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# SEM STUDIES ON ARTHROPOD EXOSKELETONS

## Part 1: Decapod Crustaceans, *Homarus gammarus* L. and *Carcinus maenas* (L.)

By

H. Mutvei

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The spatial arrangement of the chitin-protein fibres and the morphology of the pore-canals are described in the exoskeleton of two decapod crustaceans, the lobster (*Homarus gammarus* L.) and the shore-crab (*Carcinus maenas* (L.). The description is based on scanning electron microscope (SEM) studies.

The chitin-protein fibres in the exo- and endocuticles are arranged into horizontal lamellae which give rise to and are connected with numerous vertical lamellae. Hence, the vertical lamellae are not optical artefacts as maintained by several previous writers. The vertical lamellae have a highly variable arrangement in being vertically and frequently also horizontally curved.

Both the horizontal and the vertical exoskeletal lamellae are pierced by the pore-canals. Each pore-canal has a wall of its own which is composed of vertical fibres. This fibrous wall is firmly attached to the exoskeletal lamellae. The pore-canal walls represent to the vertically continuous, fibrous system which apparently has the function to increase the strength and flexibility of the exoskeleton. The vertical course of the pore-canals is straight or slightly undulating, and this course is not influenced by the complicated spatial arrangement of the exoskeletal lamellae.

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## INTRODUCTION

The possession of a rigid exoskeleton which covers the entire soft body is one of the main reasons of the evolutionary success in the arthropods. In most arthropods the exoskeleton seems to have a basically uniform structure. Studies of this structure have been carried out with the aid of the light microscope and the transmission electron microscope (TEM). In spite of these studies, many structural features have still remained obscure. This can probably be explained by the fact that the skeletal components form a complicated three-dimensional architecture which on the basis of two-dimensional sections for the light microscope and TEM studies cannot be easily analyzed and reconstructed. An ideal instrument for three-dimensional studies of the exoskeletal structure is the scanning electron microscope (SEM). However, this instrument has a limited use because in most arthropods the exoskeletal components are too small for its resolving power.

In the exoskeleton of recent decapod crustaceans four layers have been generally distinguished (see e.g. Richards, 1951; Dennell, 1960; Travis, 1970; Hackman, 1971): (1) the epicuticle; (2) the exocuticle (pigmented layer); (3) the endocuticle; and (4) the membraneous layer. The exo- and endocuticles form the main part of the thickness of the exoskeleton. These two layers are composed of calcified fibrous lamellae which are pierced by

vertical pore-canals. The fibres consist of chitin and protein, and they are embedded in an amorphous, organic ground component.

The present paper, Part 1, deals with the organic structure, of the exo- and endocuticles in two recent decapod crustaceans: *Homarus gammarus* L. and *Carcinus maenas* (L.). In these forms the exoskeletal components are large and therefore well suited for SEM studies. Particular attention is paid on the spatial arrangement of the chitin-protein fibres and the morphology of the pore-canals. The mineralization of these fibres has been described in detail by Travis (1963, 1970), and is therefore not dealt with in the present paper.

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## MATERIAL AND METHODS

The exo- and endocuticles in the exoskeleton of two decapod crustaceans, the lobster (*Homarus gammarus* L.) and the shore-crab (*Carcinus maenas* (L.), were studied. The material was collected in the Gullmar Fjord on the west coast of Sweden. Immediately after the animals were captured, the exoskeleton was cleaned from the soft tissues, rinsed in sea water, then left to dry in air.

The exoskeleton was fractured horizontally or vertically. The fracture planes were studied either without any chemical treatment, or they were demineralized in glutaraldehyde solution or in glutaraldehyde-acetic acid solution. The glutaraldehyde solution is a well known fixative for proteins, linking primarily the amino groups (Flitney, 1966; Bowes and Carter, 1966). The commercially available solution, which contains glutaric acid, is 25 % and its pH varies from pH 2.7 to pH 3.4. Due to its low pH the glutaraldehyde solution dissolves the $CaCO_3$ in the exoskeleton. For demineralization of the exoskeleton the glutaraldehyde solution was used either concentrated, diluted with distilled water, or in mixture with acetic acid. After demineralization the preparations were dehydrated in alcohol and amyl acetate, or in acetone. Most preparations were left to dry in the air, whereas others were dried in the "Model E 3 000 Critical Point Drying Apparatus", manufactured by the Polaron Equipment Ltd., Hertfordshire, England. I am grateful to Prof. J. R. Rowley for permission to use this apparatus at the Palynological Laboratory, Stockholm.

The preparations were coated with evaporated gold, and studied with a SEM Stereoscan (Cambridge Instrument Ltd.) at the Swedish Museum of Natural History, Stockholm. More than five hundred SEM micrographs of about a hundred preparations were taken.

## OBSERVATIONS

### *The chitin-protein fibres and their arrangement*

The exo- and endocuticles of the crustacean exoskeleton are composed of chitin-protein fibres which according to TEM studies by Travis (1963, 1970), are embedded in an organic, amorphous ground component.

In my material, the chitin-protein fibres are clearly visible in all demineralized exoskeletons of the lobster (Pl. 7, Figs. 1, 2, 3, 4) and the shore-crab (Pl. 2, Fig. 4; Pl. 8, Figs. 2, 3, 4). Moreover, the fibres are also clearly seen in those parts of the non-demineralized exoskeletons where the calcification has been less extensive (Pl. 1, Figs. 3, 4; Pl. 2, Figs. 2, 3; Pl. 4, Figs. 2, 3, 4; Pl. 5, Figs. 1, 2, 3, 4; Pl. 6, Figs. 1, 2, 3, 4). On the other hand, the amorphous ground component of Travis could not be detected in my SEM preparations. The chitin-protein fibres form horizontal and vertical lamellae which in my preparations have the following structure and spatial arrangement (Fig. 1).

The individual horizontal lamellae are in places subdivided into longitudinal regions both in the lobster and in the shore-crab. In each region, the chitin-protein fibres either have a more or less regular, horizontally curved course (Pl. 1, Figs. 1, 2; Pl. 4, Fig. 1), or a feather-like course (Pl. 8, Figs. 3, 4; in the upper-right corner of Pl. 1, Fig. 1). Other parts of the horizontal lamellae lack a subdivision into longitudinal regions, and the fibres have here a parallel or a sub-parallel course (Pl. 2, Figs. 1, 2, 3, 4; Pl. 5, Figs. 1, 2).

Numerous vertical lamellae emerge from both

sides of each horizontal lamella (Pl. 2, Fig. 1) and pass into the adjacent horizontal lamellae (Fig. 1). The chitin-protein fibres between the horizontal and vertical lamellae are directly continuous (Pl. 1, Figs. 3, 4; Pl. 2, Fig. 2; Pl. 3, Fig. 4; Pl. 4, Figs. 2, 3, 4; Pl. 5, Figs. 3, 4; Pl. 6, Figs. 1, 2, 3, 4). The vertical lamellae are more or less vertically curved (Pl. 1, Figs. 3, 4; Pl. 3, Figs. 1, 2, 3, 4; Pl. 4, Figs. 1, 3). In addition, these lamellae are often curved along their horizontal course. The latter condition is indicated in Pl. 5, Figs. 2, 3, 4, and Pl. 6, Figs. 1, 2, 3, 4.

The arrangement of the horizontal and vertical lamellae in the exocuticle is the same as in the endocuticle (compare Figs. 1, 2 and Figs. 3, 4 in Pl. 8). However, both in the lobster and in the shore-crab the horizontal lamellae have a much closer spacing in the exocuticle than in the endocuticle. This condition agrees with that observed in the fresh-water crayfish by Travis (1963, 1970).

In the shore-crab, the vertical lamellae are exceedingly numerous (Pl. 4, Fig. 1; Pl. 5, Figs. 2, 3, 4; Pl. 6, Figs. 1, 2, 3, 4) and in places they seem to be fused to each other (Pl. 8, Figs. 2, 3, 4). In the lobster, on the other hand, the vertical lamellae are considerably less numerous and, therefore, broad interspaces are left between them (Pl. 1, Figs. 3, 4; Pl. 2, Fig. 1; Pl. 3, Figs. 1, 2, 3, 4; Pl. 4, Figs. 2, 3, 4). Both in the lobster and in the shore-crab the vertical lamellae are always pierced by the pore-canals in the same manner as the horizontal lamellae (Pl. 1, Figs. 3, 4; Pl. 2, Figs. 1, 2; Pl. 3, Figs. 1, 2, 3, 4; Pl. 4, Fig. 3; Pl. 8, Figs. 2, 3, 4).

In the previous literature, the observations on the spatial arrangement of the chitin-protein fibres have been controversial. The principal arrangement of these fibres into horizontal and vertical lamellae was observed for example by Richards (1951), Dennell (1960), Locke (1961, 1967) and Travis (1963, 1970). However, the observations were made in sections which did not permit detailed studies of this arrangement. On the other hand, Bouligand (1965, 1971) maintained that the vertical lamellae do not exist and are optical artefacts. He believed that the chitin-protein fibres in the horizontal lamellae undergo constant changes in orientation from lamellae to lamellae. The hypothesis of Bouligand has been adopted and extended by Neville *et al.* (1969) and Neville and Luke (1969*a*, *b*). However, on the basis of light microscopic investigations Dennell (1973) found that the structural model of Bouligand is incorrect. Dennell was of the opinion that the horizontal lamellae are connected by interlamellar fibres, and that long disoriented macrofibres also occur passing from one interlamellar zone to another.

As pointed out in the present paper, the course of the chitin-protein fibres in the horizontal lamellae varies considerably both in the lobster and in the shore-crab. Still greater variations were encountered in the arrangement and course of the vertical lamellae. In view of these conditions, the controversy between the previous writers about the existence and arrangement of the vertical lamellae is understandable. These variations made previous structural analyses from sections extremely difficult.

### *The pore-canals*

The exo- and endocuticles are traversed by the pore-canals. Usually the canals pierce the horizontal exoskeletal lamellae at right angles, but in the places where the exoskeleton is curved their orientation may be from oblique to almost parallel to the lamellar structure. The pore-canals have a considerably larger diameter in the lobster (Pl. 2, Figs. 1, 2, 3; Pl. 3, Figs. 1, 2, 3, 4; Pl. 4, Figs. 2, 3, 4; Pl. 7, Figs. 1, 2, 3, 4) than in the shore-crab (Pl. 2, Fig. 4; Pl. 5, Fig. 2; Pl. 8, Figs. 2, 3, 4). In both crustaceans the course of the pore-canals through the exoskeleton is straight or slightly undulating (Pl. 8, Figs. 2, 3, 4; Pl. 9, Figs. 1, 2, 3, 4). Hence, their course is not influenced by the complicated spatial arrangement of the vertical exoskeletal lamellae. Therefore, not only the horizontal lamellae but also the vertical lamellae are pierced by the pore-canals (Pl. 1, Figs. 3, 4; Pl. 2, Figs. 1, 2; Pl. 3, Figs. 1, 2, 3, 4; Pl. 4, Fig. 3; Pl. 8, Figs. 2, 3, 4). At these places where the pore-canals perforate the exoskeletal lamellae the chitin-protein fibres in these lamellae are curved around the pore-canals (Pl. 2, Fig. 3; Pl. 7, Figs. 1, 2, 3, 4).

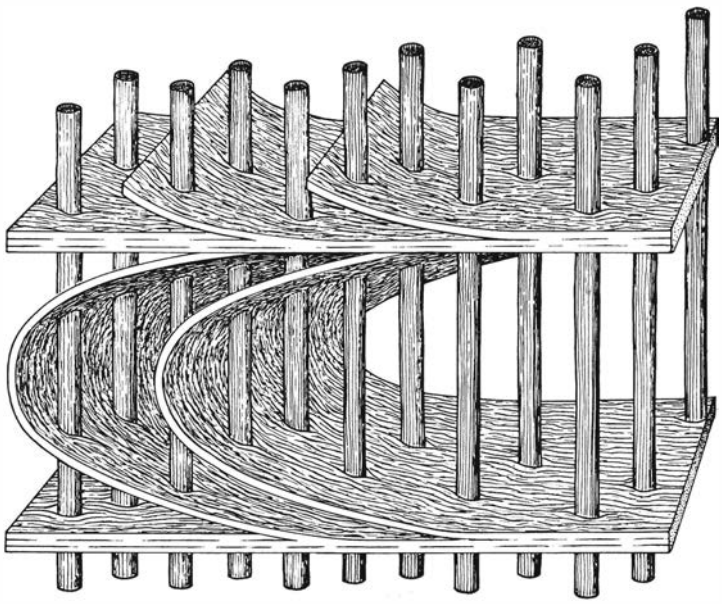

*Fig. 1.* Block diagram of the exoskeleton in a decapod crustacean showing two horizontal lamellae connected by vertical lamellae, and numerous pore-canals with vertically continuous, fibrous walls.

Both in the lobster and in the shore-crab, the diameter of the pore-canals decreases in the exocuticle towards the epi-exocuticular junction (compare Figs. 3, 4 and Fig. 2 in Pl. 8). This is in agreement with the observations by Travis (1963, 1970) in the fresh-water crayfish.

Each pore canal has a wall of its own which is composed of vertical chitin-protein fibres (Fig. 1). These fibres are of the same appearance as the fibres in the exoskeletal lamellae (Pl. 7, Figs. 1, 2, 3, 4). The pore-canal walls are observed in all preparations, both demineralized and non-demineralized (Pl. 2, Fig. 4; Pl. 6, Fig. 4; Pl. 7, Figs. 1, 2, 3, 4; Pl. 8, Figs. 3, 4; Pl. 9, Figs. 1, 2, 3, 4). At their passage through the horizontal and vertical exoskeletal lamellae the pore-canal walls are firmly attached to these lamellae (Pl. 7, Figs. 1, 2, 3, 4). By fracturing the exoskeleton vertically or horizontally the pore-canal walls may detach from the lamellae. Long portions of the detached walls appear frequently on the fracture planes (Pl. 6, Fig. 4; Pl. 9, Figs. 1, 2, 3, 4). The latter condition demonstrates clearly that the pore-canal walls are vertically continuous. Hence, they are not formed from branches of the exoskeletal lamellae, but represent an independent vertical fibrous system.

The following structures described in the previous literature are probably related to the pore-canal walls. In her TEM studies on the fresh-water crayfish, Travis (1963, 1970) observed in the walls of some pore-canals vertical fibres and interpreted them as vertical branches from the fibres of the exoskeletal lamellae which "wall in" the pore-canal system. It is obvious that these vertical fibres actually belong to the pore-canal walls. Dennell (1973) described in light microscopic preparations of the shore-crab "long sinuous macrofibres" which may be the pore-canal walls. In the insects an interpretation of the pore-canal structures is still hypothetical. The "pore canal filaments" described by Locke (1961), Neville and Luke (1969 *a*, *b*), Filshie (1970) and others, may be parts of the pore-canal walls which during the preparation may have been detached. A preparation of the shore-crab resembling this condition is illustrated in Pl. 2, Fig. 4. Neville and Luke (1969 *a*) described "cytoplasmic membranes bounding the locust pore canals". Also these membranes may correspond to the pore-canal walls in the lobster and shore-crab.

The interpretations of the morphology of the pore-canals have hitherto been controversial. Richards (1951) considered the pore-canals as heli-

cally coiled. Locke (1961) said that the basic shape of the pore-canals in insects is "probably that of a cylinder bent in a helix or in some other way". According to Locke the shape of the pore-canals may be related to the arrangement of the fibres in the exoskeletal lamellae. Neville *et al.* (1969) described the pore-canals in insects as helices or as helically twisted ribbons, rotating 360° or 180° per lamella. These writers thought the rotation to be determined by the molecular architecture of the chitin-protein complex. A slower rotation rate of the pore-canals was noted by these writers in the crustacean *Astacus*. Similar opinions on the morphology of the pore-canals were expressed by Neville and Luke (1969 *a, b*). Bouligand (1971), on the other hand, considered the course of the pore-canals in crabs to be slightly undulating.

The results dealt with in the present paper show clearly that, at least in the decapod crustaceans, a rotation of the pore canals does not take place. The pore-canals are here straight or slightly undulating, resembling crabs diagrammatically illustrated by Bouligand (1971, Fig. 14). Consequently, the pore-canals in the lobster and shore-crab have a vertical course entirely independent from the complicated spatial arrangement of the exoskeletal lamellae.

The pore-canals probably have several functions. One important function is the transportation of various substances necessary for hardening, repair, and protection of the exoskeleton (see e.g. Richards, 1951; Dennell, 1960; Travis, 1963; 1970; Hackman, 1971; Neville *et al.*, 1969; Neville and Luke, 1969 *a, b*; Filshie, 1970). Locke (1961) suggested that "the pore canal filaments may also function as anchors to stick the epithelium to the endocuticle". However, another important function of the pore-canals not dealt with in the previous literature, was clearly indicated from the results gained here in the decapod crustaceans. As seen in my SEM preparations, the pore-canal walls are vertically continuous and firmly attached to the horizontal and vertical exoskeletal lamellae (Fig. 1). This means that these lamellae in the decapod crustaceans are efficiently bound together by an independent, vertical fibrous system, whose existence has hitherto been unknown. Such an ultra-architectural pattern results in an increase of the strength and elasticity of the exoskeleton.

## DISCUSSION

Because of large sized structural components, the exoskeleton in decapod crustaceans is particularly well suited for SEM studies. By direct observations it was established that the chitin-protein fibres form numerous horizontal lamellae which are connected by vertical curved lamellae. Hence, the vertical lamellae are not optical artefacts as suggested by Bouligand (1965, 1971), Neville *et al.* (1969) and Neville and Luke (1969 *a, b*), but, as indicated by several previous light microscope and TEM studies (e.g. Richards, 1951; Dennell, 1973; Travis, 1963, 1970; Locke, 1961) they exist in reality.

As far as can be judged from the literature, this basic type of exoskeletal structure occurs in most arthropod groups, such as insects, crustaceans and arachnids. However, deviations from this type are found in certain ostracods, where the chitin-protein fibres form an irregular reticulation (Bate and East, 1972).

In decapod crustaceans, there occurs another fibrous system made up of numerous, vertically continuous pore-canal walls. This fibrous system seems to increase the strength and flexibility of the exoskeleton. It is still unknown whether or not the fibrous pore-canal walls may be found in other arthropod groups. As suggested above, the "pore-canal filaments", described in insects and ostracods, may represent detached portions of the pore-canal walls.

The course of the pore-canals in the decapod crustaceans, dealt with here, is straight or slightly undulating. Hence, it is entirely independent from the spatial arrangement of the exoskeletal fibrous lamellae. On the other hand, according to Neville *et al.* (1969) and Neville and Luke (1969 *a, b*), the pore-canals in insects are helically coiled. To what extent this coiling is related to the arrangement of the chitin-protein fibres is still imperfectly known.

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# PLATES

## Plate 1

*Homarus gammarus* L.

*Fig. 1.* Fracture plane to show three consecutive horizontal lamellae and variations in the course of the chitin-protein fibres in these lamellae (× 250).

*Fig. 2.* Similar preparation (× 600).

*Figs. 3,4.* Obliquely vertical fracture plane to show several horizontal lamellae which are connected by numerous, curved, vertical lamellae (× 2 800 and × 7 000, respectively).

All preparations in this plate come from the endocuticle and they are chemically untreated.

## Plate 2

*Homarus gammarus* L.

*Fig. 1.* Surface of a horizontal lamella, showing the emergence of several vertical lamellae (× 1 300).

*Fig. 2.* A horizontal lamella (upper and right side) passing into a downward directed, vertical lamella (lower left side). Note that both the horizontal and vertical lamellae are perforated by the pore-canals (×2 500).

*Fig. 3.* Two superimposed lamellae, the upper of which has emerged from the lower one. Note the arrangement of the chitin-protein fibres in these lamellae (× 6 000).

*Carcinus maenas* (L.)

*Fig. 4.* Surface of a horizontal lamella showing the arrangement of the chitin-protein fibres; the vertically oriented fibres have detached themselves from the pore-canal walls (× 7 000).

All preparations in this plate represent the endocuticle. The preparations in *Figs. 2* and *3* are chemically untreated; in *Fig. 1* they are demineralized in glutaraldehyde solution, dehydrated in alcohol and amyl acetate, then dried in air; in *Fig. 4* they are demineralized in glutaraldehyde-acetic acid solution, dehydrated in acetone, then dried in critical point apparatus.

## Plate 3

*Homarus gammarus* L.

*Figs. 1, 2.* Several consecutive horizontal lamellae which are connected by vertical lamellae with somewhat varying arrangement (× 1 100 and × 2 200, respectively).

*Fig. 3.* Similar preparation. Note variations in the arrangement of the vertical lamellae (× 1 100).

*Fig. 4.* Surface of a horizontal and a vertical lamella to show the emergence of the latter lamella (× 2 600). All preparations in this plate come from the endocuticle and they are chemically untreated.

## Plate 4

*Carcinus maenas* (L.)

*Fig. 1.* Horizontal and vertical fracture planes showing the arrangement of the horizontal and vertical lamellae (× 1 200).

*Homarus gammarus* L.

*Fig. 2.* Surface and vertical fracture plane of a horizontal lamella to show the emergence of several vertical lamellae. Note the direct continuation of the chitin-protein fibres between the horizontal and vertical lamellae (× 6 000).

*Figs. 3, 4.* Horizontal lamellae and their relation to the vertical lamellae. The emergence of the vertical lamellae from the horizontal lamellae is clearly seen (× 2 200 and × 5 500, respectively).

All preparations in this plate come from the endocuticle. The preparation in *Fig. 1* has been decalcified in glutaraldehyde-acetic acid, dehydrated in acetone, then dried in critical point drying apparatus; the remaining preparations are chemically untreated.

## Plate 5

*Carcinus maenas* (L.)

*Figs. 1, 2.* Surface of several consecutive horizontal lamellae. Note the curved course of the chitin-protein fibres in these lamellae (× 220 and × 2 400, respectively).

*Figs. 3, 4.* Horizontal and obliquely vertical fracture planes showing the arrangement of the horizontal and vertical lamellae (× 1 100 and × 2 200, respectively).

All preparations in this plate represent the endocuticle and are chemically untreated.

## Plate 6

*Carcinus maenas* (L.)

*Figs. 1, 2, 3, 4.* Horizontal and obliquely vertical fracture planes through the endocuticle to show the arrangement of the horizontal and vertical lamellae. The preparations in *Figs. 3* and *4* also show the detached portions of the pore-canal walls (× 1 100, × 2 200, × 2 200 and × 2 200, respectively).

All preparations in this plate are chemically untreated.

## Plate 7

*Homarus gammarus* L.

*Figs. 1, 2.* Surface of horizontal lamellae to show the pore-canals and their fibrous walls. Note a well exposed pore-canal wall in the middle of both figures (× 6 000 and × 12 000, respectively).

*Figs. 3, 4.* Similar preparations (both × 6 000).

All preparations in this plate represent the endocuticle and have been decalcified in glutaraldehyde solution, dehydrated in alcohol and amyl acetate, and then dried in air.

## Plate 8

*Carcinus maenas* (L.)

*Fig. 1.* Vertical fracture plane through the epicuticle and adjacent part of the exocuticle (× 13 000).

*Fig. 2.* Vertical fracture plane through the exocuticle. Note the arrangement of the lamellae and the course of the pore-canals (× 7 000).

*Figs. 3, 4.* Vertical fracture planes through the endocuticle. Note the orientation of the chitin-protein fibres in the horizontal and vertical lamellae, and the shape and vertical course of the pore-canal walls (both × 6 500).

All preparations in this plate have been decalcified in glutaraldehyde-acetic acid solution, dehydrated in alcohol and acetone, and dried in critical point drying apparatus.

## Plate 9

*Homarus gammarus* L.

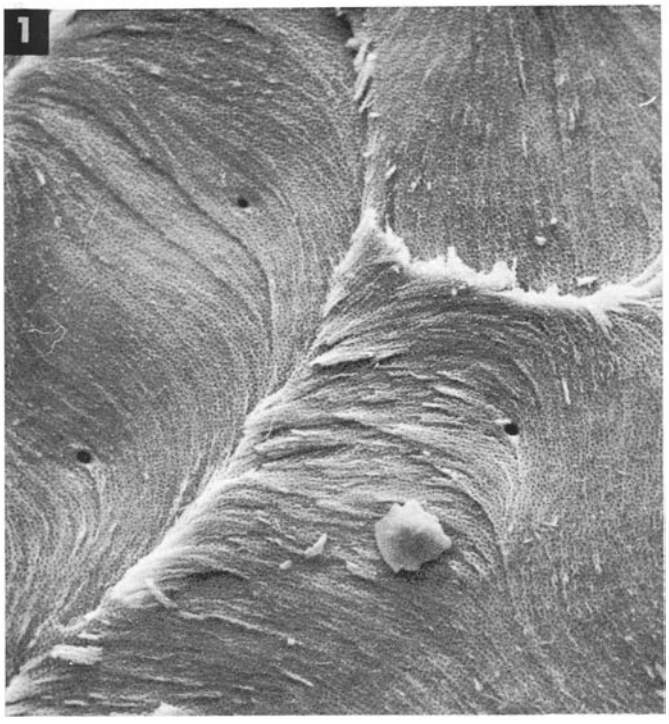
*Fig. 1.* Surface of horizontal and vertical lamellae showing detached, long portions of the vertical pore-canal walls which appear as numerous needles (× 1 100).

*Fig. 2.* Vertical fracture plane showing numerous vertical pore-canal walls which traverse a horizontal lamella; the vertical lamellae are mostly broken off (× 2 800).

*Fig. 3.* Horizontal lamellae traversed by vertical pore-canal walls (× 5 000).

*Fig. 4.* Inner face of a horizontal lamella showing the pore-canals and long detached portions of their fibrous walls (× 6 000).

All preparations in this plate represent the endocuticle. Preparations in *Figs. 1, 2* and *3* have been decalcified in glutaraldehyde solution, dehydrated in alcohol and amyl acetate, then dried in air; preparation in *Fig. 4* is chemically untreated.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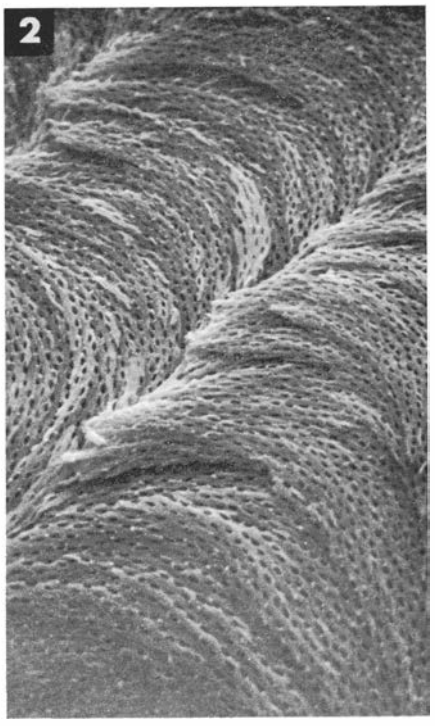
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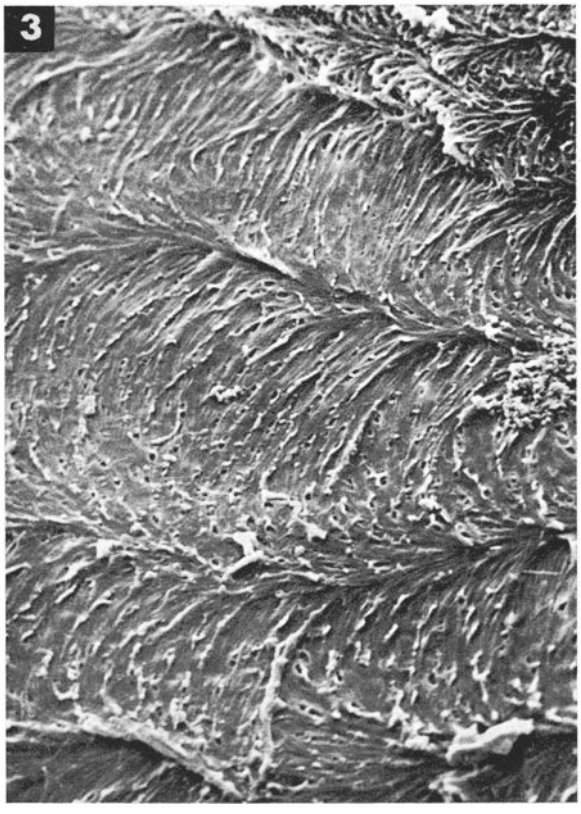
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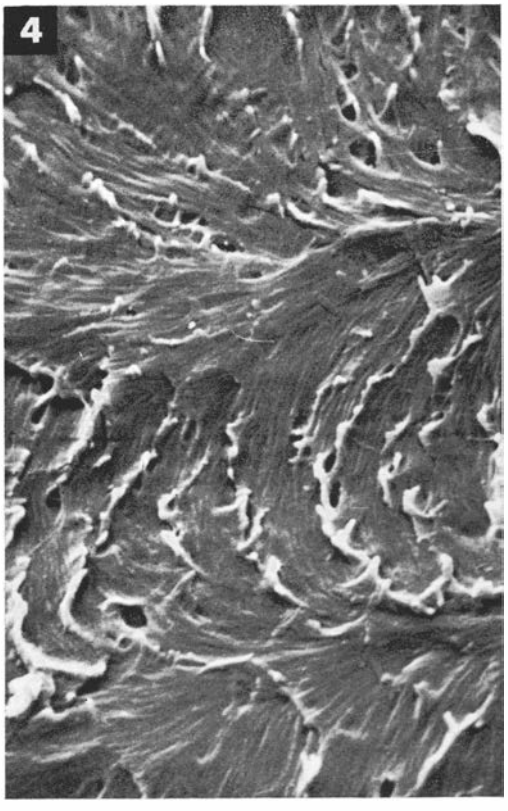
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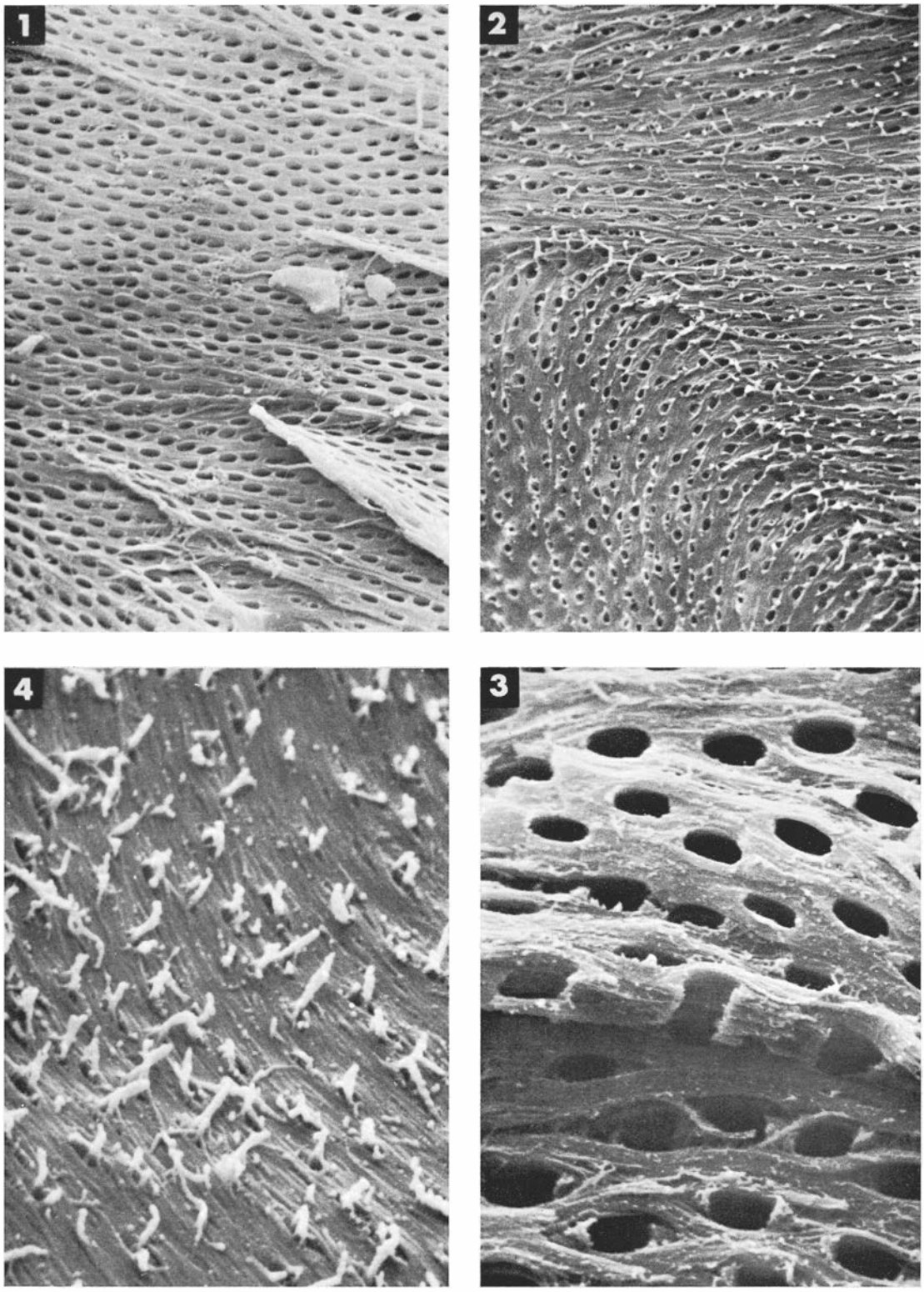
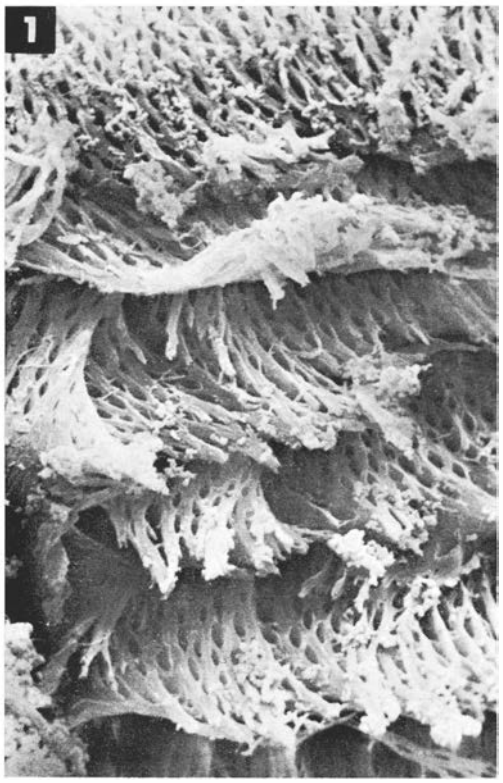
Plate II

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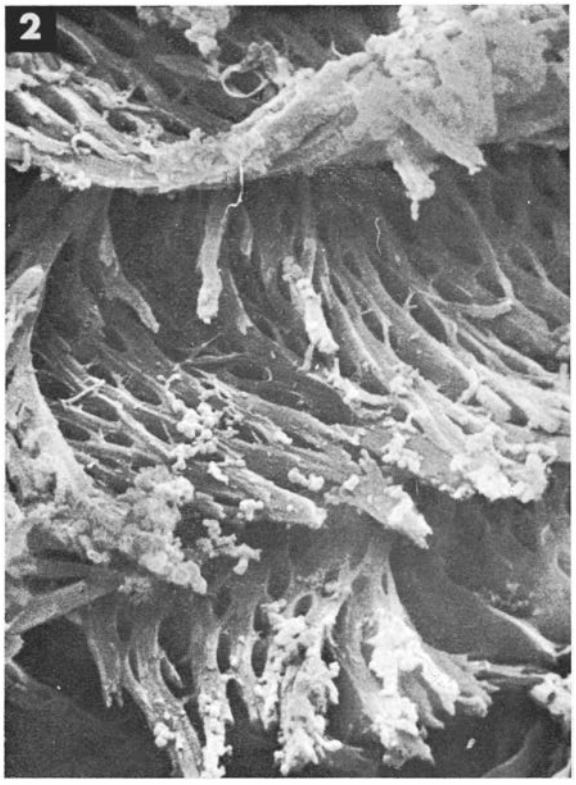
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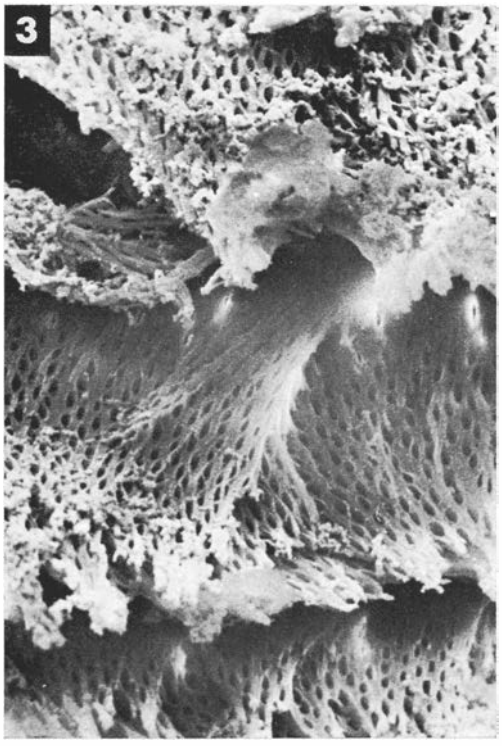
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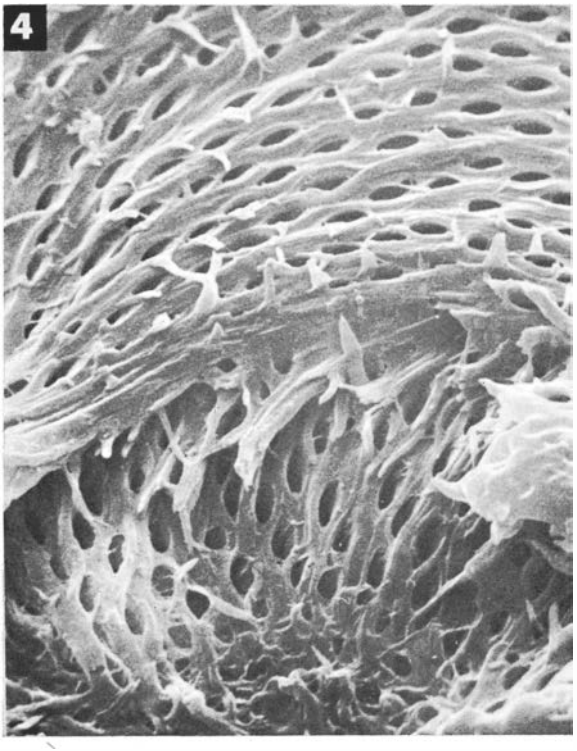
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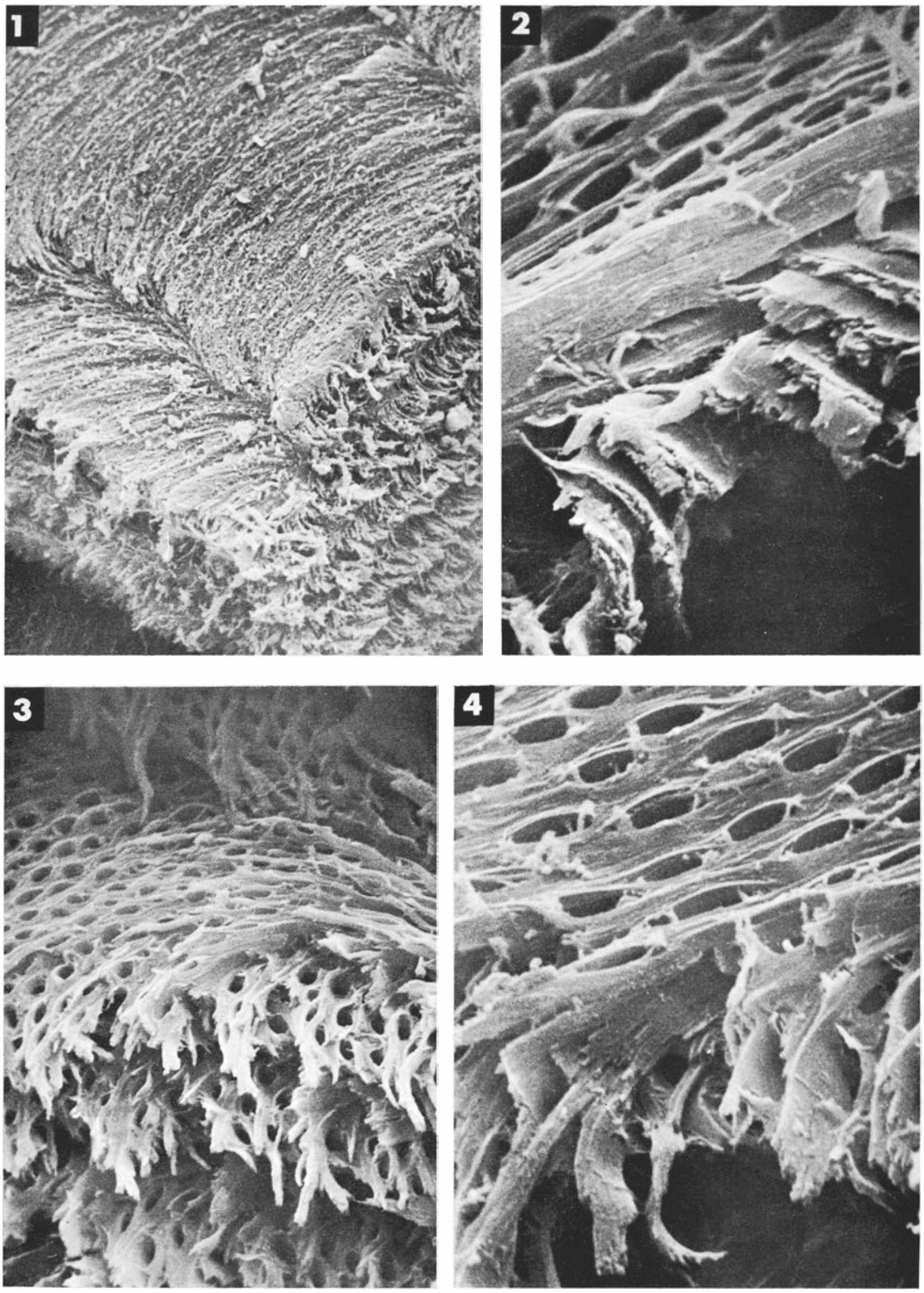


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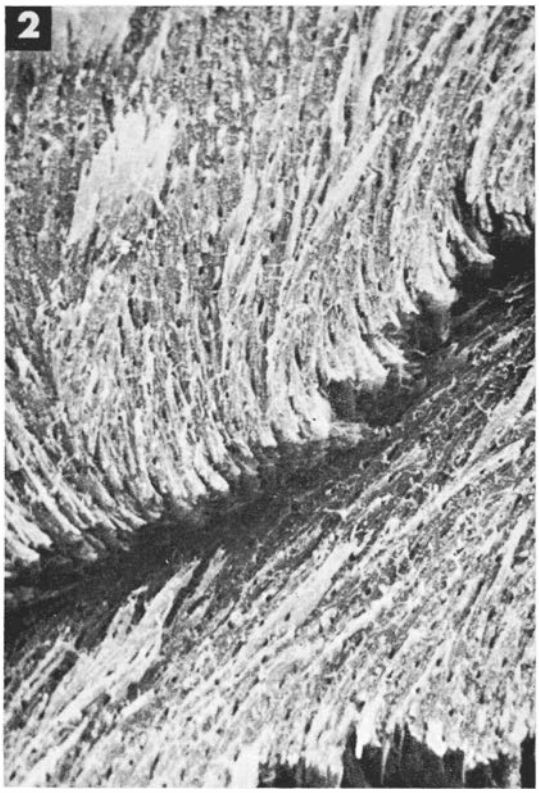
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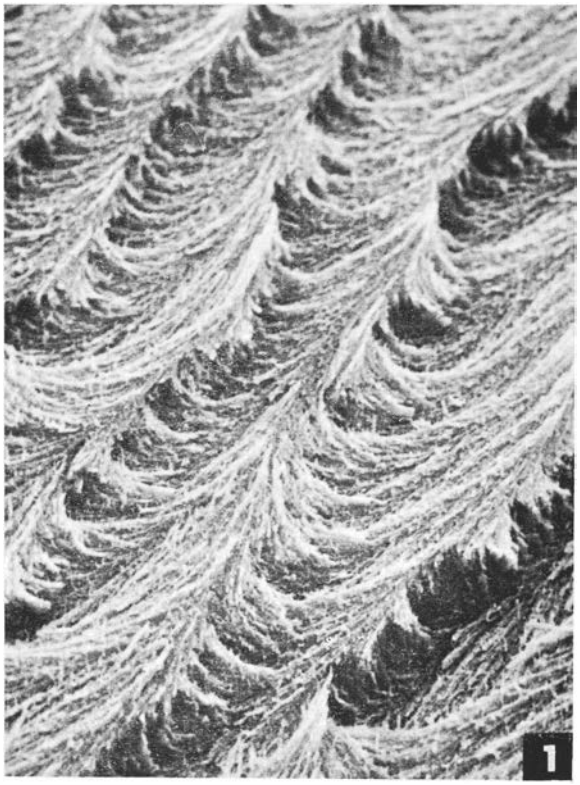
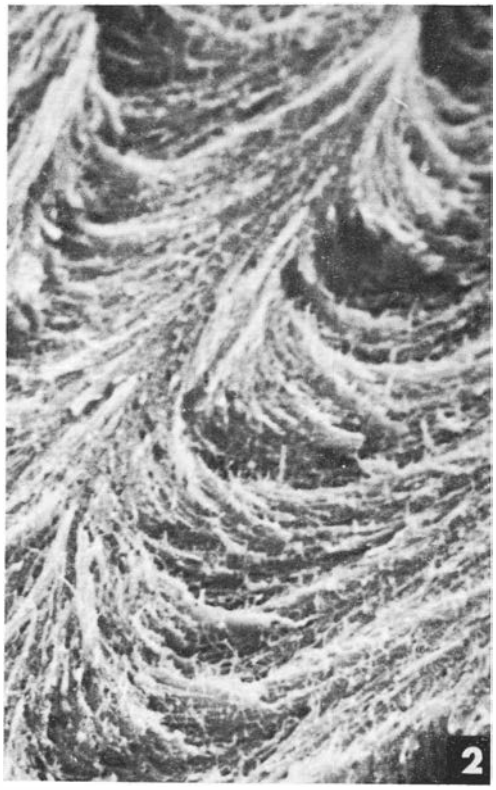
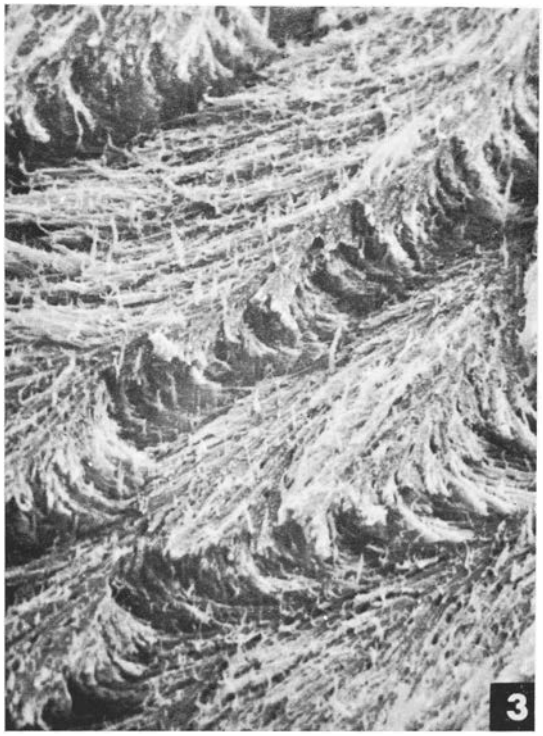
Plate VI



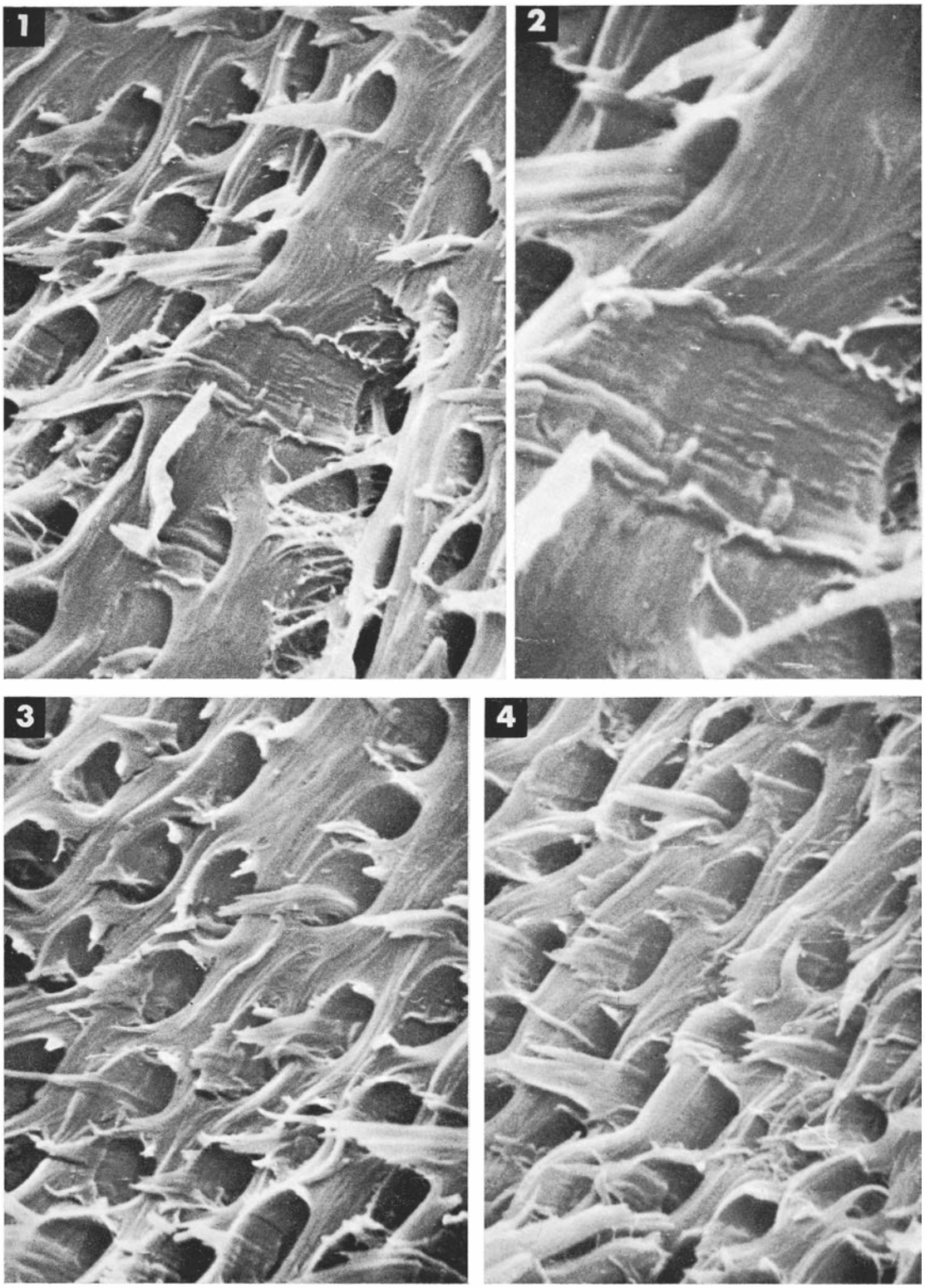


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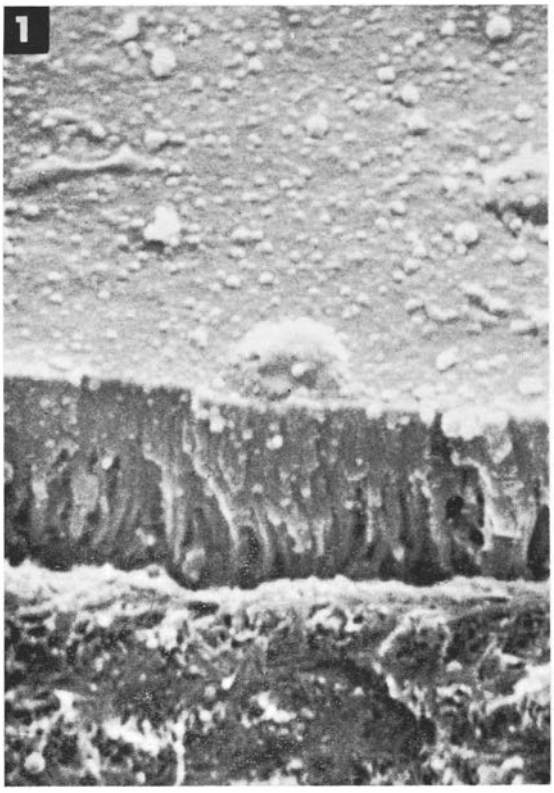
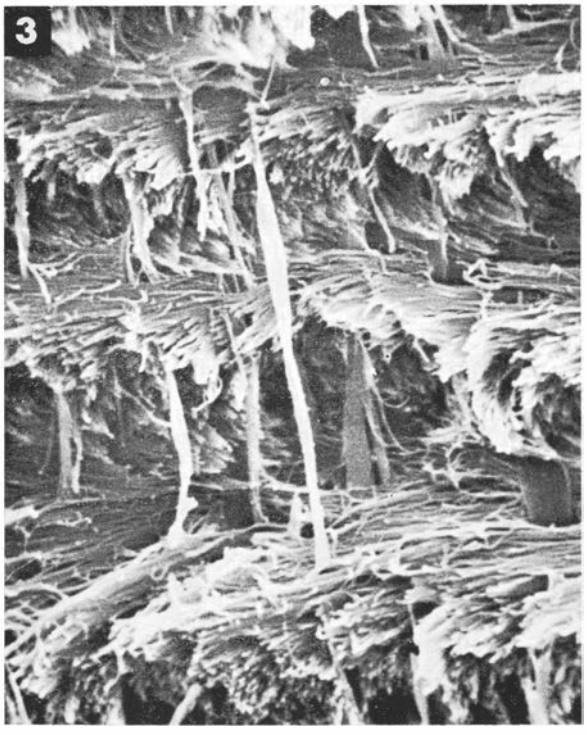
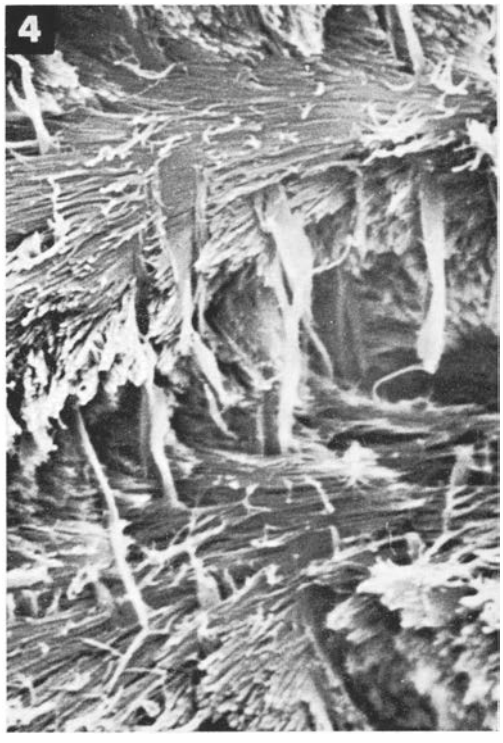
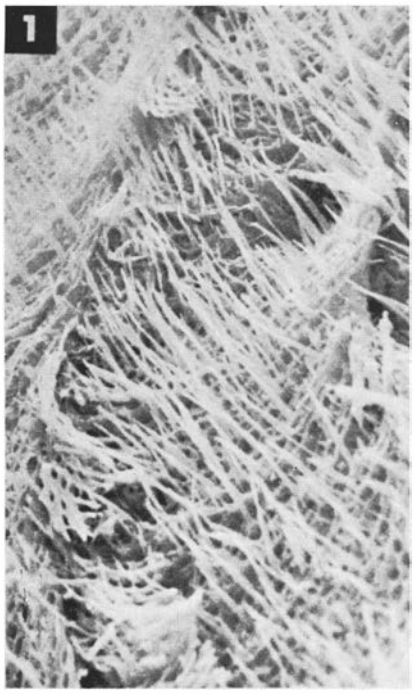
Plate VIII





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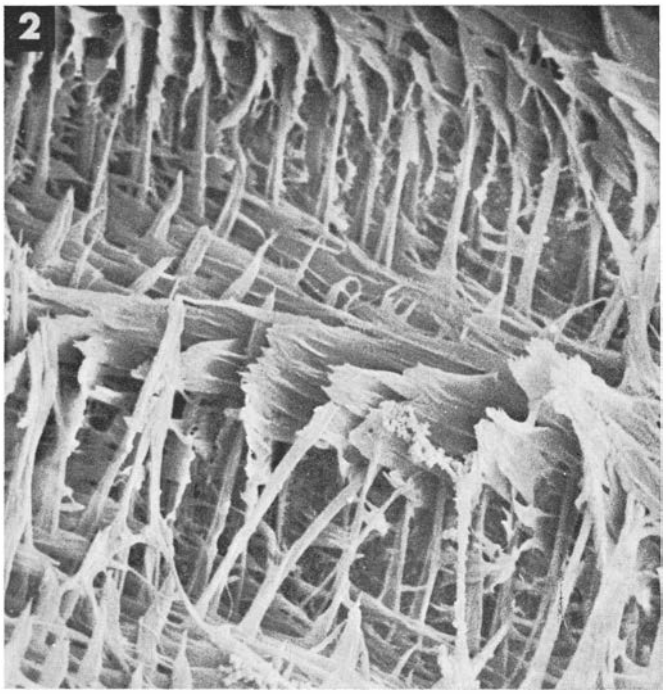
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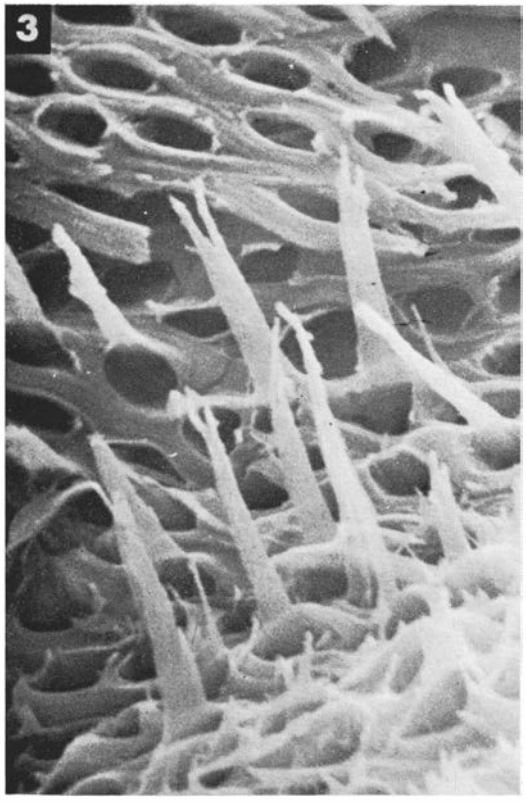
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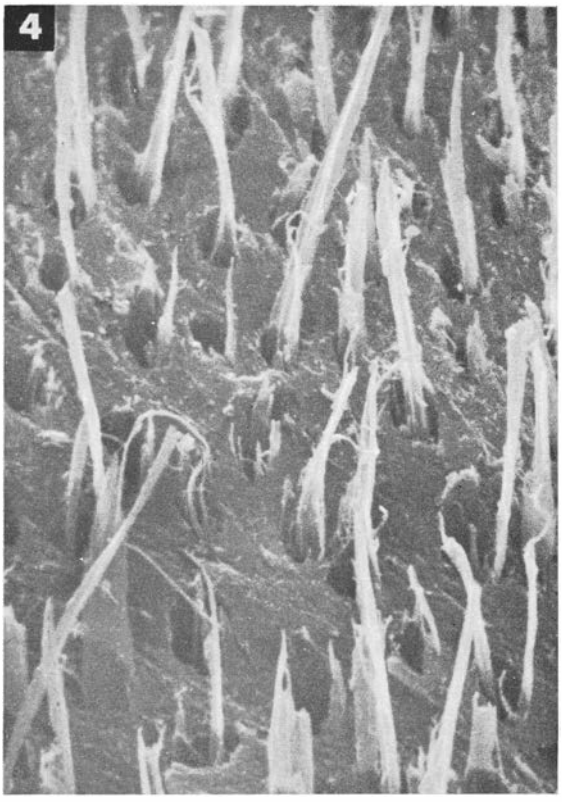
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