show a more broken appearance. The interface between the perpendicular face and the horizontal undersurface of the outer layer was also examined (Pl. 2, fig. 3). In all three micrographs the laminae appear to be composed of arrays of rods, with more or less circular cross-sections, linked together in sheets; some sheets seem to arc across the inter-laminae. A detailed view of the undersurface of the outer layer (Pl. 2, fig. 4) shows that the fingerprint-like patterns seen in Text-figure 2 are produced by arced sheets of fibrous material. A near-horizontal view of a lamina unit in an unetched break (Pl. 2, fig. 5) reveals a herringbone-like pattern of rods. In contrast, a near-horizontal slice, despite being subjected to flattening and polishing (or perhaps because of this) shows a mosaic of fibrous and rod-like material from different levels of the cuticle (Pl. 2, fig. 6, which is a detail of Pl. 3, fig. 5).

Polygonal patterns. In sub-surface areas of the unetched preparation viewed from above, polygonal areas about 40 μm across and delimited by slightly raised ridges can be detected (Pl. 3, fig. 1).

Cavities. Round or elliptical cavities have already been mentioned as a consistent feature of the outer layer of cuticle. At low magnifications, arrays of these cavities can be seen, with the broken upper portions of perpendicular canals below them (Pl. 3, fig. 4). In preparations sliced at an angle of a few degrees from the horizontal, the outer layer is perhaps somewhat disrupted by the effect of the etching process on the principal layer; the latter can be seen through the cavities (Pl. 3, fig. 5). One preparation in which the principal layer has been etched inwards to a considerable extent, leaving the outer layer roofing a miniature cave (Pl. 3, fig. 6), shows the stumps of canals as stalactic projections from the cave roof, clearly connecting to the cavities in the outer layer which are 'illuminated' by the electron beam striking the top surface of the cuticle and 'shining through it'. On the right of the micrograph, a rather stouter perpendicular canal is the only one left extending from the inner matrix to the outer layer.

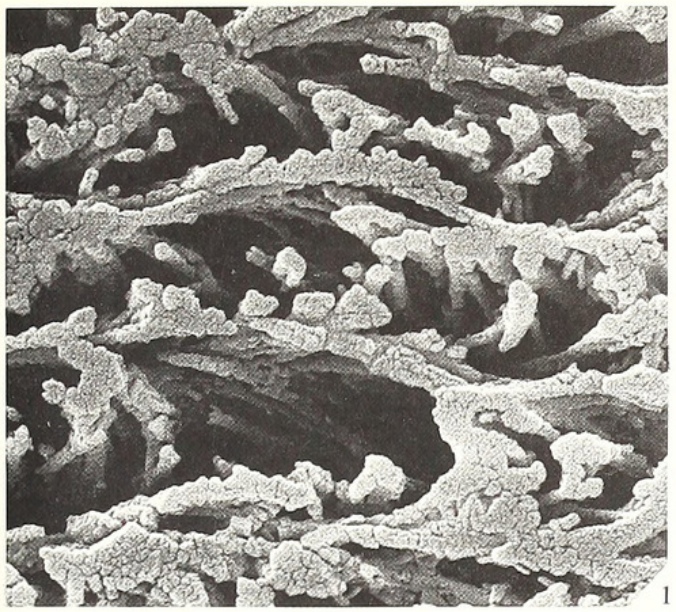
Other structures. The undersurface of the outer layer in some preparations seems to be covered by a thin coating skin through which some details of that undersurface can still be seen. This skin often peels back or breaks open to reveal clearer details. This phenomenon can just be seen on the bottom right of Plate 1, figure 1. Roughly star-shaped arrays of fibrous or crystalline material (Pl. 3, fig. 2) stand out below the general level of the undersurface of the outer layer in some preparations. In some areas of nearly all preparations, horizontal tubular structures 1–2 μm in diameter criss-cross the undersurface of the outer layer, sometimes forming node-like structures where they intersect (Pl. 3, fig. 3).

COMPOSITION

Semi-quantitative elemental analysis, using the LINK system of X-ray microanalysis attached to the SEM gave peaks for calcium and phosphorus in the outer layer, whereas the principal layer showed a strong peak only for calcium with lesser peaks for silicon and iron and only a trace of phosphorus.

EXPLANATION OF PLATE 2

Figs 1–6. *Ellipsocephalus polytomus* Linnarsson, Middle Cambrian, Enerum, Öland, Sweden. Scanning electron micrographs of etched sections (except 5) of cranidial cuticle showing details of outer layer, all $\times 9000$. 1 and 2, lamina units in preparations RM Ar 46218*e*-E9, *b*-E4. 3, interface between vertical section and undersurface, preparation RM Ar 46218*b*-E4. 4, undersurface, preparation RM Ar 46218*b*-E4. 5, unetched low angle break, preparation RM Ar 46218*e*-E8. 6, low angle slice, preparation RM Ar 46218*b*-E6.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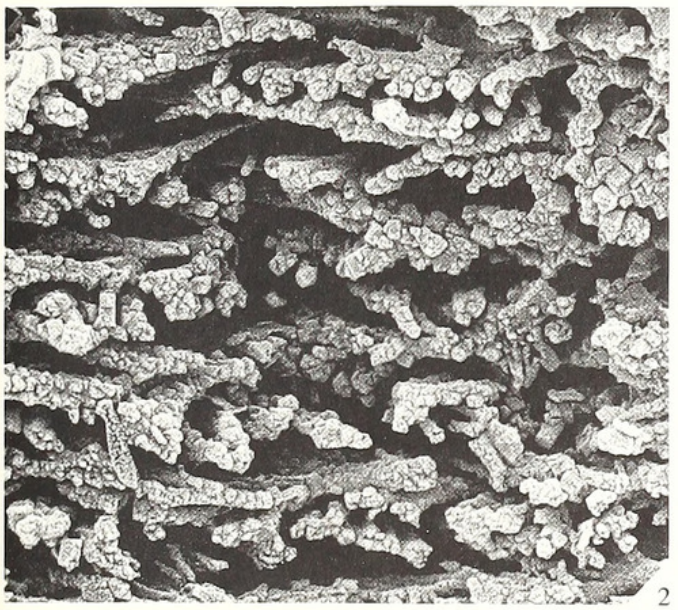
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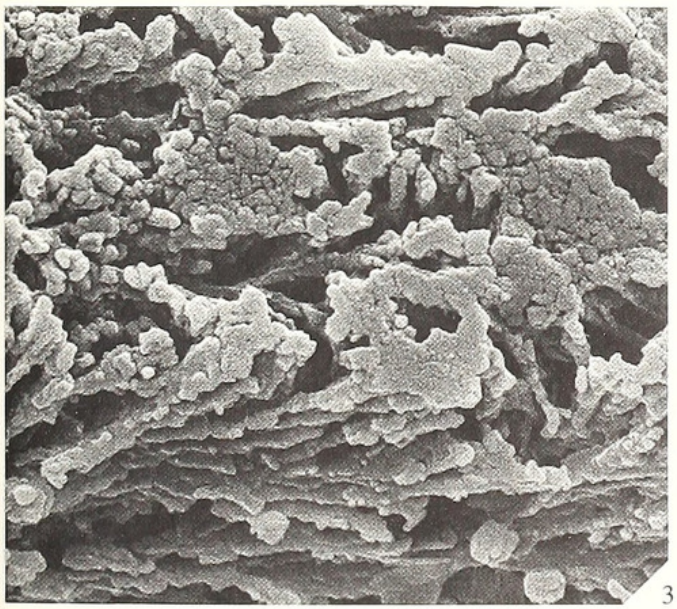
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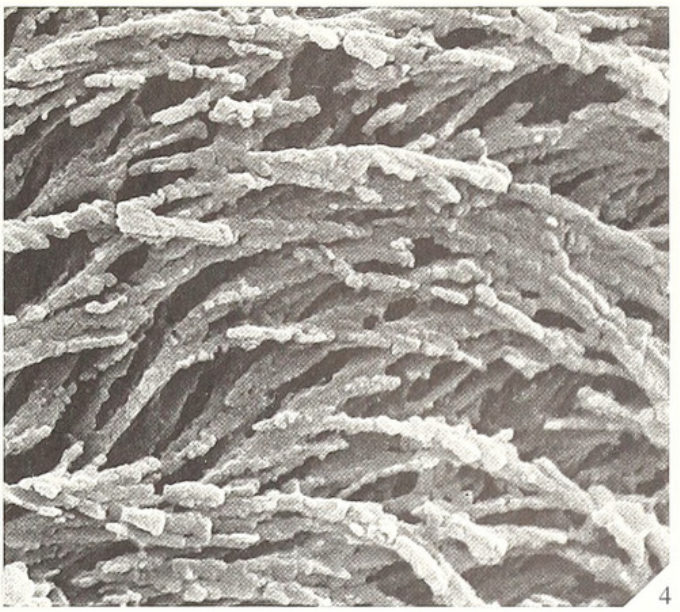
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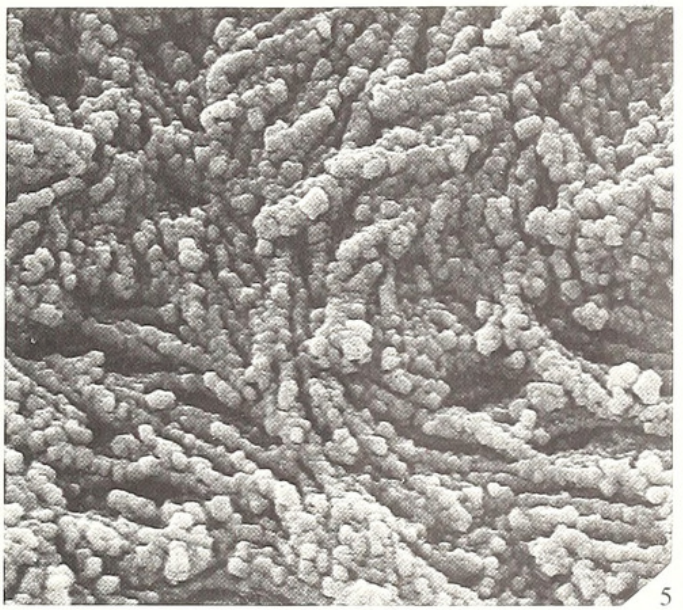
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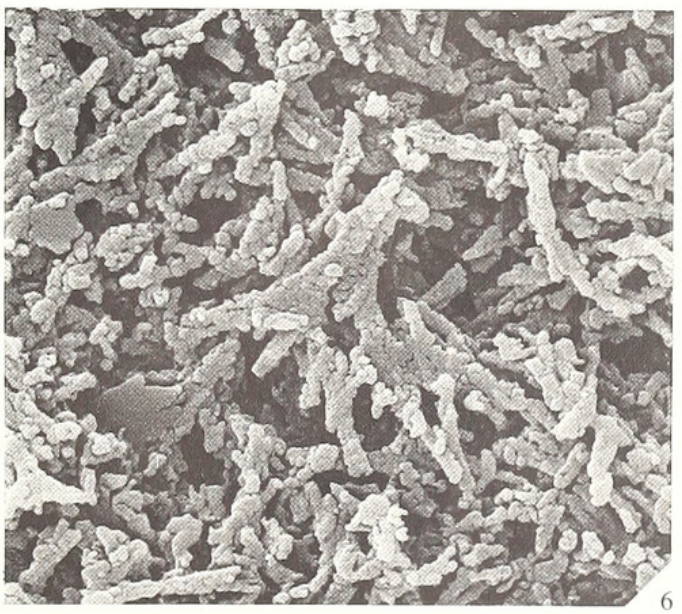
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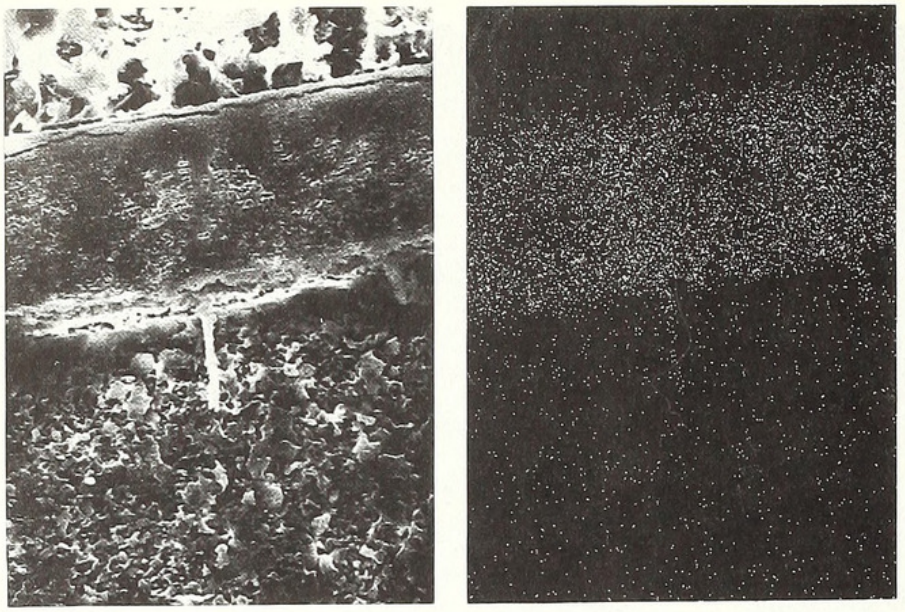

TEXT-FIG. 3. Etched perpendicular section of *Ellipsocephalus polytomus* Linnarsson cuticle outer layer. Left, scanning electron micrograph; right, spot X-ray microanalysis for phosphorus. Preparation RM Ar 46218-A2, ×450.

A spot analysis for phosphorus showed an exact co-incidence of the concentration of phosphorus with the outer layer (Text-fig. 3) and also suggested that the 3 μm perpendicular canals contain high concentrations of phosphorus.

DISCUSSION

Subdivisions of trilobite cuticle

Størmer (1980) discussed the broad divisions of the trilobite cuticle and generally supported Dalingwater and Miller's (1977) view that it consisted of an outer prismatic layer and a principal layer with three distinct laminate zones – an outer zone with narrow lamina units, a middle zone with a few relatively wide units and an inner zone with a few narrow units. Størmer also recognized that rarely are all regions of the cuticle equally well represented or well preserved in any one example. Teigler and Towe (1975) have argued for two basic layers of cuticle, suggesting that the thin outer layer may be prismatic or pigmented or apatitic.

Our interpretation of *Ellipsocephalus* cuticle is that in life the outer laminated layer had only the thin apparently structureless outermost layer above it, the latter possibly representing an epicuticle. Furthermore, the lateral transition between laminated cuticle and prismatic cuticle in one preparation and the invasion of the laminated layer by calcite crystallites in another suggests that the prismatic layer observed in the cuticle of many trilobites may not be an original layer. However, much more evidence is needed before we can firmly draw this conclusion.

Ultrastructural detail of lamina units

Calcified cuticles of Recent arthropods, for example those of decapod crustaceans, have organic

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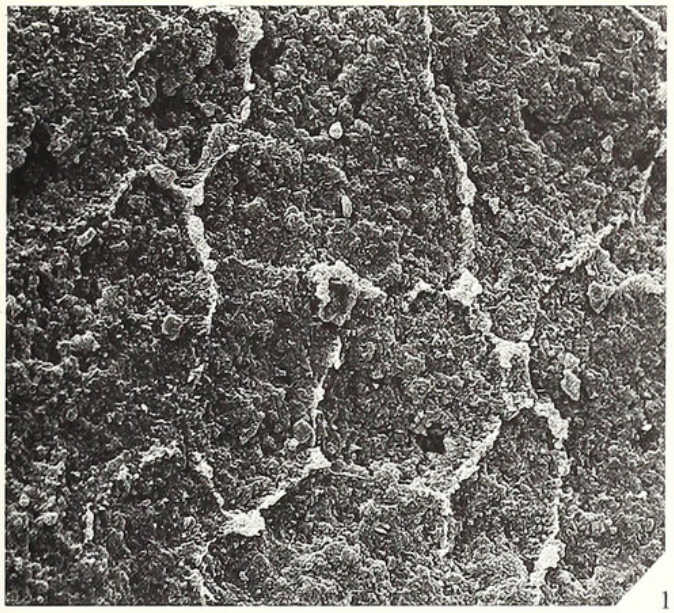
EXPLANATION OF PLATE 3

Figs 1–6. *Ellipsocephalus polytomus* Linnarsson, Middle Cambrian, Enerum, Öland, Sweden. Scanning electron micrographs of etched sections (except 1) of cranidial cuticle. 1, low angle break, showing prismatic structures, preparation RM Ar 46218*e*-E8, ×500. 2, arrays of fibrous material on outer layer undersurface, preparation RM Ar 46218*b*-E4, ×9000. 3, tubular structures on undersurface of outer layer, preparation RM Ar 46218*e*-E9, ×1200. 4–6, cavities in the outer layer; 4, perpendicular sectional view, preparation RM Ar 46218*b*-Ei, ×300; 5, from above, preparation RMAr 46218*b*-E4, ×1000; 6, from slightly below, 'illuminated' by beam striking top surface, preparation RM Ar 46218*e*-E9, ×300.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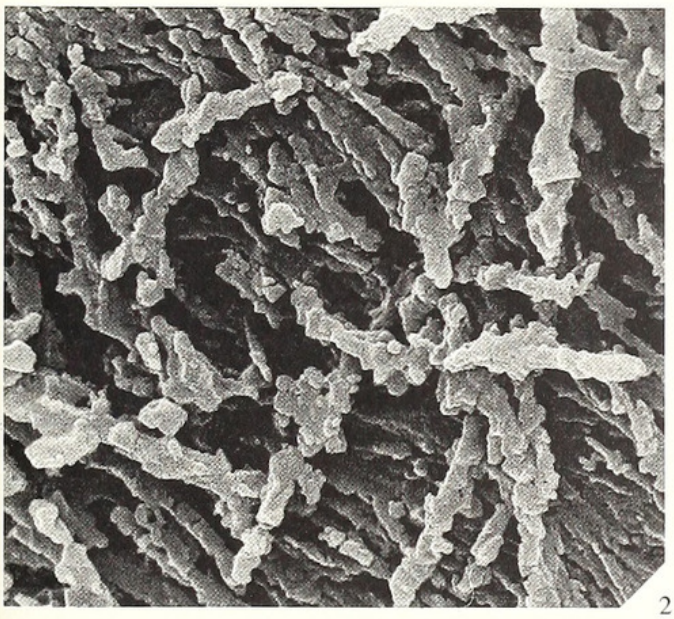
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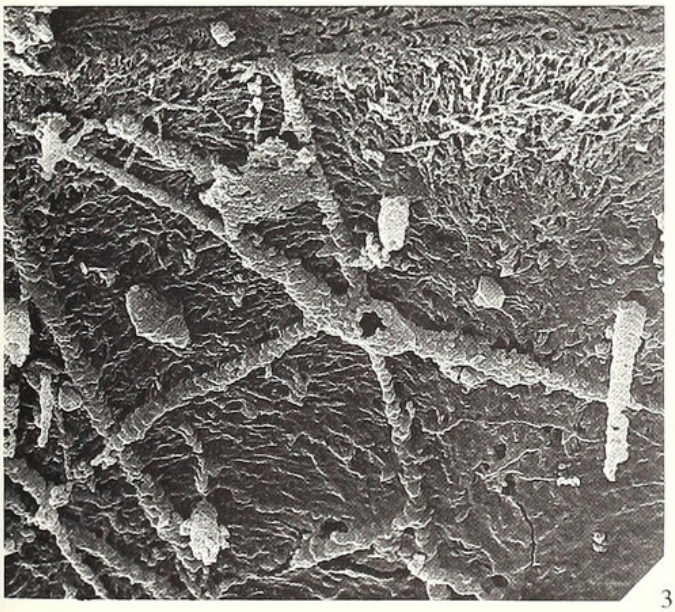
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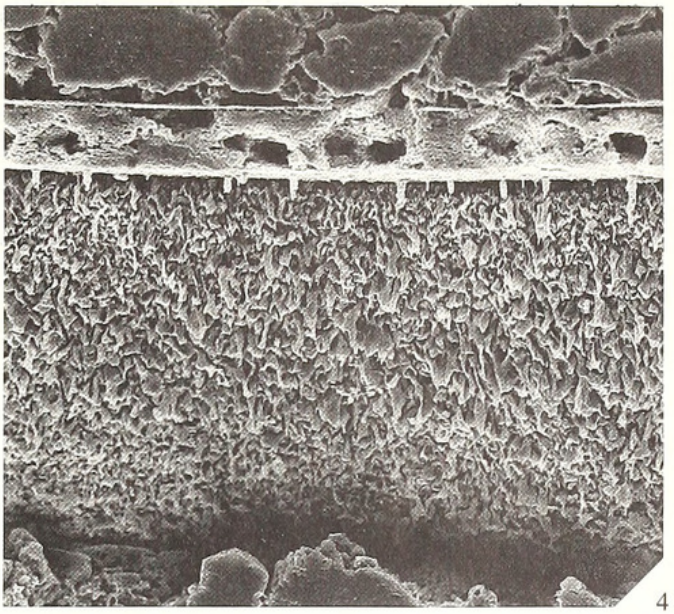
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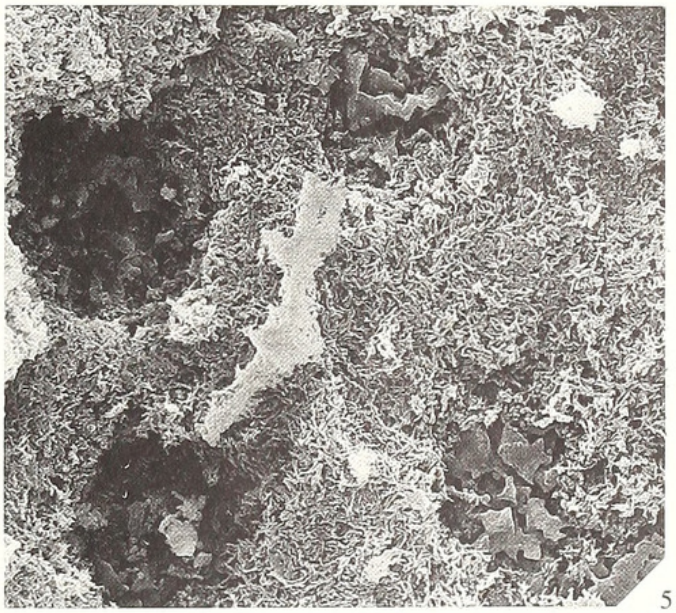
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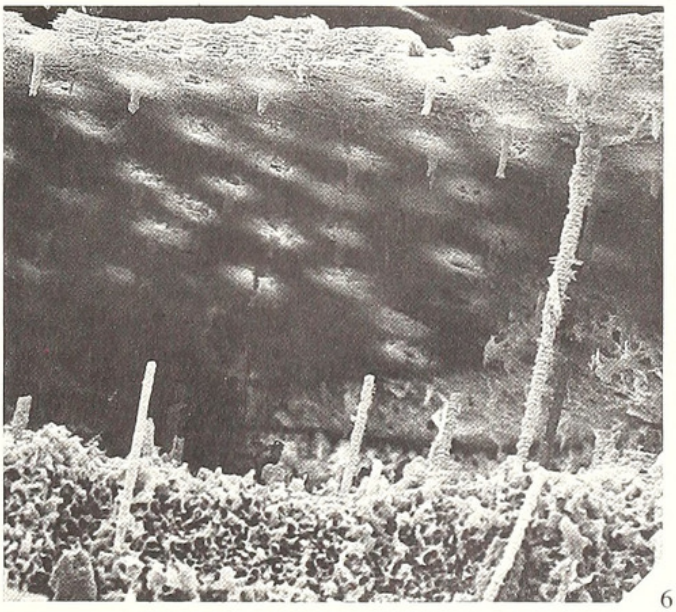
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templates upon or within which inorganic salts are deposited. On analysis, these templates show at least three levels of structural organization (Giraud-Guille 1984*a*). Near-molecular associations of chitin and proteins to form microfibrils represent the first level; associations of microfibrils in reticulate, macrofibrillar or homogenous arrays form the second level; and spatial arrangements of level two associations (e.g. macrofibrils in helicoidal arrays) give the third level. Minerals are probably deposited within the reticulate arrangement of microfibrils in the decapod crustacean exocuticle and around the macrofibres of the calcified zone; homogenous arrays of microfibrils effectively fill all available space in the uncalcified endocuticle. It is quite possible to envisage the three-dimensional arrangement both of organic template and deposited minerals in the decapod crustacean calcified zone if one accepts the Bouligand–Neville interpretation of laminated cuticles. In fact, the model was originally proposed after examination of *Carcinus* calcified zone macrofibrils (Bouligand 1965), but later shown to be more widely applicable to microfibrillar arrangements, for example in insect cuticles (Neville 1975).

It is, however, at the third level of cuticular architectural organization that the Dennell–Mutvei–Dalingwater view (see Dalingwater and Mutvei 1990 for a more detailed discussion) does not accord with the Bouligand–Neville model: the former suggest that laminae are real structures and that sheets of fibres arc across the inter-laminae. In this context it is interesting to note that it is difficult to produce a satisfactory three-dimensional helicoidal arrangement for the reticulate associations of exocuticular microfibrils: Giraud-Guille (1984*a*, p. 81, fig. 6) has illustrated a semi-helicoidal pattern with fibre direction changing in blocks, but even that is not easily reconciled with the reality of her excellent micrographs.

In Recent decapod crustacean material examined with the SEM, it is difficult to distinguish between organic template and deposited minerals, even with the help of transmission electron micrographs of the same material in which all inorganic material has been removed by decalcification prior to sectioning. So interpretation of lamina unit ultrastructure of the trilobite material described here is extremely difficult, because in addition to great structural complexity we also have to consider the effects of replacement and diagenesis. We tentatively suggest that in *Ellipsocephalus* the laminae are composed of horizontal sheets of rods with further sheets of material arcing at low angles across the inter-laminae and connecting adjacent laminae. The rods probably represent the original inorganic material of the cuticle, and possibly also reflect the original organic template.

Significance of a finely laminated outer cuticular layer

Many extant arthropods from all the major groups (crustaceans, insects, chelicerates) have an outer cuticular layer with fine lamina units – invariably much finer than those in central regions of their cuticles. This outer layer is very likely to have been formed pre-ecdysially, i.e. under the old cuticle prior to moulting, whereas central and inner regions of the cuticle are usually formed after ecdysis. Possibly slower pre-ecdysial formation in some ways results in the formation of narrow lamina units, but perhaps a functional explanation is more likely. A region of narrow lamina units on the outside of a cuticle will have considerable crack-stopping ability. This holds good with lamination interpreted either according to the Bouligand–Neville model or the Dennell–Mutvei–Dalingwater explanation (for further discussion see Dalingwater 1985, p. 360).

Cavities and canals

The cavities in the outer layer appear circular or egg-shaped in many perpendicular sections, but a few are pear-shaped with the narrower end pointing upwards. Only in sections close to their mid-line do pear-shaped structures reveal their true shape; glancing slices will appear round or oblong. We therefore suggest that the most complex aspect seen reflects the true shape – resembling that of an upwardly pointing pear. The 3 μm canals which characterize the principal layer connect the cavities to the inner surface of the cuticle and therefore originally to the epidermis. On the other hand, the cavities do not quite extend to the surface of the cuticle, nor do they appear to be connected to the surface. However, they extend so close to the surface, that in hand specimens

illuminated from above they can be seen through the thin (less than 10 μm) overlying layer of cuticle. Furthermore, slight abrasion will easily remove this overlying layer and expose the tops of cavities.

These cavities and canals are similar in position and dimensions to the Osmólska cavities described and discussed in great detail by Størmer (1980). Størmer considered that this type of cavity occurred below the prismatic layer, but Wilmot (1990*b*) has clearly shown that they usually occur within the prismatic layer. Størmer (1980) suggested a chemosensory function for the Osmólska cavities, whereas Wilmot (1990*b*) preferred to interpret the cavities and canals as some type of modified pore canal. However, the most closely analogous structures to the cavities and canals that we have encountered in an extensive search through the literature are the gland ducts of the millipede *Glomeris convexa* which have dilated tips within a finely-laminated outer region of cuticle (Richards 1951, p. 55, fig. 32c). Gland ducts may be concerned with the secretion and maintenance of the epicuticle. The dilated tips of the gland ducts in *Glomeris* are shown to connect to the surface of the cuticle by minute canals. As mentioned above, we have not detected such openings in *Ellipsocephalus*, but connections to the surface by minute canals would show up only very rarely in sectional slices.

The canals in the endocuticle of *Flexicalymene* which Mutvei (1981, p. 230, fig. 5) termed pore canals have a diameter of about 0·3 μm, similar to that of the pore canals in Recent arthropod cuticles, and do not connect to cavities. They do, however, show a feature of similarity with the canals in *Ellipsocephalus*: disc-like lateral extensions which Mutvei called horizontal lamellae or laminae. They almost certainly reflect ultrastructural elements of the principal layer, but whether they represent the laminae themselves or structures within lamina units is uncertain.

Mutvei (1981, p. 229, fig. 4) described wider ducts, 3–7 μm in diameter, in *Flexicalymene* cuticle. There may also be two types of canal in *Ellipsocephalus* cuticle: the great majority are the 3 μm diameter canals which connect to cavities, but slightly wider canals which do not connect to cavities (e.g. to the right in Pl. 3, fig. 6) may account for the irregularities in the spacing of punctations as seen in surface views of hand specimens and the presence of light circular areas on worn surfaces of hand specimens.

Absence from the outer layer of any structures that can definitely be regarded as pore canals is puzzling. In an outer (and presumably pre-ecdysially formed) layer of cuticle a supply-line for minerals and for other materials required for mineralization would be needed after ecdysis. In Recent decapod crustacean cuticles pore canals almost certainly carry out this function (Roer and Dillaman 1984). However, pore canals are essentially organic structures, so they may not necessarily be preserved as *canals*. Elliptical openings are present on the undersurface of the outer layer (Pl. 2, fig. 4) reminiscent of pore canal pathways: thus pore canals may indeed originally have passed upwards through the *Ellipsocephalus* cuticle outer layer.

Significance of other primary microstructures

Polygonal structures observed at a sub-surface level in the outer laminated layer (Pl. 3, fig. 1) may be equivalent to the interprismatic septa of calcified cuticles of Recent decapod crustaceans. The walls of the septa in these Recent cuticles represent cell margins transformed into cuticular material and show concentrations of cation-binding glycoproteins and maximum carbonic anhydrase activity (Giraud-Guille 1984*b*). Thus the walls represent sites of calcification initiation. It is important to note that they do not extend to the surface of the cuticle and are therefore distinct from polygonal surface ornament whose shapes and sizes are not necessarily related to epidermal cell shapes. Giraud-Guille (1984*b*) has clearly shown that interprismatic septa coincide precisely with underlying epidermal cells.

The thin coating skin (Pl. 1, fig. 1) on the undersurface of the outer layer may represent a deposit of relict organic material from the dissolution of the principal layer. Relict organic material has been identified in other trilobite cuticles by Dalingwater (1973) and Teigler and Towe (1975).

The roughly star-shaped arrays also on the undersurface of the outer layer (Pl. 3, fig. 2) may be the remains of fibrous structures binding together this and the underlying principal layer. Dennell

(1973) identified horizontal arrays of fibres in decapod crustacean cuticles which he suggested might bind together adjacent lamina units.

Secondary microstructures

The 1–2 μm horizontal tubules on the undersurface of the outer layer (Pl. 3, fig. 3) are interpreted as secondary structures because they are irregular in appearance and inconsistent with other cuticular structures in their arrangement. They are remarkably similar in dimensions and appearance to borings described by Runnegar (1985) from shells of the gastropod *Yuwenia bentleyi* from the Lower Cambrian Pavara Limestone of South Australia. Runnegar concluded that these borings were made by cyanobacteria rather than by fungi. Although the nodal structures in the tubules in *Ellipsocephalus* could be interpreted as fungal reproductive bodies, in other aspects the resemblance to the borings described by Runnegar is so close that it seems reasonable to consider the tubules in *Ellipsocephalus* also as infilled borings of cyanobacteria.

Composition of the cuticle in Ellipsocephalus

Although the outer layer is now almost certainly composed of calcium phosphate in the form of apatite and the principal layer (except for the 3 μm canals) of calcium carbonate in the form of calcite, it is uncertain if this reflects the original composition. Teigler and Towe (1975) did, however, demonstrate a high concentration of phosphorus in an outer layer of a Recent crab cuticle. One suggestion that we can make at this stage is that the outer layer may originally have had a different composition from the principal layer, since detailed microstructures are preserved in the former but not in the latter except as discs around perpendicular canals. Alternatively, the outer layer could have had a different structure from the principal layer which was more predisposed to replacement; preferential replacement could have resulted in better preservation of microstructural detail.

We intend to make further studies of the composition of the cuticle employing a range of techniques including cathodoluminescence.

Concluding remarks

Ultrastructural details described here from *Ellipsocephalus* cuticle are the finest so far from any trilobite cuticle and it is ironic that they are possibly also the oldest such details described from any arthropod cuticle. But, before any firm conclusions can be drawn about the general structure of trilobite cuticle, more work is needed on a range of cuticles using careful preparation techniques and taking advantage of the increased resolution of the current generation of SEMs. Parallel studies of Recent crustacean cuticles are also needed to elucidate the precise positional relationships of organic template and inorganic impregnating minerals. There are signs that arthropod cuticle workers are at last breaking out of the straightjacket imposed by the Bouligand–Neville model of cuticular architecture (Neville 1975). Compère and Goffinet (1987*a*, *b*), for example, have described new and exciting structural details, from decapod crustacean cuticles, which do not fit the model. We need to know about and to be able to explain the reasons for differences between the cuticles of different species and of higher taxa, as well as attempting to identify features of similarity.

The significance of the work described and discussed here is not only in the discovery of such exceptionally fine details in a trilobite cuticle, but also in heralding a new phase of fossil arthropod cuticle research made possible by new techniques and new instruments.

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