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## *Original Papers*

# The Question of von Korff Fibres in Mammalian Dentine

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The distribution and orientation of the "fibres of von Korff" have been studied in developing dentinal surfaces and in fractured and in polished acid etched surfaces of mature dentine both with the scanning electron microscope and indirectly via a carbon replica technique for the transmission electron microscope. It has been found that von Korff fibril bundles can be visualized in prepared surfaces of mature dentine and that their specific location and conformation in the finished tissue can be related to the developing surface. Von Korff fibrils are characteristic of the first-formed (mantle) dentine and are not found in the bulk of the dentine formed later (circumpulpal dentine). Structures superficially similar to "von Korff fibres" occur at the developing surface during the later stages of dentine development but are incorporated into the dentinal matrix in an entirely different manner.

There is a third or intermediate zone between the very irregular and fibrillar mantle dentine and the very regular and relatively homogenous circumpulpal dentine. This zone is distinguishable on the basis of the distribution and orientation of von Korff fibril bundles, although the three zones merge gradually with one another and vary in relative thickness in different species. The majority of von Korff fibril bundles in mature dentine are truly radial for only a small part of their course. The presence of von Korff bundles in the wombat can be related to the absence of enamel tubules in this marsupial. Areas of mantle dentine lacking von Korff fibrils may alternate with areas containing many fibrils in some placental mammals. This presence or absence of von Korff fibrils can be related to the presence or absence respectively of "tubules" in the overlying enamel.

La distribution et l'orientation des «fibres de von Korff» ont été étudiées au niveau de surfaces dentinaires en voie de développement et surfaces fracturers ainsi qu'au niveau de surfaces polies de dentine adulte, puis attaquées à l'acide, à la fois avec un microscope électronique «scanning» et indirectement à l'aide de répliques de carbone, examinées à l'aide d'un microscope électronique par transmission. Les faisceaux de fibrilles de von Korff peuvent être mis en évidence au niveau de la dentine adulte et leur situation spécifique ainsi que leur structure sont caractéristiques au niveau de la surface de développement. Les fibrilles de von Korff sont situées dans la première couche de dentine formée et ne se trouvent pas dans la masse dentinaire, formée plus tardivement (dentine circumpulpaire). Des structures ressemblant à des «fibres de von Korff» se rencontrent au niveau de la surface en voie de développement, au cours des stades tardifs de la dentinogenèse, mais elles sont incorporées dans la matrice dentinaire d'une façon totalement différente.

Entre la première couche de dentine fibrillaire et irrégulière, formée, et la couche de dentine circumpulpaire, régulière et homogène, on observe une troisième zone intermédiaire. Cette zone se différencie par la distribution et l'orientation des faisceaux de von Korff. Cependant ces zones fusionnent progressivement les unes avec les autres et présentent une épaisseur variable d'une espèce à l'autre. La majorité des faisceaux fibrillaires de von Korff dans la dentine adulte ne présente un trajet radial vrai que sur une infime partie de leur parcours. La présence de faisceaux de von Korff chez le wombat d'Australie peut être mise en rapport

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avec l'absence de canalicules de l'émail chez ce marsupial. Des régions de dentine périphérique, sans fibrilles de von Korff peuvent alterner avec des régions contenant de nombreuses fibrilles dans certains mammifères placentaires. La présence ou l'absence de fibrilles de von Korff peut correspondre respectivement à la présence ou l'absence de «canalicules» dans l'émail sus-jacent.

Die Verteilung und Lagerung der „von Korffschen" Fasern ist auf sich entwickelnden Dentinoberflächen und auf frakturierten und polierten, säuregeätzten Oberflächen von reifem Dentin untersucht worden, und zwar auf dem Scanning-Elektronen-Mikroskop und indirekt mit der Kohleabdruck-Technik auf dem Transmissions-Elektronen-Mikroskop. Dabei wurde festgestellt, daß von Korffsche Fibrillen-Bündel auf vorbehandelten Oberflächen von reifem Dentin sichtbar gemacht werden können, wobei ihre spezifische Lage und Verteilung im ausgewachsenen Gewebe mit der Wachstumszone in Zusammenhang gebracht werden kann.

Die „von Korffschen" Fibrillen sind charakteristisch für das zuerst gebildete, sog. Mantel-Dentin. Dagegen finden sie sich in der Masse des später gebildeten Circumpulpal-Dentins nicht. Den „von Korffschen" Fasern in ihrer Struktur ähnliche Fasern treten an der sich entwickelnden Oberfläche im letzten Stadium der Dentinbildung auf, werden jedoch auf ganz andere Weise in die Dentinmatrix verwoben.

Eine dritte oder Intermediärzone konnte zwischen dem recht unregelmäßigen, fibrillären Mantel-Dentin und dem ausgesprochen regelmäßigen, relativ homogenen Circumpulpal-Dentin gefunden werden. Dieser Anteil läßt sich von den beiden andern auf Grund der Lagerung der „von Korffschen" Faserbündel unterscheiden, obschon alle drei Zonen ineinander übergehen und in ihrer Dicke bei den verschiedenen Gattungen variieren. Die Mehrzahl der „von Korffschen" Faserbündel im reifen Dentin ist in ihrem Verlauf nur auf einer kurzen Strecke wirklich radiär gelagert. Das Vorkommen „von Korffscher Faserbündel" beim Wombat läßt auf fehlende Tubuli im Zahnschmelz dieser Beuteltiere schließen. Bereiche von Mantel-Dentin mit fehlenden „von Korffschen" Faserbündeln können bei einigen placentären Säugetieren mit solchen, die viele Fibrillen enthalten, abwechseln. Dieses Vorhandensein oder Fehlen der „von Korffschen" Fibrillen kann mit vorhandenen, resp. fehlenden Tubuli im darüberliegenden Zahnschmelz in Zusammenhang gebracht werden.

## Introduction

Raschkow (1835) appears to have been the first to picture "sinuous" longitudinal fibres at the periphery of the developing pulp where the first dentine formation was taking place. Similar structures were subsequently illustrated by Hoehl (1896) and described by Hansen (1899) in sectioned material. The detailed light microscope appearance of the development and structure of the very first-formed or mantle dentine (Weidenreich, 1925) was however, described by von Korff (1905) and it was von Ebner (1906a, 1906b) who first referred to the "von Korff fibres". Disse (1909) called them "Höhl's fibres".

The presence of coarse fibre bundles (now generally known as the "corkscrew fibres of von Korff") extending from the developing pulp, across the developing front of the mantle dentine and continuous within it in a fan-like arrangement has since been confirmed by many workers. There is disagreement however, as to the presence and the role of these fibre bundles in subsequent dentine formation. Structures at the developing front similar to those described by von Korff have been reported by some to persist unchanged throughout the formation of the bulk of the dentine [1, 2, 16—24, 31, 33, 46, 47], while Symons (1956) noted a reduction in their diameter and others have found them either difficult to detect or absent altogether [8—10, 13, 17, 25, 30, 38—40, 44, 45]. There is reasonable agreement however, that von Korff fibres are present during secondary dentine formation (for example, Kantorowicz, 1910; Zerosi, 1966).

Weidenreich (1925) first applied the terms "mantle" and "circumpulpal" to the early and later formed dentine respectively, on the basis that von Korff's fibres could not be traced beyond, and contributed only to, the first-formed dentine. This view has gained general acceptance as has the concept of the fibre orientation in the mantle dentine being predominantly perpendicular to the enamel-dentine junction. However, little evidence has been advanced to confirm these points through the use of modern techniques and the improved resolution which they afford. The aim of the present study was, therefore, to visualise von Korff fibres both in mature dentine and at the developing front with the electron microscope and, in so doing, to gain information on their relative distribution, their orientation and structure.

## Materials and Methods

Sound, formalin-fixed teeth from the following mammalian species were examined: coypu (*Myocastor coypus*), jird (*Meriones sp.*), grey squirrel (*Sciurus sp.*), rabbit (*Oryctolagus cuniculus*), dog (*Canis familiaris*), pig (*Sus scrofa*), lamb (*Ovis aries*), calf (*Bos taurus*), African elephant (*Loxodonta africana*), bottle-nosed dolphin (*Tursiops truncatus*), Geoffroy's dolphin (*Inia geoffrensis*), manatee (*Trichecus manatus*), monkey (*Macaca mulatta*), human (*Homo sapiens*), kangaroo (*Macropus sp.*), opossum (*Metachirus nudicaudatus*) and wombat (*Vombatus ursinus*) — latin names are from Morris (1965). The pulps were removed from the teeth, some of which were then fractured by closing the jaws of a small vice in which they were held, whilst others were sectioned, polished and acid-etched (0 · IN HCl for 3 sec). The exposed developing and mature dentinal surfaces were examined by two methods:

1. For direct examination in a scanning electron microscope (Cambridge Instrument Co. Stereoscan, 10 kV), a conducting layer, of ca. 200 Å carbon and ca. 300 Å gold, was evaporated *in vacuo* on to the surface to be studied.

2. For indirect examination in a transmission electron microscope (Siemens Elmiskop I, 40 kV), surfaces were replicated in carbon using a single-stage replica technique described by Boyde (1967).

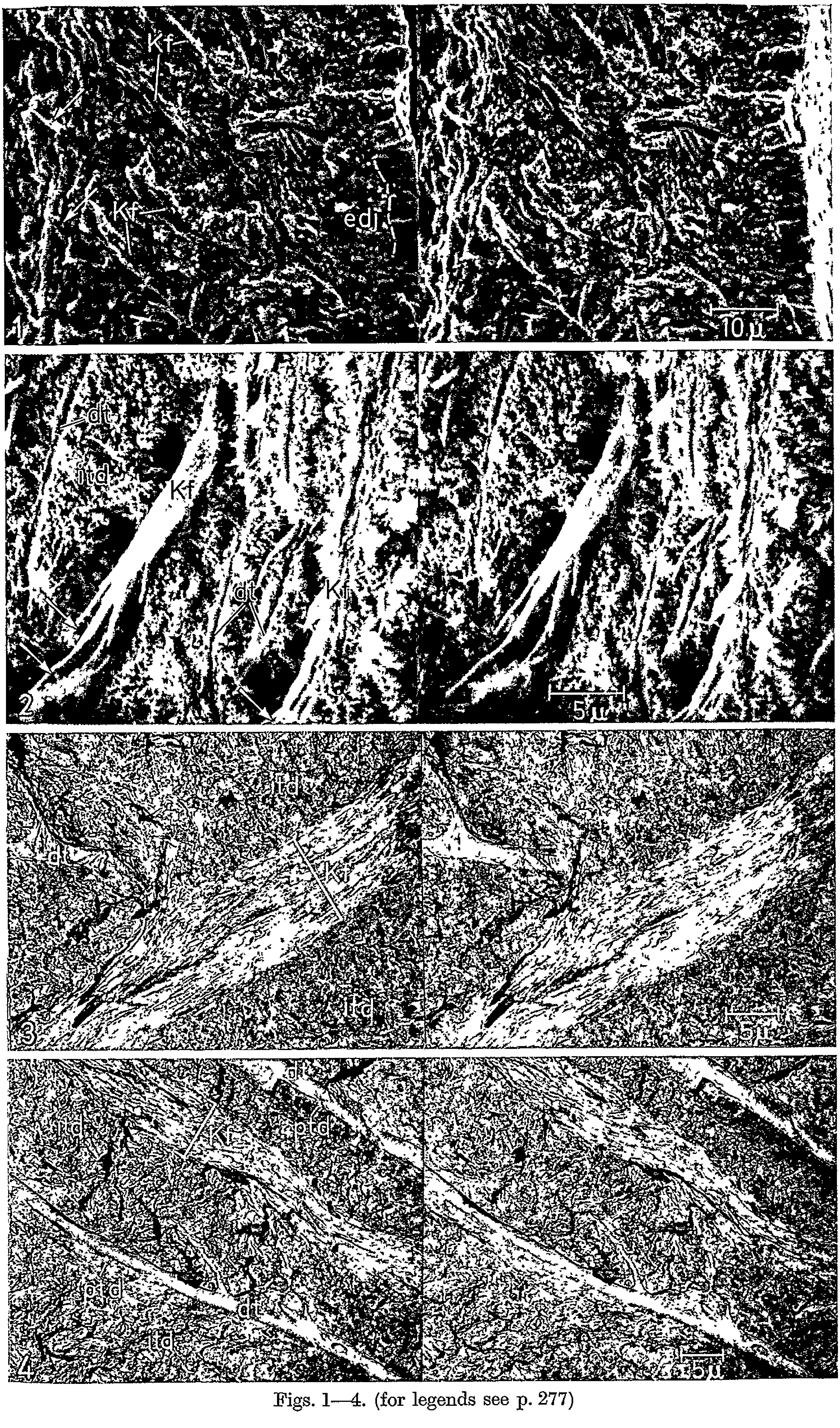
Stereo-pair images were recorded by both methods and subsequently examined with the aid of a mirror stereo-comparator (Hilger and Watts Stereometer).

## Results

### *Fractured Surfaces of Mature Dentine*

Structures identified as the "corkscrew fibres of von Korff" (Figs. 1, 2, 14 and 16) were present in the dentine close to the enamel-dentine junction in all the placental species examined and also in *Vombatus ursinus*. The teeth of the two cetaceans examined showed the widest and most easily accessible mantle dentine layer. Thus, certain features which are seen with difficulty in other species are very easily discerned in *Tursiops truncatus* and *Inia geoffrensis* (Figs. 1—5) and although the following description will be largely illustrated by reference to these two dolphins, the same may be held to be true in a general way of the other mammals.

The structures identified as "von Korff" fibres are large bundles of finer fibrils which are coherent for the greater part of their length and which are relieved from the fracture surfaces during the fracturing process. The individual fibrils of these bundles, although not completely demarcated have an average diameter of ca. 500—1000 Å (Figs. 2, 4) in distinction to the fibrils of the surrounding closely interwoven matrix feltwork with diameters of ca. 400—700 Å (Figs. 2, 3).



Figs. 1—4. (for legends see p. 277)

The fibrils of the von Korff bundles are fanned out in an irregular manner in the dentine close to the enamel-dentine junction (Figs. 1, 2, 13, 14). The fibril course appears uninfluenced by the terminal branches of the dentinal tubules, suggesting that the von Korff bundles take precedence over the dentinal tubules in terms of positioning during dentine development. Von Korff bundles are sometimes found in a peritubular location in this region (Fig. 6). The bundles are distinguished easily from the "peritubular dentine" since the latter has its own peculiar fine structure and does not exhibit a fibrillar appearance (Boyde and Lester, 1967).

The von Korff bundles run a rootwards course from their "fanned" origin, passing vertically (apically) at the sides of the tooth and thus nearly parallel to the enamel-dentine junction for a distance (Figs. 14, 16). A radial orientation of von Korff fibrils is confined to cuspal and intercuspal (pulp chamber roof) regions; this has been recognised in the earlier work in this field [8—10, 36, 38] but it is possibly not appreciated widely at the present day and for this reason the term "radial fibres" (for example, Schmidt and Keil, 1958) is, in our opinion, misleading. The terminal parts of the von Korff bundles do however, characteristically change direction to run parallel to the dentinal tubules in the area intermediate between mantle dentine and the circumpulpal dentine. Only here do the von Korff bundles run strictly and typically parallel to the dentinal tubules (Fig. 4).

Further still from the enamel-dentine junction is the typical appearance of circumpulpal dentine, viz: — fine parallel tubules, finer lateral branches and a relatively homogeneous and afibrillar intertubular dentine (Fig. 5). Only the very occasional coherent fibril bundle runs parallel with the tubules, but these can not be followed for any distance.

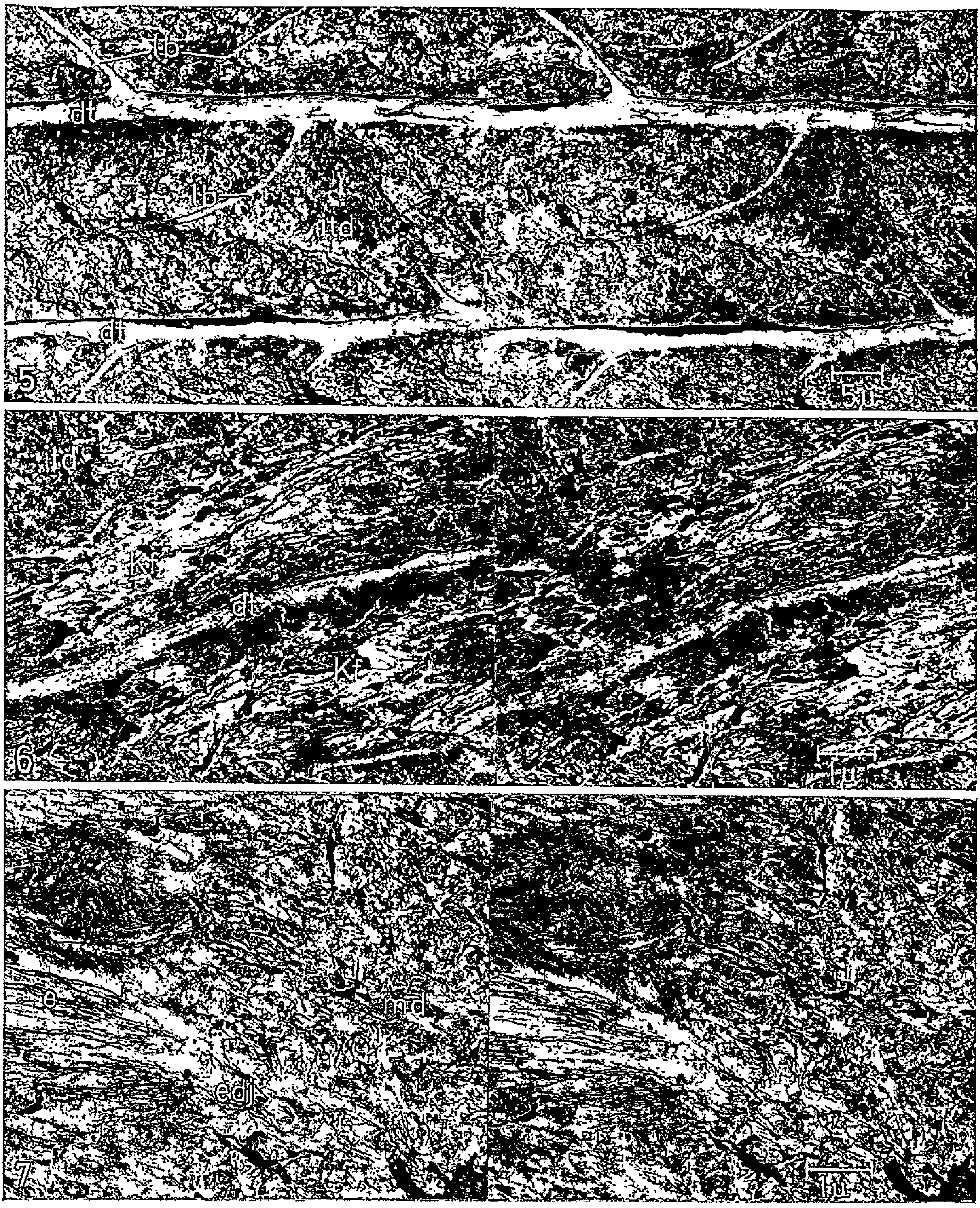
Von Korff fibres were not seen in a very thin layer of dentine immediately adjacent to the enamel-dentine junction (Fig. 7), except where they crossed the junction (see below and Figs. 10 and 11). No membranous structure was observed at the enamel-dentine junction in any of the species studied. The junction appears as an inconspicuous and intimate fusion of the first-formed fibrillar dentine with the closely packed crystallites of the first-formed enamel (Fig. 7).

Fig. 1. Stereo-pair scanning electron micrograph of longitudinally fractured developing *Tursiops truncatus* tooth. Note the irregular fibrillar mantle dentine and the von Korff fibril bundles which are also visible at the developing dentinal front. *Legends of Illustrations* 1—20. *Kf* von Korff fibril bundles; *md* mantle dentine; *itd* inter-tubular dentine; *ptd* peritubular dentine; *dt* dentinal tubule; *lb* lateral branch of dentinal tubule; *edj* enamel-dentine junction; *e* enamel; *c* crack. Tilt angle for scanning electron micrograph stereo-pairs = 10°. Tilt angle for transmission electron micrograph stereo-pairs = 8° 40′

Fig. 2. Stereo-pair scanning electron micrograph of longitudinally fractured *Tursiops truncatus* mantle dentine showing von Korff bundles and component fibrils (see arrows)

Fig. 3. Stereo-pair transmission electron micrograph of carbon replica of fractured *Tursiops truncatus* mantle dentine

Fig. 4. Stereo-pair transmission electron micrograph of carbon replica of fractured *Tursiops truncatus* dentine showing "intermediate zone" where the von Korff bundles are oriented parallel to the dentinal tubules



Figs. 5—8. (for legends see p. 279)

### *Developing Dentinal Surfaces*

Von Korff fibril bundles can be seen upon developing dentinal surfaces with the scanning electron microscope (Figs. 17, 18, 19). The fibril bundles are aligned and oriented with respect to one another so as to constitute longitudinal ridges in the very early stages of dentine formation (Figs. 17, 18). The further from the developing edge of dentine (the mantle dentine), the less marked are the ridges and they are absent altogether from the developing surface of circumpulpal dentine. These "ridges" of von Korff fibril bundles are most marked and cover a greater area of developing surface in those species which have a thick layer of mantle dentine (for example, the rabbit and the dolphins) and are less marked in those species with a very thin layer (for example, pig and calf).

Smaller and more widely separated fibril bundles, the individual fibrils of which spread out *over* the developing dentinal surface, were occasionally seen in those areas where the greater bulk of the dentine had been formed. These fibrils contributed to the fibrillar feltwork oriented parallel to the developing front at that level, and constituted an integral part of it (Fig. 20).

### *Etched Surfaces*

The dentine shows a disappointing lack of structural detail in the acid-etched, polished surfaces. However, very large von Korff fibril bundles often run to the peaks of the scalloped outline typical of the enamel-dentine junction in longitudinal section. Such peaks in the outline of the junction occasionally mark the point of origin of clefts continuous for some distance into the substance of the enamel (Figs. 10, 11). These clefts are identified with the "enamel spindles" because of their size and preferred localization towards the incisal edge or dentinal cusp region and because they bear no relation to the prism direction.

The cross-banding generally regarded as typical of collagen is clearly demonstrated by the von Korff fibrils identified in replicas of the fractured and etched material (Fig. 8). Their collagenous nature has been reported previously in developing material prior to mineralization [3, 16, 31].

## Discussion

The so-called 'von Korff fibres' were the subject of a great deal of discussion in the early part of this century. This discussion, much of it emotional and often bitterly personal, revolved essentially about two points. The first was the question of whether the "fibres" were present throughout all stages of dentine formation,

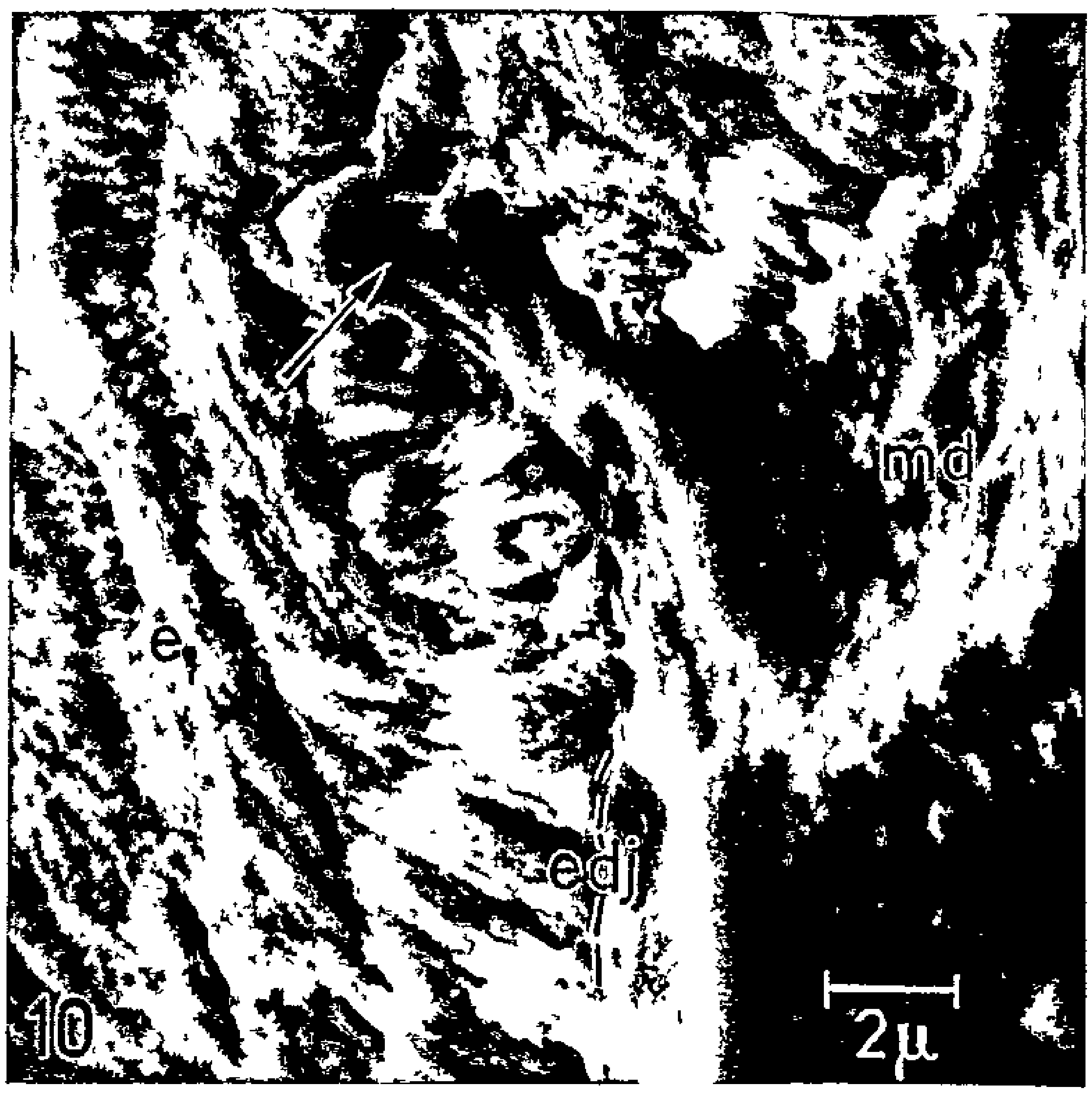
Figs. 5—8. Transmission electron micrographs of carbon replica of fractured *Tursiops truncatus* teeth

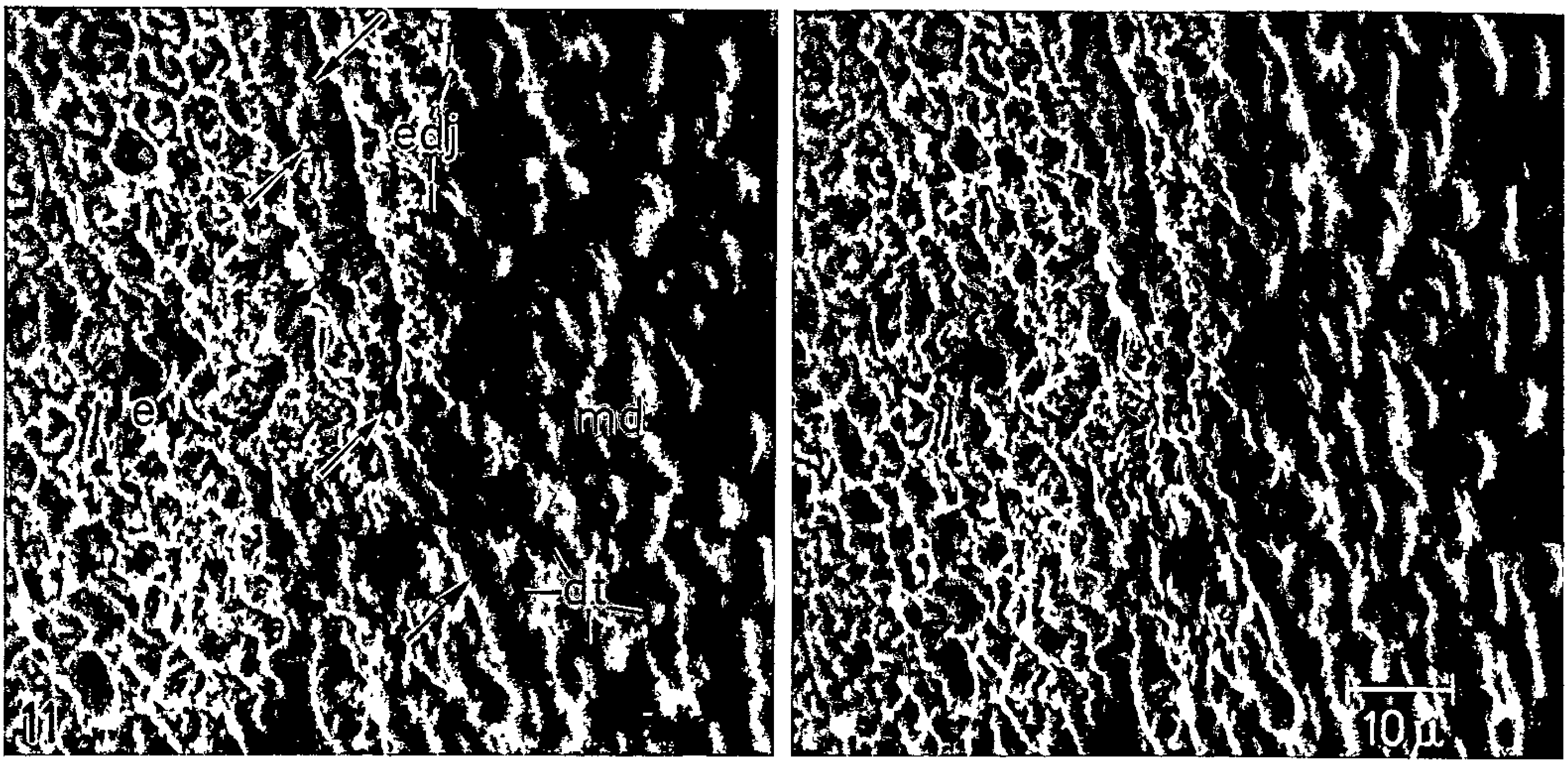
Fig. 5. Circumpulpal dentine with its parallel tubules, fine lateral branches and afibrillar inter-tubular dentine

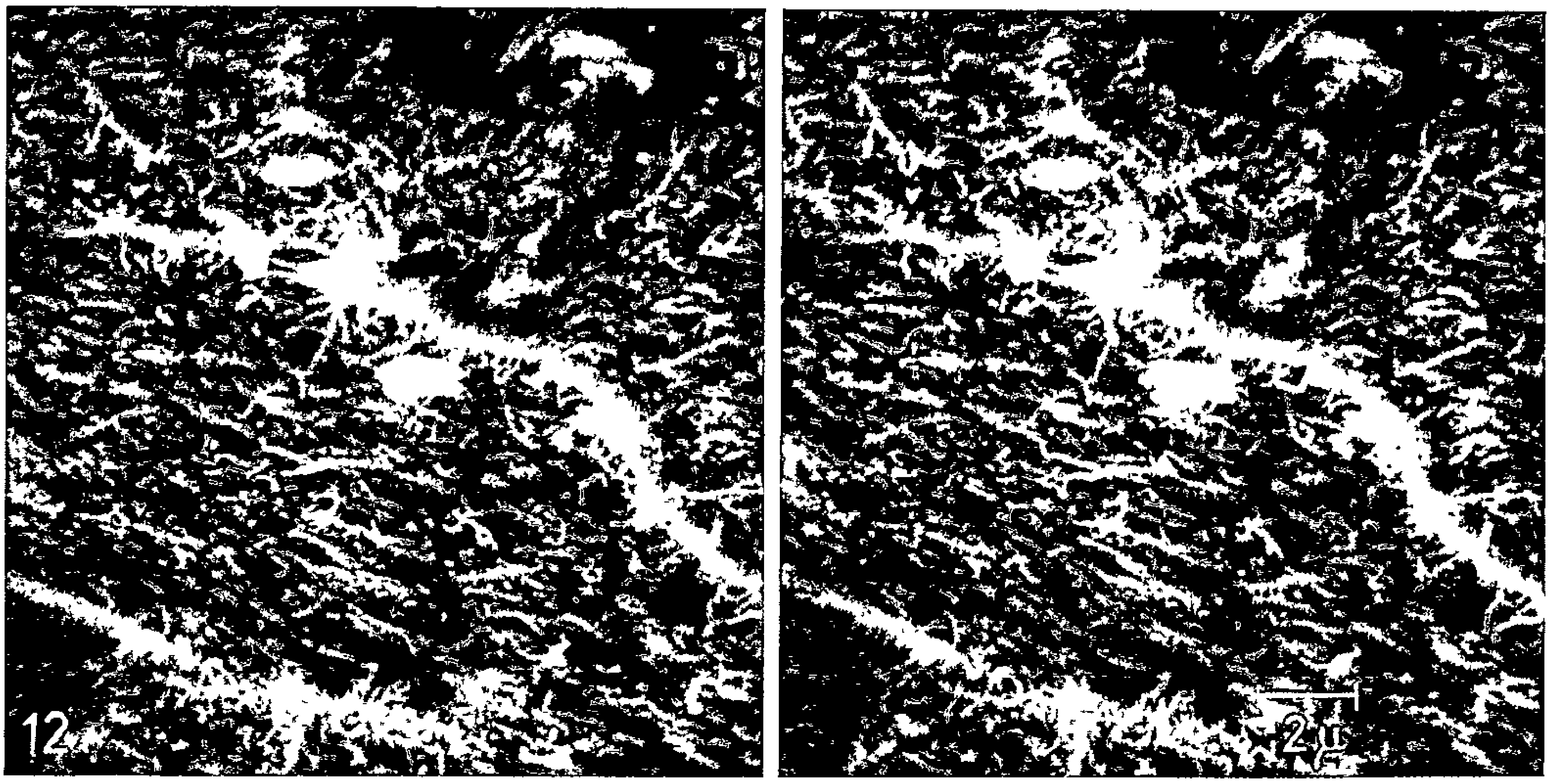
Fig. 6. Mantle dentine showing tubule running within von Korff bundle

Fig. 7. Enamel-dentine junction region showing the inconspicuous and intimate fusion of enamel and dentine

Fig. 8. Polished, acid-etched mantle dentine. The von Korff fibrils show typical collagen cross banding







Figs. 9—12. (for legends see p. 281)

and the second was the question of the significance of the contribution that these fibres (as pulpal derivatives) made to the dentinal matrix as opposed to the (at that time not directly observable) contribution made by the odontoblasts. The whole question of von Korff fibres was thus inextricably bound up with early opinions on the role of the odontoblast in dentinogenesis, that is, whether the odontoblast simply formed the odontoblast process (JASSWOIN, 1924; VON KORFF, 1927) or whether it played a part in the elaboration of the dentine matrix (as, for example, the "precollagen" of VON EBNER (1906a, 1906b, 1909) and STUDNIČKA (1907)). On reflection, it is clear that these authors were all largely correct in their observations despite the apparent controversy. Their arguments appear to rest largely on differences in interpretation both of their own observations and of the work of others (VON KORFF's later isolation in South America [23, 45] and the uncertainty of silver staining techniques no doubt confused the situation). For example, VON EBNER (1909), who was perhaps VON KORFF's most vehement critic, found that in developing pig teeth the fibres of VON KORFF could be entirely lacking even from the earliest stages but that they had entirely disappeared once the dentine reached a thickness of 80 μ. At the same time, he commented upon the unreliability of silver staining and confirmed the presence of structures basically similar to those described by VON KORFF during later stages of dentine formation, but interpreted these as artefact "baulks of ground substance (matrix)". Despite this statement, VON EBNER (1909) did not entirely rule out the possibility of similarly-arranged fibril bundles being incorporated into the dentinal matrix. Further, in the case of irregular or secondary dentine formation, he referred to fibril bundles which were continuous from the pulp into the predentine and he drew an analogy with the Sharpey fibres of bone. It is clear that these authors were closer to agreement in their observations than their writings might otherwise indicate.

The present study has shown that the "fibres of von Korff" can be visualized with the electron microscope in prepared surfaces of mature dentine and that their location and conformation in the finished tissue can be related to the developing surfaces. Von Korff fibres are characteristic of the first-formed dentine and are not found in the bulk of the dentine formed later: thus the basis for WEIDENREICH's (1925) nomenclature is confirmed.

That neither von Korff fibres nor related structures could be identified in a previous study of predentinal surfaces using a replica technique for the transmission microscope [26] is in no way inconsistent with the present results. The

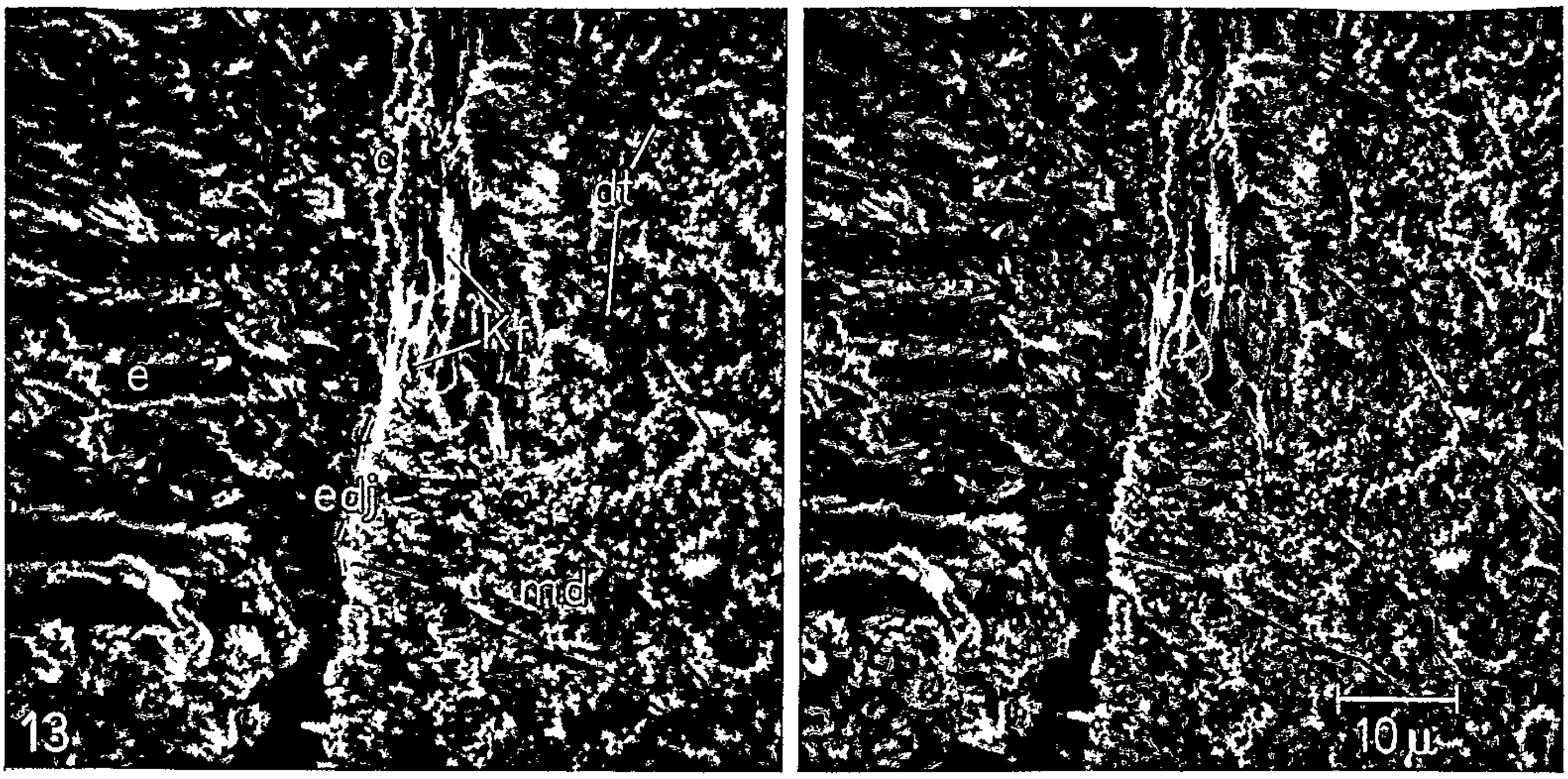
Figs. 9—12. Scanning electron micrographs

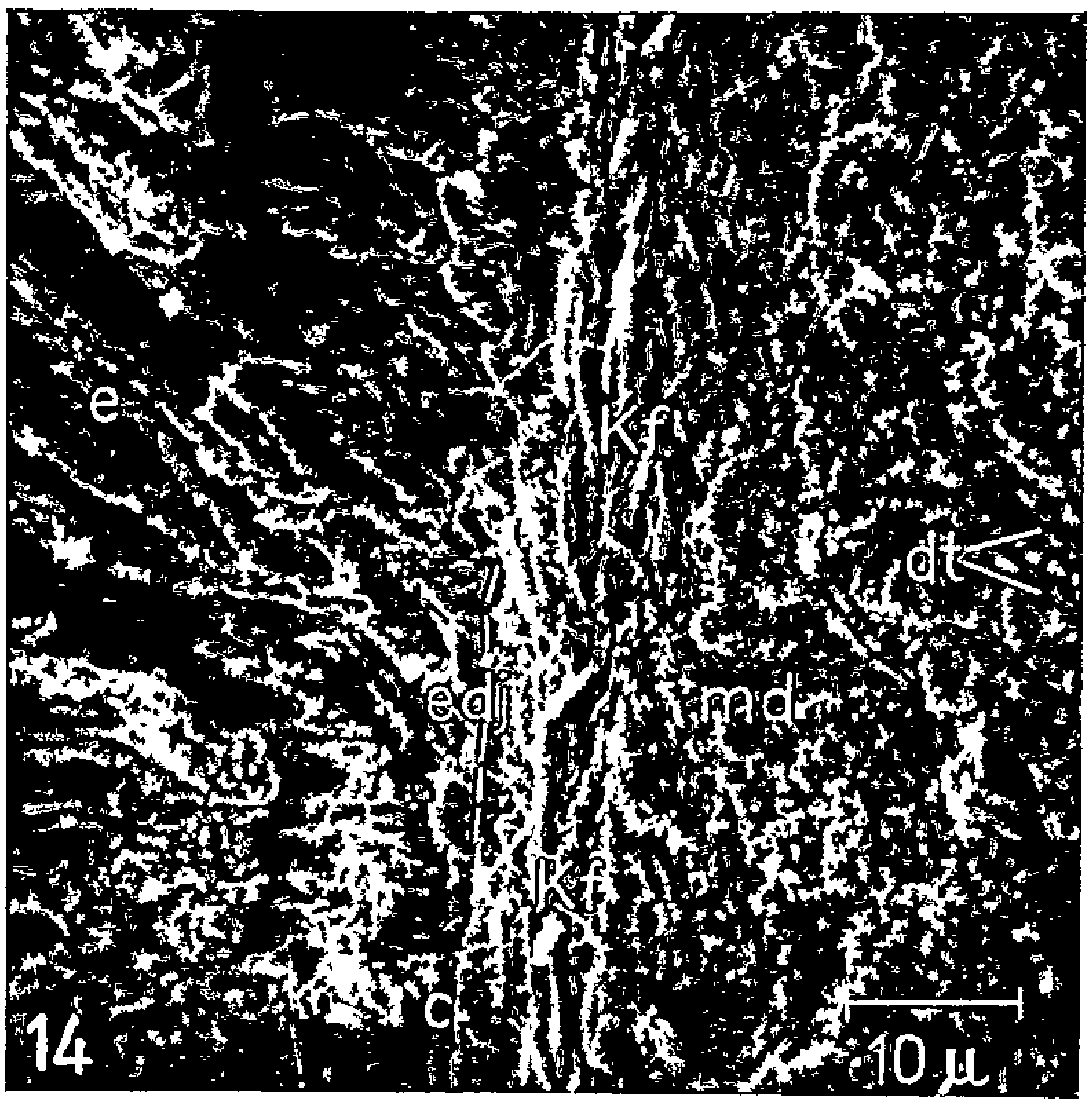
Fig. 9. Transverse fracture (*Sciurus sp.*) in region of enamel-dentine junction. Note the "rows" of von Korff bundles in the first-formed dentine

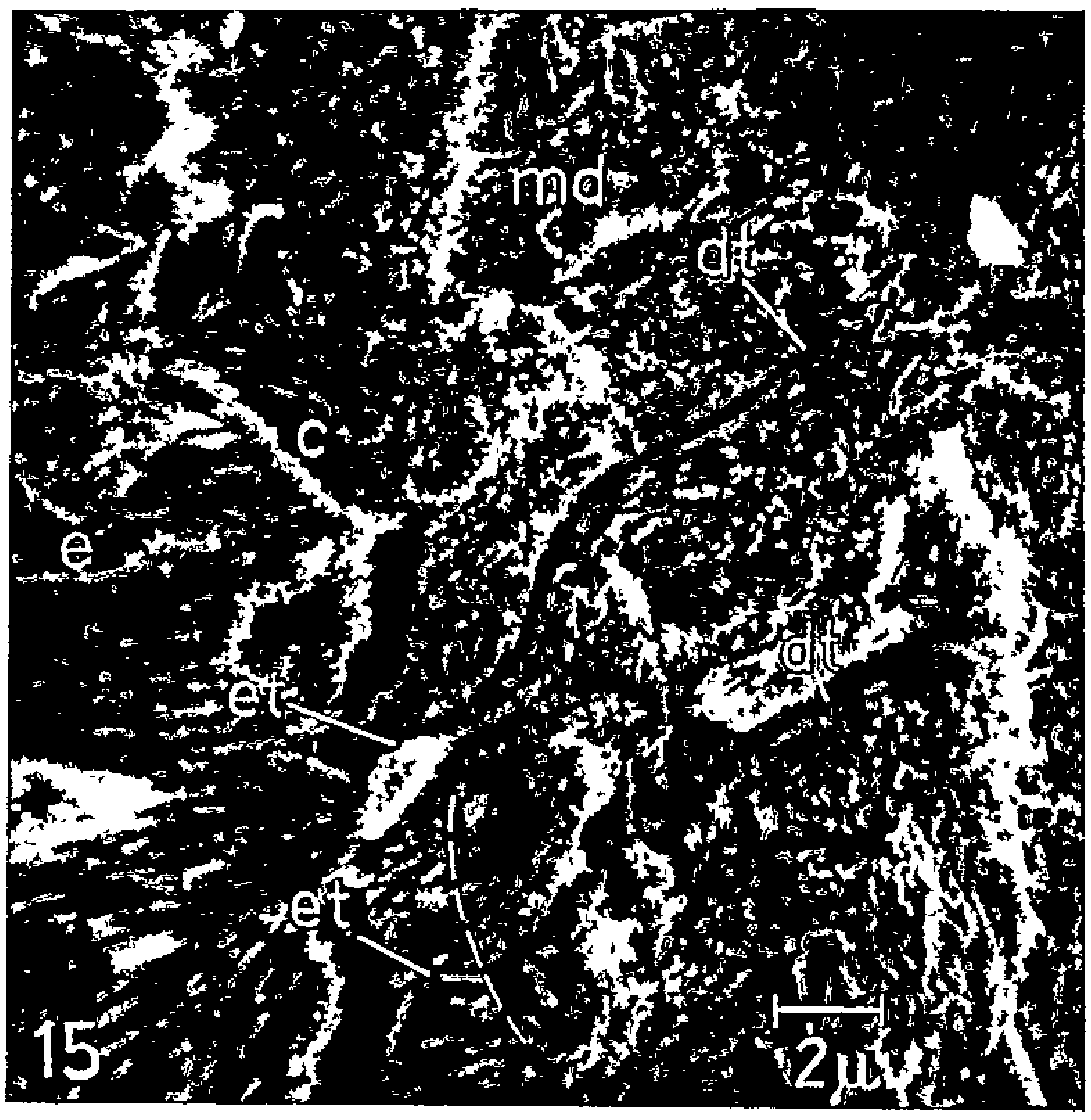
Fig. 10. Longitudinally sectioned and polished human tooth in enamel-dentine junction region. Note the von Korff bundle projecting into the enamel at a peak of the "scalloped" junction

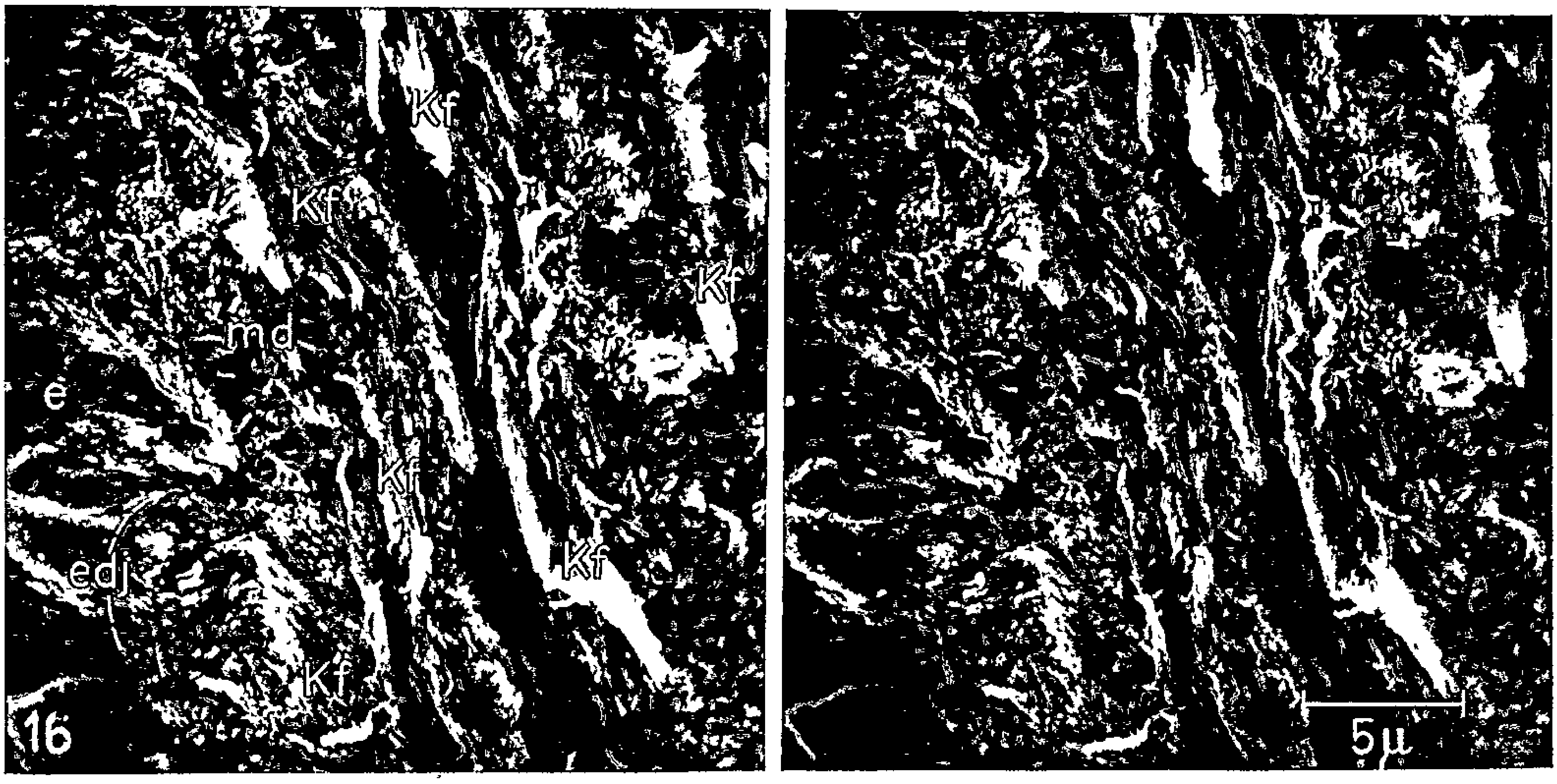
Fig. 11. Stereo-pair of longitudinally sectioned and polished human tooth in enamel-dentine junction region showing dentinal projection continuous across junction with enamel "defect" (see arrows)

Fig. 12. Stereo-pair of the very fibrillar, poorly mineralized vaso-dentine of *Mesoplodon layardi*









Figs. 13—16 (for legends see p. 283)

scanning electron microscope makes possible a very high sampling rate quite out of the range of the replica technique. Again, the recovery of the replica is probably limited to relatively smoother surfaces.

Von Korff bundles have not been found in the bulk of the circumpulpal dentine, yet there is no doubt that a profusion of structures with an at least superficial resemblance to the "corkscrew fibres of von Korff" can be seen between the odontoblasts throughout dentine formation in light microscopic material. In the present study, however, it has been possible to trace the life history of von Korff fibrils and related structures. We find that the fibrillar origin and the coherent pulpal part of the "true" von Korff fibres are completely embedded in the developing dentine as they appear at the developing front (Fig. 19), and we would place the thinner structures at the developing surface of the later-formed dentine (Fig. 20) in an entirely separate category, since they resolve into their individual fibrils at the developing front to contribute to the typical feltwork of the "intertubular dentine". In this way, the structures occurring between the odontoblasts during the later stages of dentine development ("inter-odontoblastic circumpulpal fibres") have both a separate origin and a different fate from that of the true von Korff fibres and should not be confused with them. Studnička (1907), Symons (1956) and Nylen and Scott (1958) traced "von Korff fibres" into forming circumpulpal dentine where the fibres paralleled the tubules and neither branched nor spread out. It is likely, however (and their illustrations support the view) that they studied the 'intermediate zone' so clearly demarcated in the dolphin dentine of both the dolphins that we have examined and existing to a lesser extent in other species (including man).

The 'intertubular dentine' in the circumpulpal dentine region consistently presents an afibrillar appearance on fracture. This is in direct contrast to the clear demarcation of fibrils in mantle dentine, which is known to be less mineralised than circumpulpal dentine [11, 27, 35, 42]. The relation that might therefore be suspected between the fibrillar nature of a dentine on fracture and the degree of mineralization has been borne out by comparative study. An extreme example is the almost totally fibrillar nature of the vaso-dentine portion of the tooth of the rare ziphioid whale (*Mesoplodon layardi*) which microradiographic examination shows to be very poorly mineralized (Fig. 12).

The observation that the enamel spindles appear to be the continuation of von Korff fibril bundles which cross the enamel-dentine junction both provides a possible explanation for their site of development and also suggests a mechanism

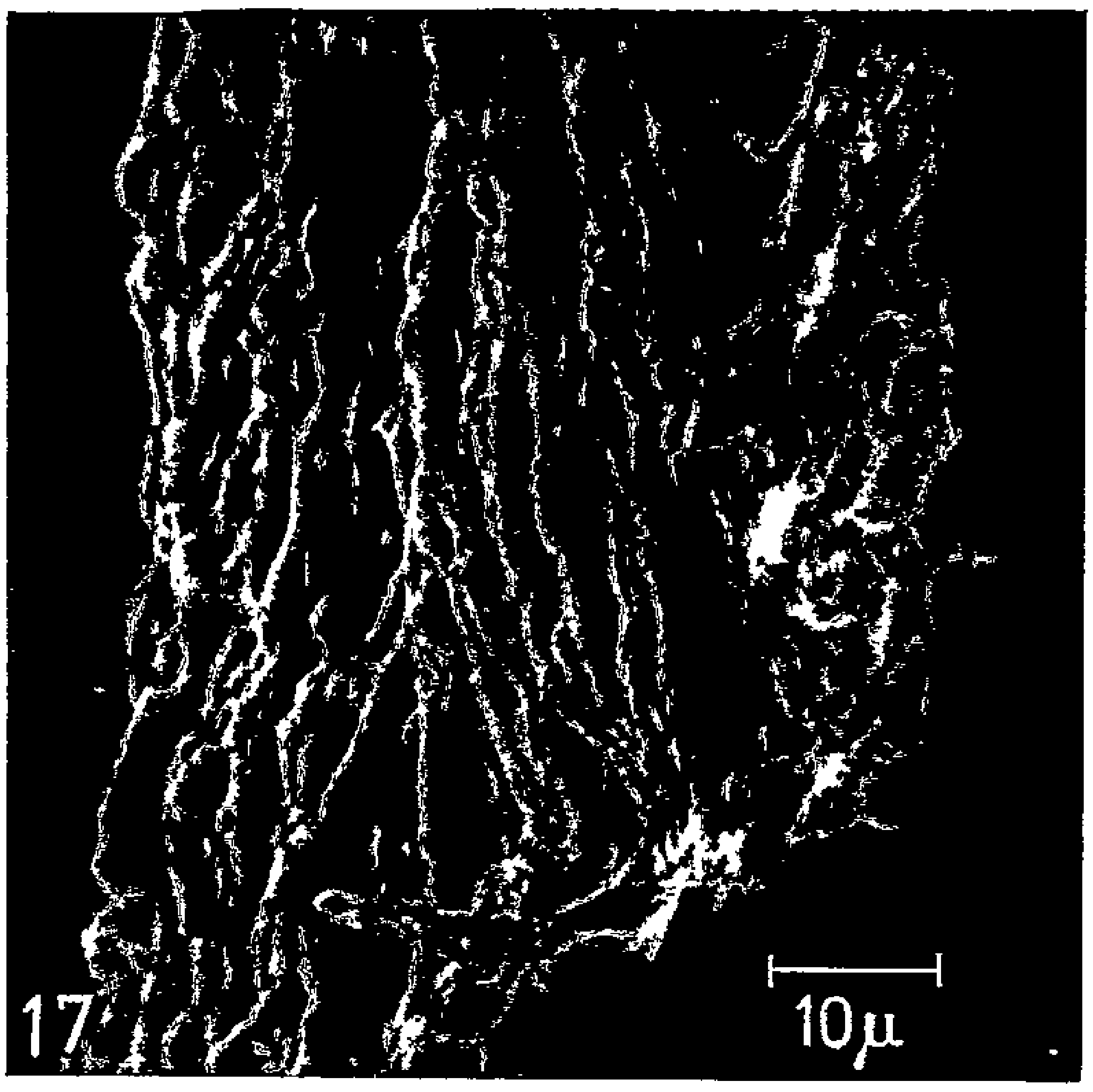
Figs. 13—16. Scanning electron micrographs of longitudinally fractured teeth in the region of the enamel-dentine junction

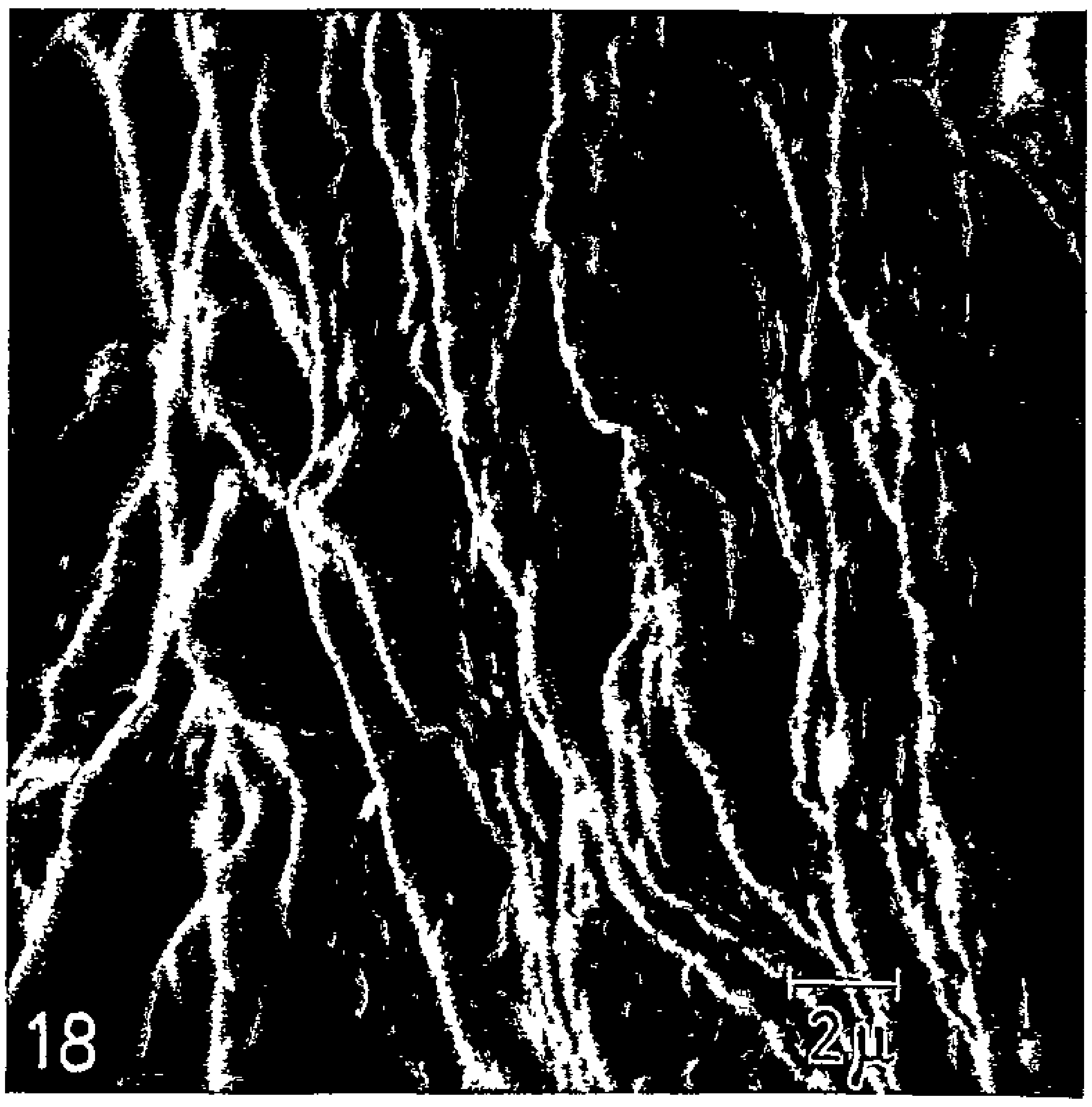
Fig. 13. Stereo-pair (*Canis familiaris*) demonstrating the "rows" of von Korff bundles and showing a layer of mantle dentine containing von Korff fibrils beneath a layer free of them

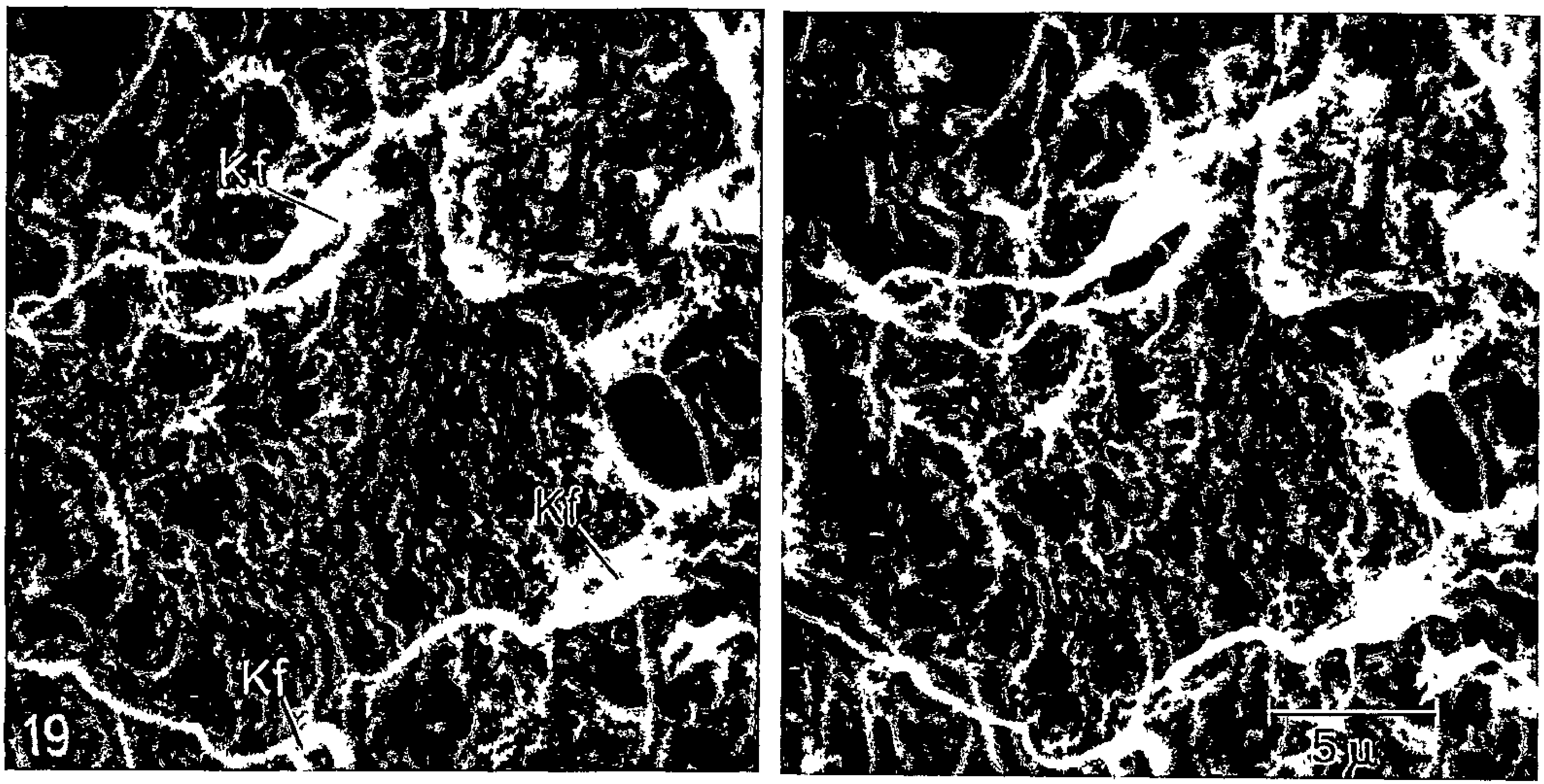
Fig. 14. From the same specimen as Fig. 13, showing von Korff fibrils oriented parallel to the enamel-dentine junction

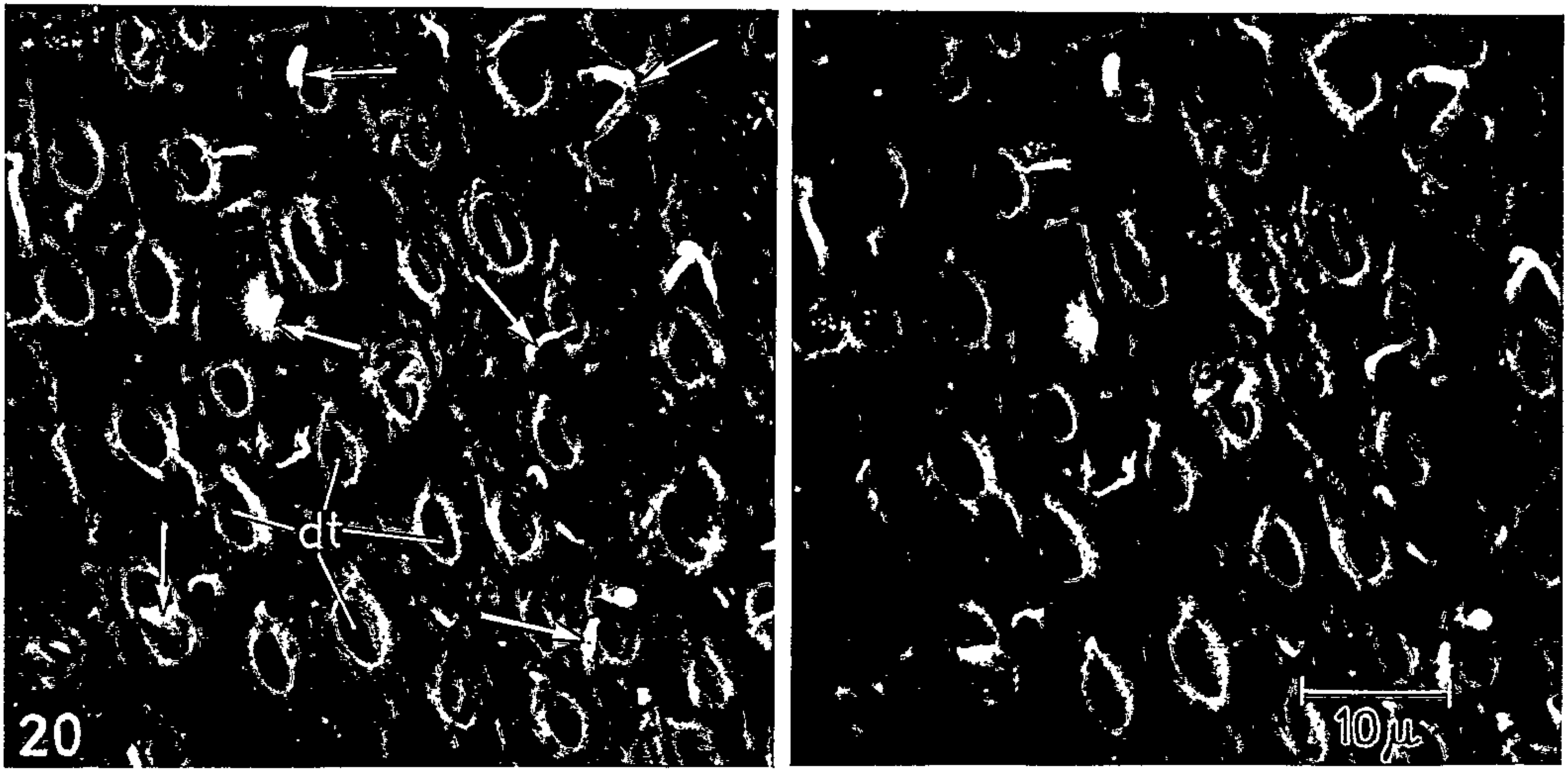
Fig. 15. *Canis familiaris* showing dentinal tubules (uninterrupted by von Korff bundles) crossing the enamel-dentine junction and continuous with an enamel "tubule" (at arrow)

Fig. 16. *Vombatus ursinus*, showing wide layer of mantle dentine containing large von Korff bundles oriented nearly parallel to the enamel-dentine junction









Figs. 17—20 (for legends see p. 285)

for their development. It is noteworthy that the spindles are most commonly found in cuspal regions where it is established that the von Korff fibril bundles stand perpendicular to the enamel-dentine junction throughout their length. Thus, this is a site at which these bundles would be suitably oriented to penetrate the basement membrane between the mesodermal papilla and the inner enamel epithelium. Such a penetration might be related to a rapid build-up of pressure due to proliferative activity on the part of the developing dental pulp. We would note also in this context that a von Korff fibril bundle would be expected to have a considerably greater mechanical stability or rigidity than the delicate fibrils of the feltwork constituting the bulk of the dentine.

Although we have not been able to delineate in enamel spindles individual collagen fibrils of the same diameter as von Korff fibrils, we are nevertheless inclined to the view that the spindles are of dentinal matrix origin. This question must be related to the sampling rate available in electron microscopy. In the present study, surfaces only were examined, whereas spindles are normally identified in thick ground sections. The chances of a fracture plane exposing a spindle where it crosses the enamel-dentine junction are remote; furthermore, the only method available to us which offers a total sampling rate for the fractured surface is the examination of the material in the scanning electron microscope. Although the resolution of the scanning microscope in our studies has generally been of the order of 300 Å, we have not been able to resolve the cross-striations of collagen fibrils in "enamel spindles". The final solution will probably lie in the chance identification in the scanning electron microscope of a von Korff fibril bundle crossing the junction, followed by the removal of a replica from such a precisely located area and its examination in the transmission electron microscope.

There seems little doubt that the absence of "enamel tubules" in the wombat (*Vombatus ursinus*) is related to the existence of a well developed mantle dentine layer. Thus, whereas the kangaroo (*Macropus sp.*) and opossum (*Metachirus nudicaudatus*) possess only a thin layer which might be called mantle dentine (and with the finer and less numerous von Korff bundles oriented nearly parallel to the dentinal tubules where these reached the enamel-dentine junction to become continuous with the "enamel tubules"), the wombat has large and numerous von Korff fibril bundles oriented parallel to the enamel-dentine junction. These bundles appear to preclude the close approach of the dentine tubules to this plane. We have frequently found in the placental mammals that areas of easily-demarcated von Korff fibrils alternate with areas lacking these but exhibiting enamel 'tubules' (Fig. 15) — enamel 'tubules' in placental mammals being more common than is generally realized [6].

Fig. 17—20. Scanning electron micrographs of developing dentinal surfaces

Figs. 17, 18. Developing edge of *Tursiops truncatus* mantle dentine showing "ridges" of von Korff bundles

Fig. 19. Stereo-pair of *Ovis aries* mantle dentine. Note the large von Korff fibril bundles inserted into the developing surface at one end and standing free at the other

Fig. 20. Stereo-pair of *Loxodonta africana* circumpulpal dentine showing the sparse, thin fibril bundles (see arrows) continuous with the developing surface. These are not von Korff fibres

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*Note Added in Proof.* Use of the terms "afibrillar" and "fibrillar" in this paper are meant to refer only to the pictures of the fractured dentine surface and are not meant to imply anything with respect to the presence or absence of the collagen in dentine, which should of course be present in all cases. We have circumstantial evidence that the texture of the fractured surface in dentine can be related, albeit crudely, to its degree of mineralisation.

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**The fine structure of peritubular dentine.** A. Boyde and K. S. Lester, *The London Hospital Medical College.*

The disposition and fine structural organisation of peritubular dentine was studied in the surfaces of fractured teeth of a number of mammalian species, both directly in a scanning electron microscope and by a single stage carbon replica technique for the transmission electron microscope. Stereo-pair micrographs were prepared in all cases.

In the walls of tubules which are oriented nearly perpendicular to the predentine surface, the peritubular dentine is either evenly distributed about the individual tubules, or where unevenly distributed, shows no overall preferred distribution of its bulk. Where the tubules slope markedly with respect to the predentine surface, however, the peritubular dentine forms predominantly on that side of the tubules inclined at an obtuse angle to the plane of the predentine surface, which happens to be the side towards the nearest part of the junction of the dentine with enamel or cement. The peritubular dentine is usually found very close to the mineralising front in the latter situation.

Fig. 5

Whether fractured or exposed as the wall of a tubule, the peritubular dentine of untreated teeth presents a surface which is remarkably smooth and featureless in comparison with the intertubular dentine. A characteristic structure, however, is revealed after extraction of the organic matrix with hot 1:2 diamino-ethane. A regular mosaic pattern of very closely-packed particles, approximately 250 Å in diameter, is found in all fracture planes as well as at the tubule wall itself (Fig. 5). It is therefore presumed that these particles, the apatite crystallites, are equidimensional, as opposed to the shape of bone and enamel crystallites which are longer in the c-axis than in the a-axis direction.

The size, shape and the close packing of the peritubular dentine crystallites deduced from this study are consistent with the high degree of mineralisation of the peritubular dentine and with the difficulty found in demonstrating its organic matrix.

# THE SURFACE MORPHOLOGY OF SOME CRYSTALLINE COMPONENTS OF DENTINE

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## INTRODUCTION.

Conventional sectioning techniques for electron microscopy must by their nature interfere with the integrity of the crystalline component of calcified tissues. The combination of a high resolution carbon replica technique and stereophotogrammetric analysis affords a unique opportunity of visualising the surface morphology of crystals relatively intact and undisturbed at their native site. This approach, broadened by the high sampling rate made possible with the use of the scanning electron microscope, has special relevance to the study of dentine where the species, morphology and organisation of the crystalline component of the peritubular dentine is uncertain and where relatively large remineralisation crystals are known to form in response to various physiological and pathological stimuli.

The aim of the present study was to investigate the fine structure, the distribution and the extent of the peritubular dentine, and to assess the occurrence and morphology of large, intratubular, " remineralisation " crystals.

## MATERIALS AND METHODS.

Prepared dentinal surfaces of teeth of the following species were studied: rat-tailed opossum *(Metachirus nudicaudatus* B61*)*; wombat *(Vombatus ursinus* B192*)*; kangaroo *(Macropus sp.* B224*)*; human (F192); rabbit *(Oryctolagus cuniculus* 165); jird (*Meriones sp.* J165); coypu *(Myocastor coypus* J330); Geoffroy's dolphin *(Inia geoffrensis* K2); sperm whale *(Physeter catodon* K19); narwhal *(Monodon monoceros* K22); bottle-nosed dolphin *(Tursiops truncatus* K47); dog *(Canis familiaris* L8); elephant *(Loxodonta africana* O1); manatee *(Trichecus manatus* Q2); horse (*Equus caballus* R2); pig *(Sus scrofa* S2); calf *(Bos taurus* S81); sheep *(Ovis aries* S188) — order of species and Latin names after Morris (1965).

The specimens examined may be divided for convenience into four main groups.

1. " Clinically sound " teeth were fractured by closing the jaws of a small vice in which they were held and the exposed dentinal surfaces were replicated in carbon using a single-stage technique described in detail by Boyde (1967).

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2. Some of the above specimens were treated prior to replication as follows :—
   (i) subjected to 1:2 diamino-ethane extraction either before or after fracture;
   (ii) subjected to 1:2 diamino-ethane extraction and etched (N/10 HCl for 5 secs).

3. Dolphin (*Tursiops truncatus)* teeth which had undergone extensive attrition (and in which the pulp cavity had become occluded as is normal in this species) and human permanent teeth with gross dentinal caries were fractured and the resulting dentinal surfaces were replicated and examined.

4. Developing dentinal surfaces of deciduous and permanent human teeth and resorbing surfaces of deciduous human teeth (some examples of all these were subjected to 1:2 diamino-ethane extraction) were examined both via the replica technique for the transmission electron microscope and directly in the scanning electron microscope. For the latter procedure, the specimens were given a conducting coat *in vacuo* of ca. 200Å carbon and ca. 300Å gold.

Stereo-pair electron micrographs were prepared in all cases. For the transmission electron microscope (Siemens *Elmiskop I* operating at 60 kV) the tilt angle was 8° 40′ and for the scanning electron microscope (Cambridge Instrument Company *Stereoscan* operating at 10 kV) the tilt angle was 10°. Stereophotogrammetric techniques were applied in both cases so that the third dimension might be appreciated and examined.

## RESULTS.

Those specimens subjected to 1:2 diamino-ethane extraction prior to examination consistently presented a " cleaner " appearance and revealed a greater degree of detail in the areas of interest.

1. *Fine structure of peritubular dentine.*

On relatively low magnification examination (ca. 15,000X), the peritubular dentine of untreated teeth has been described as " relatively homogeneous " when compared to the adjacent intertubular dentine at a fractured surface (Boyde and Lester, 1967a). However, after extraction of the organic matrix by prolonged refluxing with hot 1:2 diamino-ethane, and on more detailed examination (ca. 60,000X), a regular mosaic pattern of very closely packed particles was found in all fracture planes of the peritubular dentine as well as in the tubule wall itself (Figs. 1A, 1B, 2A, 2B, 3A, 3C). The individual particles were found to be roughly equidiametrical (ca. 250Å), that is to say they were roughly spherical or polygonal. The close-packing of the particles by its nature permitted no overall preferred orientation, although there was a tendency in some instances towards an aligning or linear coalescence of particles (Figs. 1B, 3A). Nor was this repetitive pattern confined to the peritubular dentine: indeed, the margins of the peritubular dentine were difficult to define at this magnification and the mosaic particle pattern of the peritubular dentine was found both to merge gradually with the more " fibril-like " pattern of the intertubular dentine and to be identifiable (in small areas) within it (Fig. 3A).

It was found subsequently that this pattern could also be observed in the peritubular dentine of untreated teeth (although not as clearly) provided the odontoblastic cell membrane did not remain in place over the tubule bed (wall), or, in the case of a fractured peritubular dentine surface, was not displaced and superimposed over it as a result of the fracturing process (see for example, Figs. 5D, 6A).

No evidence of collagen was found in the regions identified as "peritubular dentine." This was so despite the fact that the dentine had been pretreated in a variety of ways which might reasonably be expected to reveal the presence of collagen were it a component of the peritubular dentine. On the other hand, unmineralised collagen fibrils were commonly found (by the methods used in this study) lying within the tubules, that is between the peritubular dentine of the tubule wall and the odontoblast process (in the "periodontoblastic space" of FRANK, 1966).

### 2. *Distribution of peritubular dentine.*

The distribution of peritubular dentine bulk about patent tubules was found to be characteristic and predictable. It could be related to the angle made by the dentinal tubules with the developing dentinal (pulpal) surface. Thus, in the walls of tubules which were oriented nearly perpendicular to the predentine surface (as in human dentine), the peritubular dentine was either evenly distributed about the individual tubules, or, where unevenly distributed, showed no overall preferred distribution of its bulk. Where the tubules sloped markedly with respect to the predentine surface, however, the peritubular dentine formed predominantly on that side of the tubules inclined at an obtuse angle to the plane of the predentine surface (which happens to be the side towards the nearest part of the junction of the dentine with the enamel or cement). The latter situation is typified by artiodactyl dentine (Figs. 7A, 7B, 7C) in which the peritubular dentine is typically found very close to the mineralising front (BOYDE, 1968, in preparation).

### 3. *Mineralised tubule inclusions.*

Many of the tubules exposed in the fractured dentine of " clinically sound " human teeth (Figs. 2A, 2B, 2C, 3A, 3B, 3C, 3D, 4A, 4B, 4C), frankly carious human teeth (Figs. 5A, 5B, 5C), and occluded dolphin teeth (Fig. 4D) contained inclusions. These inclusions obviously encroached upon the lumen of the tubules, although in an irregular fashion and to varying extents in different tubules. The inclusions were broadly divisible into two main types.

One type of inclusion revealed the repetitive pattern of the peritubular dentine over its surface (the pattern was often even more clearly demarcated (Fig. 3A) than over the natural wall or fractured surface of the peritubular dentine of unaffected tubules). These inclusions were basically globular in shape (Figs. 2A, 2B, 2C), or, they were irregularly cylindrical if individual clusters had become confluent (Figs. 3A, 3B). In some instances, the tubules were completely occluded by this material (Figs. 3C, 3D) whilst adjacent tubules possessed a peritubular dentine lining of " normal " dimensions.

The other main type of inclusion was in no way similar to the peritubular dentine, consisting instead of relatively large, easily definable crystals. These crystals were divisible into three further groups with regard to their morphology and occurrence, viz:—

a. The crystals in " clinically sound " human dentine were large, tabular, and tended to show numerous growth faces on the individual elements (Figs. 4A, 4B and 4C).

b. The crystals characteristic of " sound " dolphin teeth (Fig. 4D) were rhombohedric with more regular but smaller faces.

c. Small rod-shaped crystals (Fig. 5D) occurred commonly in carious human permanent dentine. The affected tubules in carious human dentine showed all stages of progression from small isolated dendritic clusters of crystals to an almost complete occlusion of the tubule lumen by the crystals (cf. Figs. 5A, 5B, 5C). The three types (a, b and c) occurred in various combinations in carious dentine although it was usual for one crystal form to predominate.

It should be noted here that the various crystalline types enumerated above were apparently confluent with one another where they were present within the same tubule, that is, there was no clearly defined line of junction between the crystalline pattern of peritubular dentine and that of an inclusion formed of larger crystals. Further, there was a high degree of variability in the formation of inclusion material, such that any one tubule bore little or no relation to its immediate neighbour with regard to either the presence or amount of inclusion material.

4. *Remineralisation crystals at resorbing surfaces and at the mineralising front.*

Accumulations of large rod-shaped crystals were found on the projecting borders between adjacent Howship's lacunae in resorbing surfaces of deciduous dentine pretreated with 1:2 diamino-ethane. The crystals were characteristically stacked in small groups (Fig. 6B and Boyde and Lester, 1967 b).

A definite crystal-type was found to occur on the mineralising front of dentine (that is to say the surface exposed by extracting the organic material of the predentine with 1:2 diamino-ethane) when the specimen was dried down after washing out the organic solvent with water. These crystals were fusiform in shape and occasionally of considerable size (Fig. 6C). This type of crystal did not form when absolute ethanol was substituted for water in the washing procedure.

## DISCUSSION.

It has to be assumed that the (approximately 250Å equidiametrical) particles described in the peritubular dentine (we find the name " intratubular " more appropriate) truly represent its mineral component, because they remain in this form after extraction of the organic component. The close packing of the particles of the mineral component of the peritubular dentine is consistent with its high degree of mineralisation when compared to the intertubular dentine (Miller, 1954; Boyde, Switsur and Fearnhead, 1961) and with the difficulty encountered in isolating and identifying its organic matrix (Symons, 1961). However, the present results regarding the morphology of the mineral particles are obviously at considerable variance with earlier reports based on studies of ultra-thin sections with the transmission electron microscope (Frank, 1965, 1966; Höhling, Katterbach and Vogel, 1965; Höhling, 1966). These authors have described elongated, needle-shaped particles (identified as apatite by Höhling and Pfefferkorn, 1964) with their long axes approximately parallel to that of the dentinal tubules.

Before discussing the possible origin of the concept of the needle-like form of apatitic crystals in peritubular dentine, evidence against there being any significant artefact associated with the preparation of our material must be stated. Firstly, it is possible that the crystalline morphology of the peritubular dentine mineral may have been altered by contact with water, since anhydrous 1:2 diamino-ethane has not been used in these studies. However, after our finding that artefact " recrystallisation " crystals resulted from washing with water (see Fig. 6C), the procedure was adopted of washing (after 1:2 diamino-ethane extraction) only in absolute ethanol—the peritubular dentine mineral showed no discernible difference after this procedure. In any event, the equidiametrical, close-packed arrangement of the mineral particles was present (although not so clearly marked) in fresh, fractured, untreated peritubular dentine. It is difficult to conceive of radical changes in shape to a more poorly crystalline form in the peritubular dentine since there would be little space made available between the mineral particles by the removal of the organic matrix: the same is not true of the intertubular dentine, where the removal of the matrix might be expected to leave a large proportion of spaces available for ionic diffusion, and hence for the reorganisation of the mineral component. Secondly, it might be argued that the appearance of small hexagonally-packed areas may represent self-structure in the replica film itself. This possibility may be totally excluded on the grounds that a similar structure is not found in carbon film from any other site, although high resolution micrographs have been made of carbon films released from a wide variety of different types of crystal surfaces including large areas of single crystals (for example, Fig. 6B). Thirdly, it might be argued that the fracturing process does not follow the organisation of the mineral component. However, it is found that the organisation of the mineral component is always reflected in the fractured surface. Further to this point, evidence has been gained of the shape of the mineral component by direct examination with the scanning electron microscope of the mineralising front itself (that is, the forming peritubular dentine in the wall of the tubule) which is in no way altered or touched during the fracturing process. We are, therefore, left with the conclusion that these observations on peritubular dentine fine structure bear some relation to reality and with the unenviable task of trying to reconcile them with the observations of others.

It is almost certain that the concept of the apatitic nature of peritubular dentine mineral has arisen by analogy with the other vertebrate mineralised tissues. However, POSNER and co-workers have recently been paying considerable attention to a poorly crystalline component of " bone mineral " which they call " amorphous " (for example, TERMINE and POSNER, 1967). The synthetic material which they study and which they hold to have a close resemblance to the " amorphous " component of bone mineral consists of approximately spherical particles some 200Å in diameter (POSNER, 1967, personal communication) and so suggest, at least superficially, some resemblance to the mineral component of the peritubular dentine.

FRANK (1959) claimed to have identified the peritubular dentine mineral as apatitic on the basis of selected area diffraction studies. As far as we are aware, such a positive localisation of the area being diffracted is not possible with existing commercially available electron microscopes and the maximal resolution possible is of the order of 5μ which would be inadequate for the purpose of studying peritubular dentine zones approximately 1μ wide. HÖHLING and PFEFFERKORN (1964) inferred the existence of apatite crystals in the peritubular dentine from dark-field electron micrographs although their micrographs show no elongated bright-spots such as might be expected if elongated crystals were present. Further, there are no really convincing electron micrographs published in the literature which show a preferred orientation of needle-like crystals in the peritubular dentine *except* in the peripheral part of the peritubular dentine at its junction with the intertubular dentine. These particular crystals, however, could be associated with the collagen fibrils of the intertubular dentine (see for example, HÖHLING, 1966; HÖHLING, KATTERBACH and VOGEL, 1965; FRANK, 1965, 1966).

Polarising light microscope studies have demonstrated the birefringence of peritubular dentine and an orientation of the ordered elements with respect to the dentine tubules (KEIL, 1934; SCHMIDT and KEIL, 1958). This orientation has been ascribed to the mineral element. In our opinion, however, the polarising light microscope image (e.g. KEIL, 1939, Figs. 11, 15 and 20, and 1966, Fig. 44) could be explained either on the basis of the preferred orientation of the collagen fibres about or within the tubules (or the crystallites associated with these collagen fibres), or, on the basis of the difference in mass distribution of the mineral (and the high mineral content of the peritubular dentine).

The large remineralisation crystals that we have figured in dentine fall clearly into two major categories for the purpose of tentative identification.

(i) The first group comprises the large tabular crystals (Figs. 4A, 4B, 4C) and the rhombohedric crystals which (Fig. 4D) resemble those described in enamel cracks by KATTERBACH, WANNENMACHER, HÖHLING and VOGEL (1965), and HELMCKE, NEBAUER and RAU (1966), and in dentine caries by HELMCKE (1960), HÖHLING (1960), HERTING (1966) and others. These crystals are similar to those first described by HELMCKE (1955) in extraction replicas and apparently regarded by him at that time, and until some time later (see for example, HELMCKE, 1960), as calcium carbonate (calcite). Following VAHL, HÖHLING and FRANK (1964) and NEWESELY (1965) we may tentatively regard these as β-tricalcium phosphate (Whitlockite).

(ii) The short rod-like crystals, of the order of five times long as they are wide and which often form coherent or dendritic clusters, constitute the second group. These we have found to be consistently associated with gross dentine caries and (a much larger variety) at the boundaries of Howship's lacunae in resorbing hard tissues (BOYDE and LESTER, 1967 b). Again, following NEWESELY (1965), we might tentatively identify these with octacalcium phosphate.

The crystal type which we tentatively identify as β-tricalcium phosphate appears to exist in what might be regarded as physiological conditions resulting from very slow remobilisation-remineralisation processes which might be considered as an age change (consider, for example, their demonstration in enamel cracks and occluded dolphin dentine). The other type, identified as octacalcium phosphate, appears to form in conditions which we presume to be associated with a rapid remobilisation of mineral under conditions of low pH as might exist in both resorption and caries.

The present results indicate that collagen is not incorporated into the peritubular dentine proper. Collagen occurs in two situations relative to the tubule wall: (1) as the innermost layer of fibres of the intertubular dentine which are for the most part oriented circumferentially about the tubule, are visible at the developing predentinal part (LESTER and BOYDE, 1967), and become mineralised with the remainder of the intertubular dentine; and (2) as unmineralised fibres which are for the most part longitudinally oriented and lie inside the mineralised tubule wall and which may exist whether or not any bulk of peritubular dentine is present (BOYDE and LESTER, 1967 a). The presence of these latter fibres is possibly not widely recognised, although their presence has been indicated by HELWIG and MENKE (1949), MENKE (1950), HELMCKE (1953) and FRANK (1965).

It is necessary to consider this point a little further because it seems from the literature that there are considerable differences of opinion as to whether collagen forms a part of the peritubular dentine. We would disagree with FRANK's (1965) implication that the presence of collagen fibres in the "periodontoblastic space" indicates that they would eventually be incorporated in the peritubular dentine. Indeed, a preliminary report of our own studies (later published in full as LESTER and BOYDE, 1967) was misinterpreted and held to confirm the presence of collagen in peritubular dentine (JOHNSON and POOLE, 1967). In fact, although our studies show that unmineralised collagen may be found in the "periodontoblastic space," they provide no indication of when collagen appears or develops in this site, and no evidence of its incorporation in the peritubular dentine. Collagen may develop within the tubules after peritubular dentine formation, or it may conceivably have been formed before the peritubular dentine and have been pushed ahead of an advancing front of forming peritubular dentine. In the former case, we have to deduce that the odontoblast processes contain all the necessary mechanisms for delivering collagen at a point remote from the odontoblast cell body (cf. REITH, this symposium) and in the latter, that this collagen is in some way resistant to mineralisation.

If we accept that collagen fibres situated in the periodontoblastic space are not incorporated into, or encapsulated by, the peritubular dentine, then we must envisage the problem of explaining the apparent disappearance of this collagen in the case of occluded dentinal tubules. We find no trace of the odontoblast process in the dentinal tubules which have been completely filled with peritubular dentine. Is it possible that the same mechanisms which remove the odontoblast process also remove the intratubular collagen, or, is the real answer to the apparent disappearance of the collagen that our techniques are unable to detect it once it has become enveloped in peritubular dentine? The latter possibility cannot be completely excluded, although we are inclined to the view that the collagen could be demonstrated, if it were there. The questions must, therefore, be raised as to whether the intratubular collagen is removed in some way or whether tubules which contain collagen ever become completely occluded by mineralised material.

## SUMMARY.

A high resolution carbon replica method for the transmission electron microscope and stereophotogrammetric analysis have been used, together with the scanning electron microscope, to examine the fine structure and distribution of the peritubular dentine and the morphology and distribution of dentine "remineralisation" crystals in the teeth of a variety of mammalian species.

A characteristic fine structure was revealed in the peritubular dentine after it had been subjected to refluxing with hot 1:2 diamino-ethane. A regular mosaic pattern of very closely packed particles ca. 250Å in diameter was found in all fracture planes of the peritubular dentine as well as in the tubule wall itself. The particles were identified with the mineral phase of the peritubular dentine.

The distribution of the bulk of the peritubular dentine about any one tubule could be related to the angle made by the tubule with the developing front of the dentine. The peritubular dentine was found to occur very close to the mineralising front in certain species.

In "clinically sound" teeth, irregular inclusions showing the peritubular dentine pattern were often found to intrude upon the tubule lumen to varying extents and occasionally to occlude it completely. Large, rhombohedric, remineralisation crystals sometimes accompanied such inclusions in "clinically sound" teeth. A characteristic rod-shaped crystal type was found intratubularly in grossly carious

human teeth and also (although of much larger dimension) in resorbing dentinal surfaces. The rhombohedric and the rod-shaped crystal types have been tentatively identified with β-tricalcium phosphate and octacalcium phosphate respectively.

*Acknowledgments.* The Cambridge Instrument Company "*Stereoscan*" (University College London) was provided by the Science Research Council. The Siemens "*Elmiskop I*" (The London Hospital Medical College) was on loan from the Wellcome Trust. We would also like to thank Mr W. J. Backhouse, Mr C. Boyde, Dr F. C. Fraser and Professor R. J. Harrison who helped by providing us with specimens.

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### Abbreviations

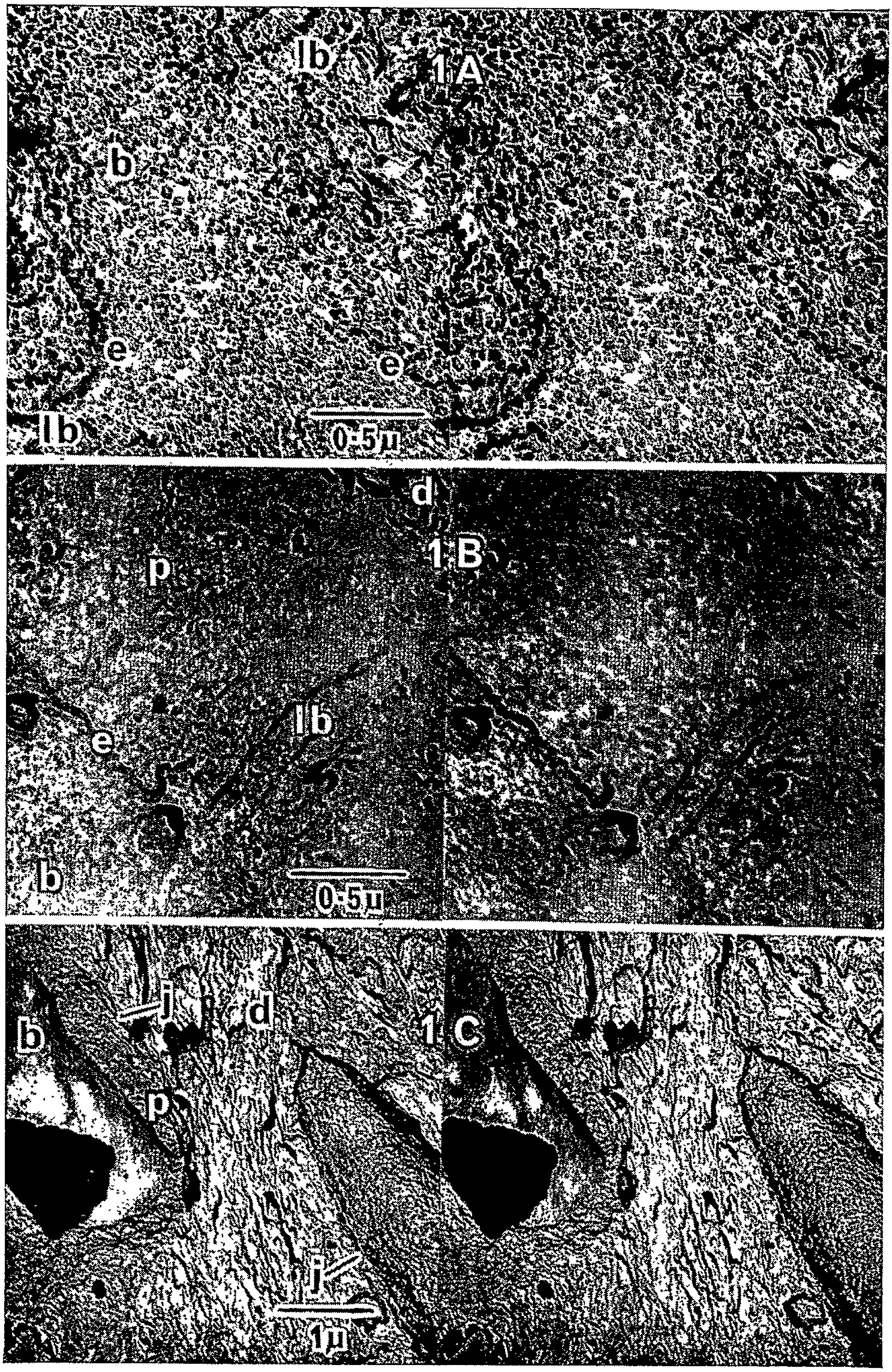
b—tubule bed (inner aspect of dentinal tubule wall).
c—" remineralisation " crystal.
d—fractured surface of intertubular dentine.
e—fractured edge of tubule wall.
l—lateral branch of dentinal tubule.
o—dentinal tubule opening.
p—fractured surface of peritubular dentine.
po—inclusion showing " peritubular dentine " pattern.

Stereo-pair transmission electron micrographs of carbon replicas of fractured dentine.

Fig. 1A: Longitudinal fracture through a tubule showing the equidiametrical particles (identified with the predominant mineral phase) of the peritubular dentine both at the free tubule surface of the peritubular dentine in the centre of the field and in the fractured walls to each side (Pig deciduous, anorganic).

Fig. 1B: Longitudinal fracture showing the close-packed, equidiametrical mineral particles of the peritubular dentine in the free tubular surface (of both major tubule and lateral branch) and the wide fractured peritubular dentine wall (Pig deciduous, anorganic).

Fig. 1C: This image demonstrates how it is possible to distinguish between the orthoscopic and the pseudoscopic appearance of the replica, that is to say, " which way round the specimen was " prior to replication. The tubule on the left is concave and its lumen is exposed. The tubule on the right is convex, the fracture plane having included the junction (at j) between the peritubular dentine and the intertubular dentine (but see Fig. 7F). (Manatee).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1 B
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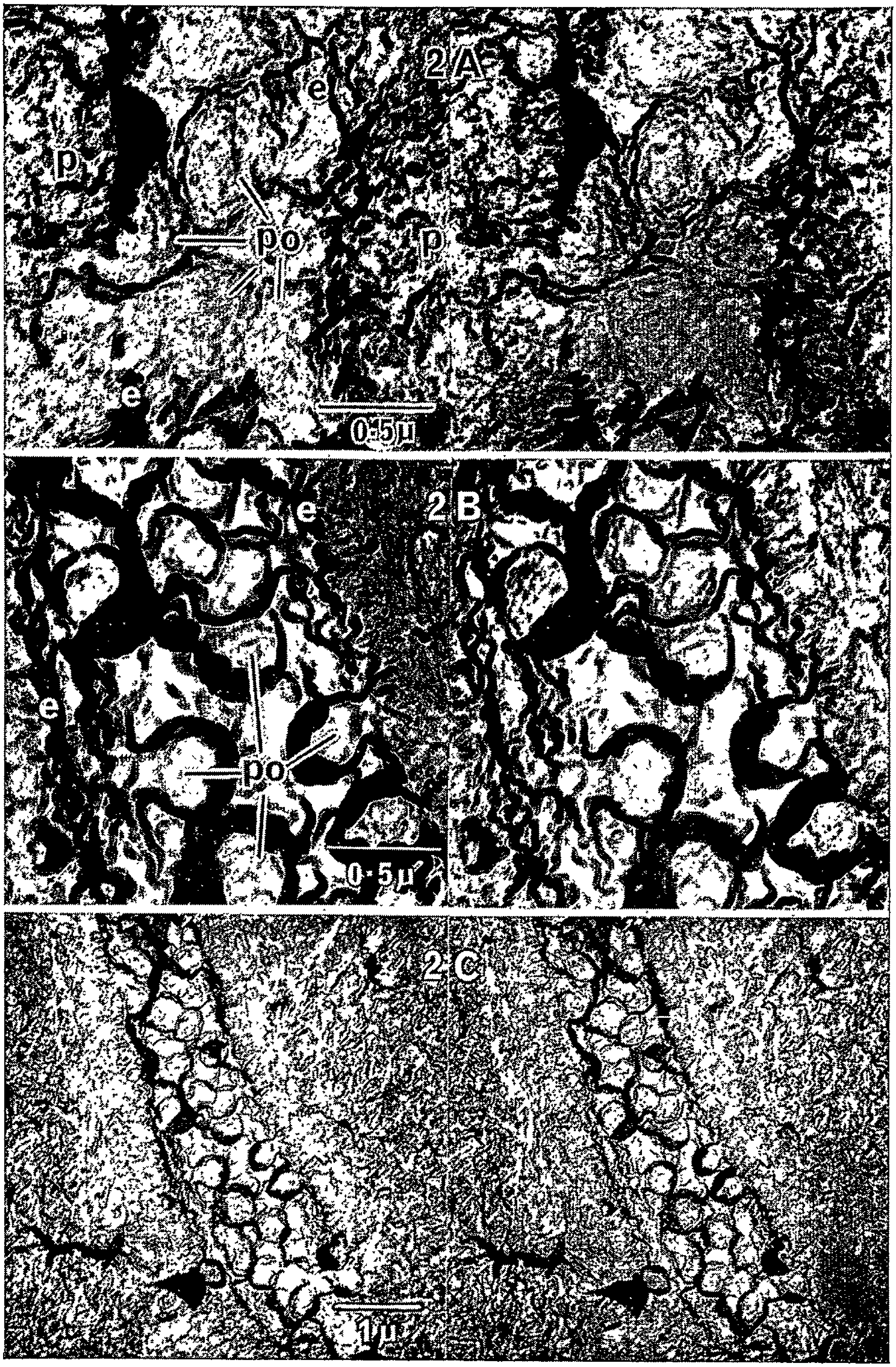
1 C
p
j
1µ

Stereo-pair transmission electron micrographs of carbon replica of longitudinally-fractured, human, cuspal, permanent dentine.

Fig. 2A: This demonstrates what we believe may represent the first stage in the occlusion of tubules with material of the same nature as the "peritubular dentine." Irregular outgrowths project from (what would otherwise be the smooth lining of) the tubule wall: these show the same close-packed equidiametrical elements as the peritubular dentine.

Fig. 2B: A more prolific development of similar spherical inclusions projecting into the tubule lumen. It is again clear that these inclusions have a similar microstructure to the peritubular dentine of the tubule wall itself.

Fig. 2C: A lower magnification of the same field as Fig. 2B.

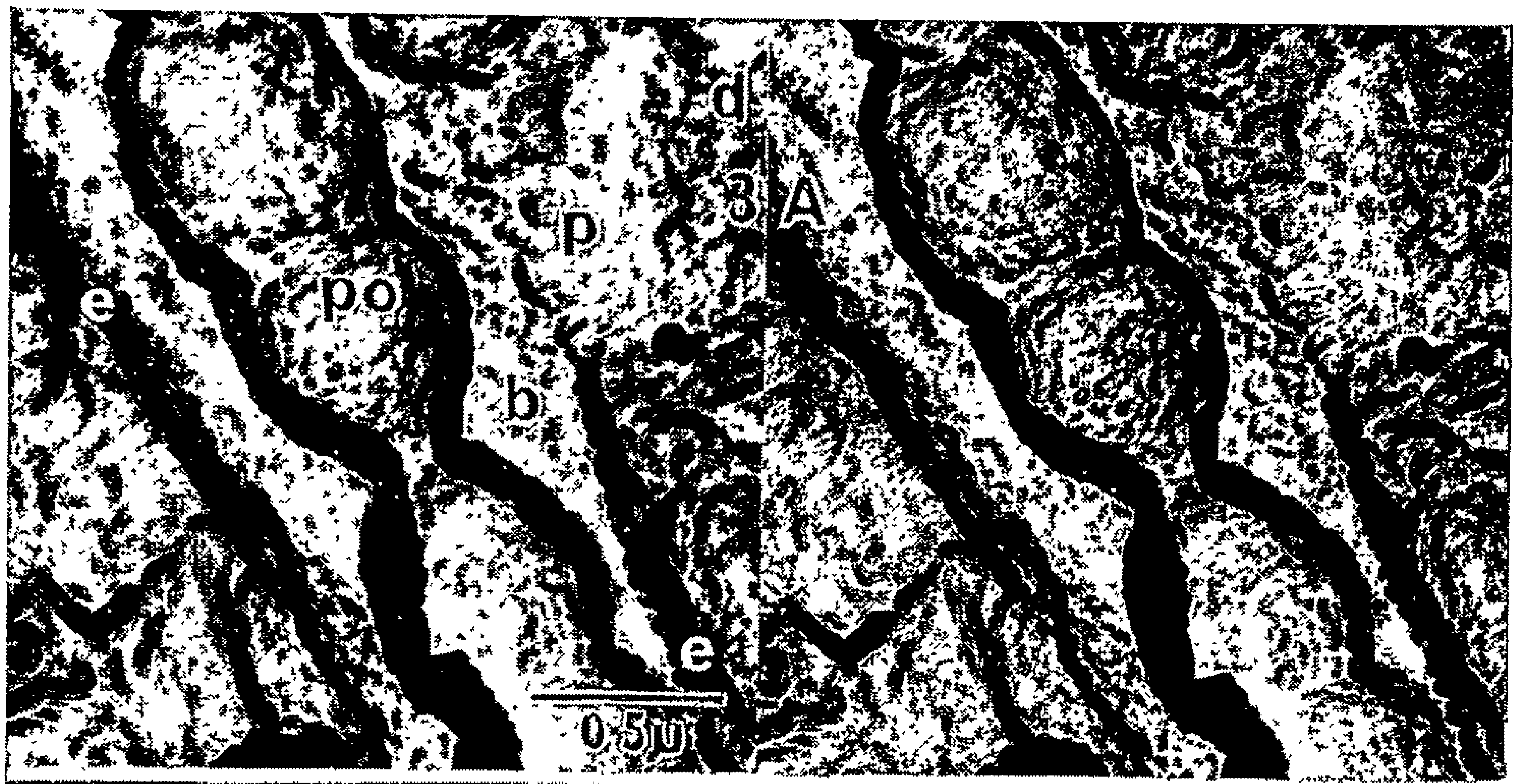


2A
e
p
po
p
e
0·5 μ
2B
e
e
po
0·5 μ
2C
1 μ

Transmission electron micrographs (Figs. 3A and 3D are stereo-pairs) of carbon replica of fractured, human, cuspal, permanent dentine.

Figs. 3A and 3B: Longitudinal fracture showing a connected row of rounded inclusions along the wall of a tubule. Fig. 3B is a lower magnification of the field in Fig. 3A.

Figs. 3C and 3D: Oblique transverse fracture of a tubule completely occluded with material characteristic of the peritubular dentine. Fig. 3C is a higher magnification of the field in Fig. 3D and shows the fine structure of the occlusion material.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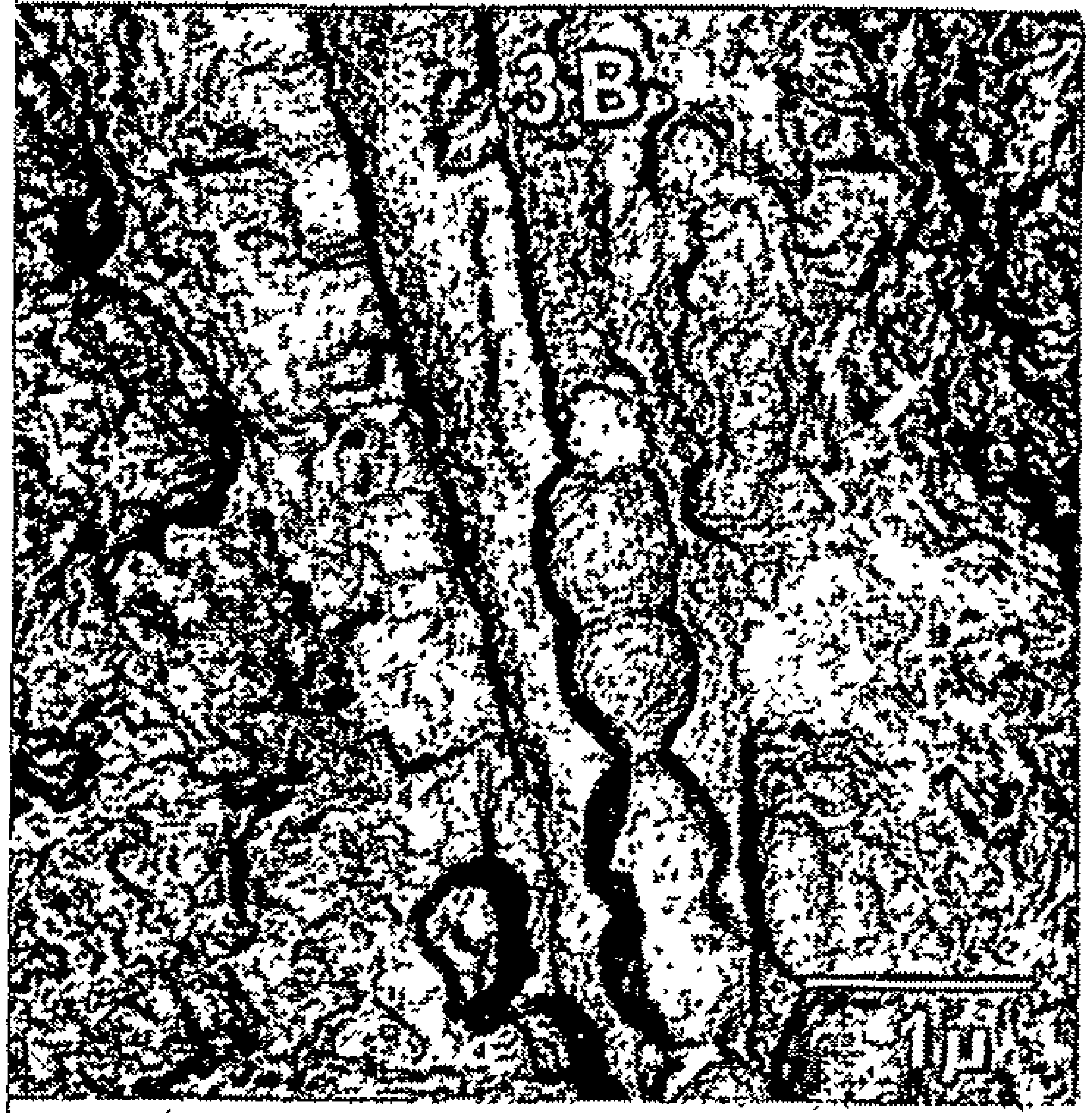
3B
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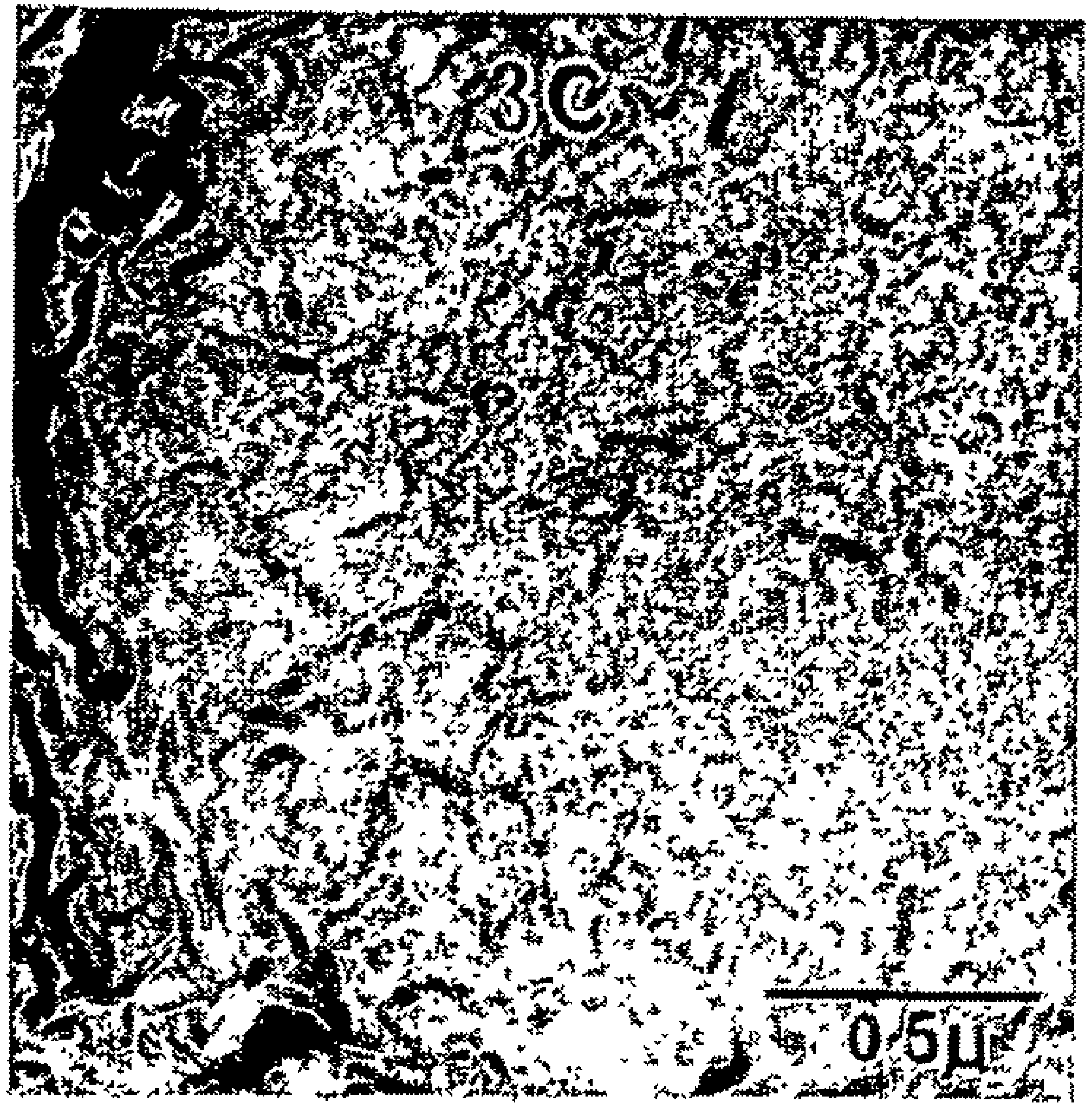
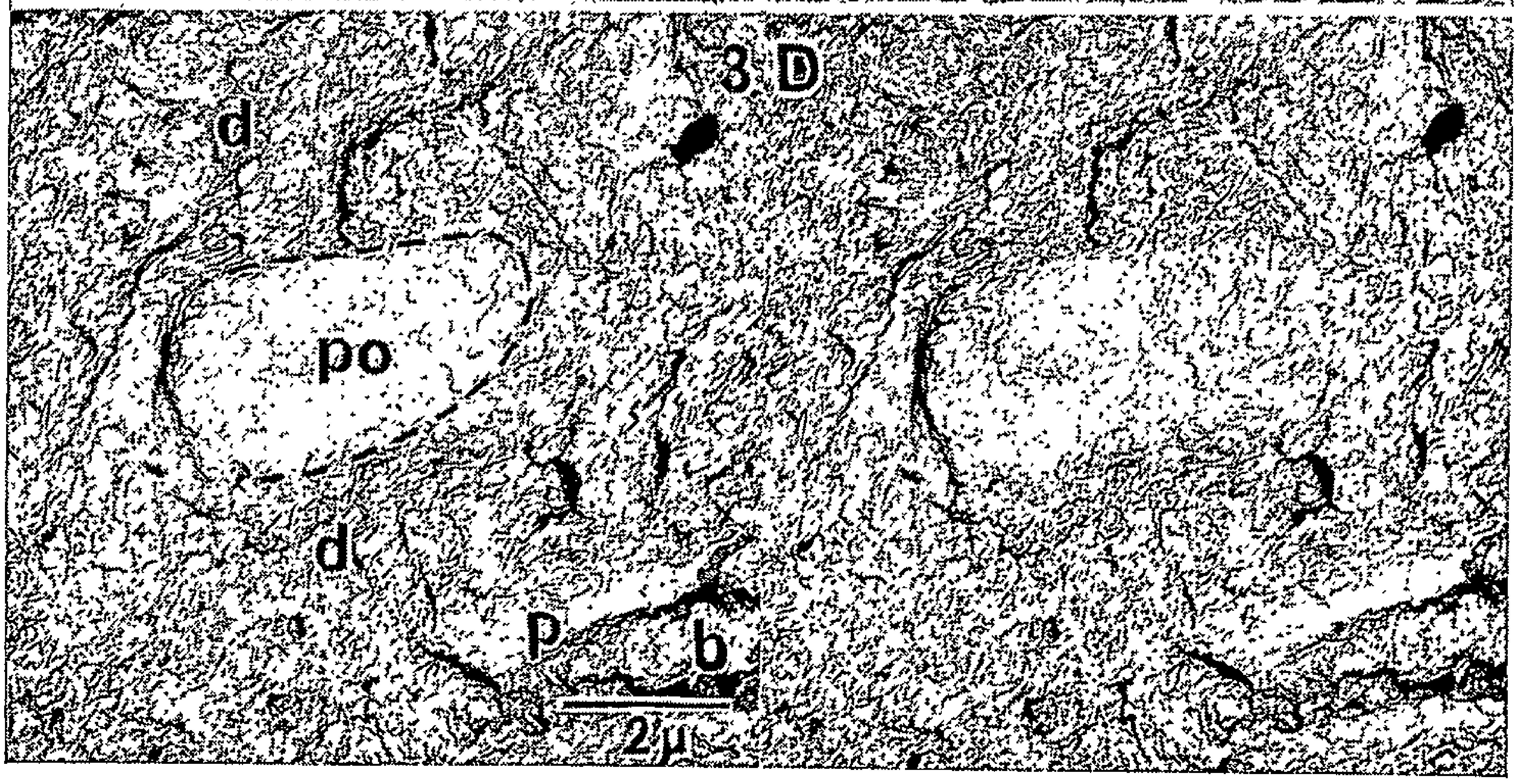
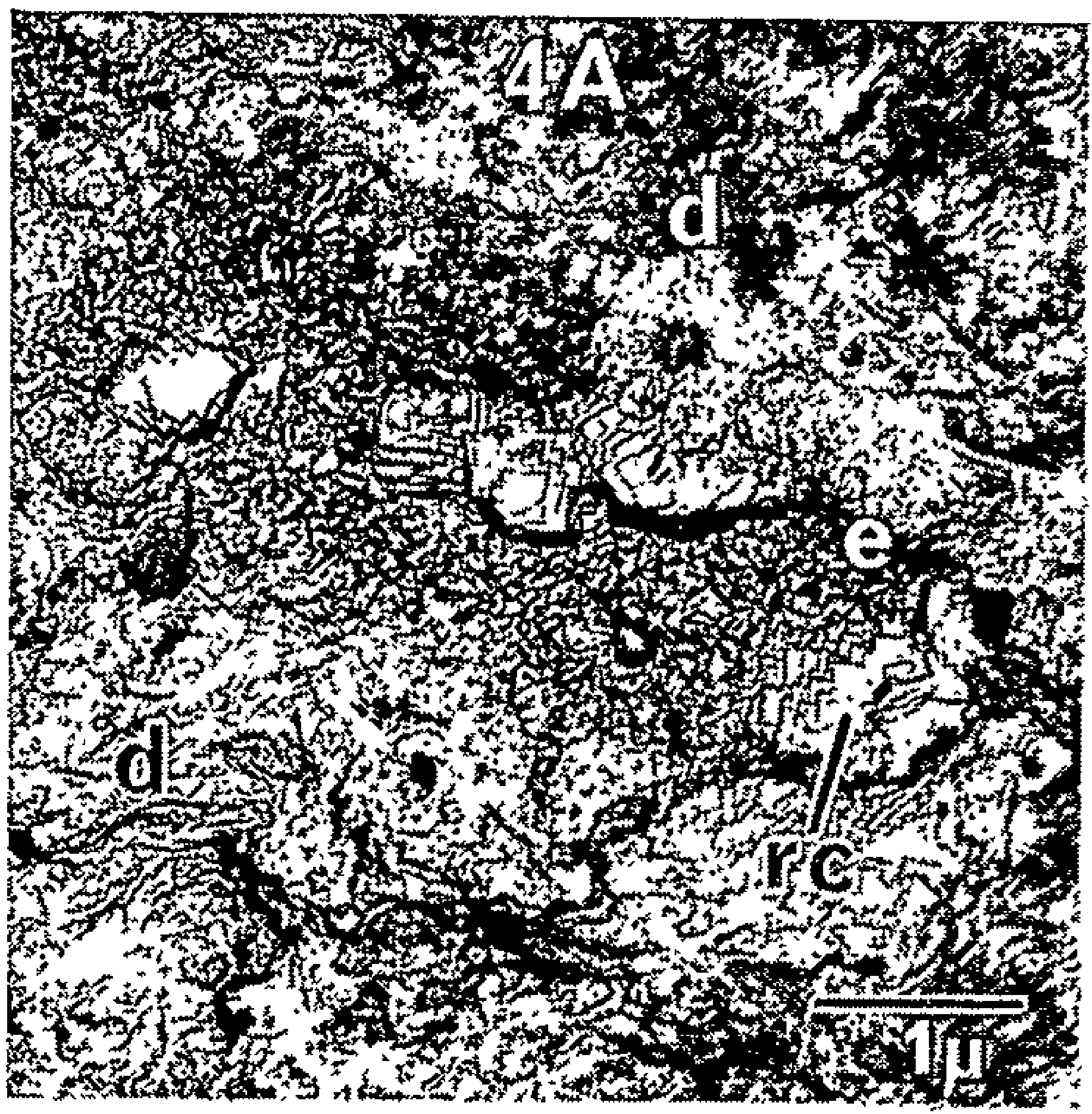

3C
0.5µ

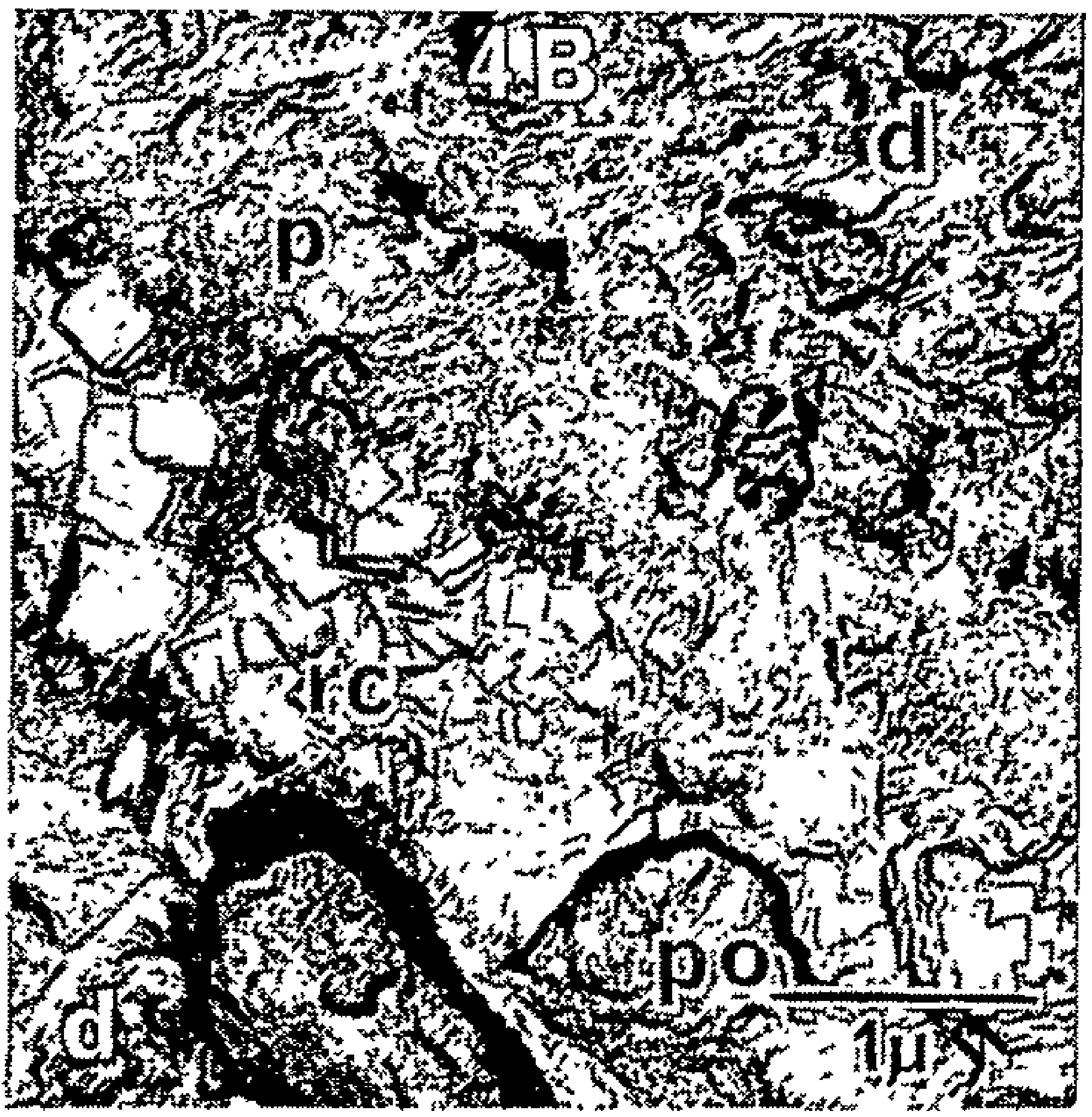

3D
d
po
d
p
b
2µ

Transmission electron micrographs (Figs. 4C and 4D are stereo-pairs) of carbon replica of longitudinally fractured "clinically sound" dentine showing large rhombohedric crystals of the type first described by HELMCKE (1955) and considered by NEWESELY (1965) to be $\beta$-tricalcium phosphate (Whitlockite).

Figs. 4A, 4B and 4C: Human cuspal permanent dentine showing large, coherent clusters of tabular crystals with growth faces evident on the individual elements at several places.

Fig. 4D: "Occluded" dolphin dentine showing separate, equidiametrical, rhombohedric crystals but with the same face angles as those in Figs. 4A, 4B and 4C.


4A
d
e
d
rc
1µ


4B
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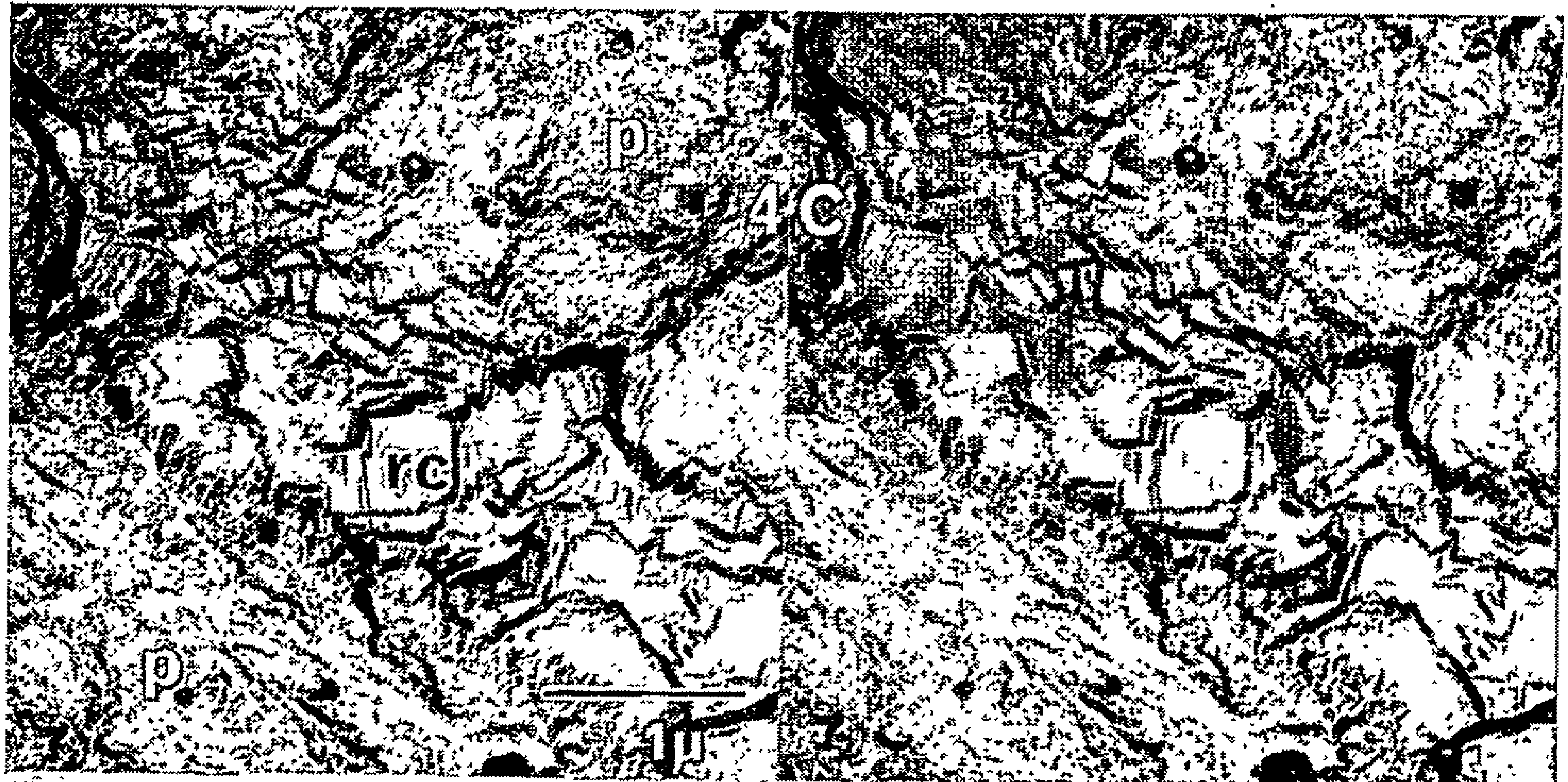
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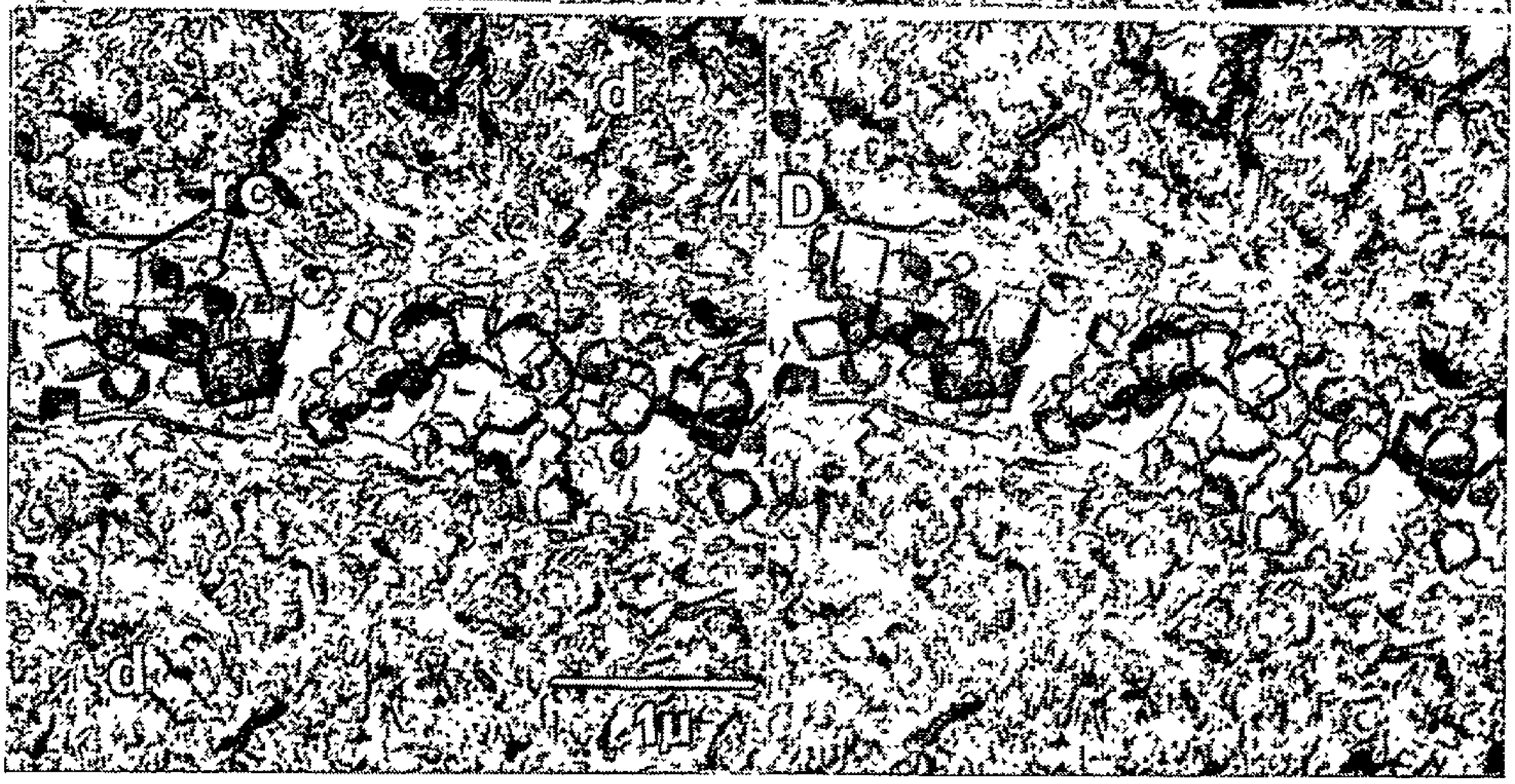
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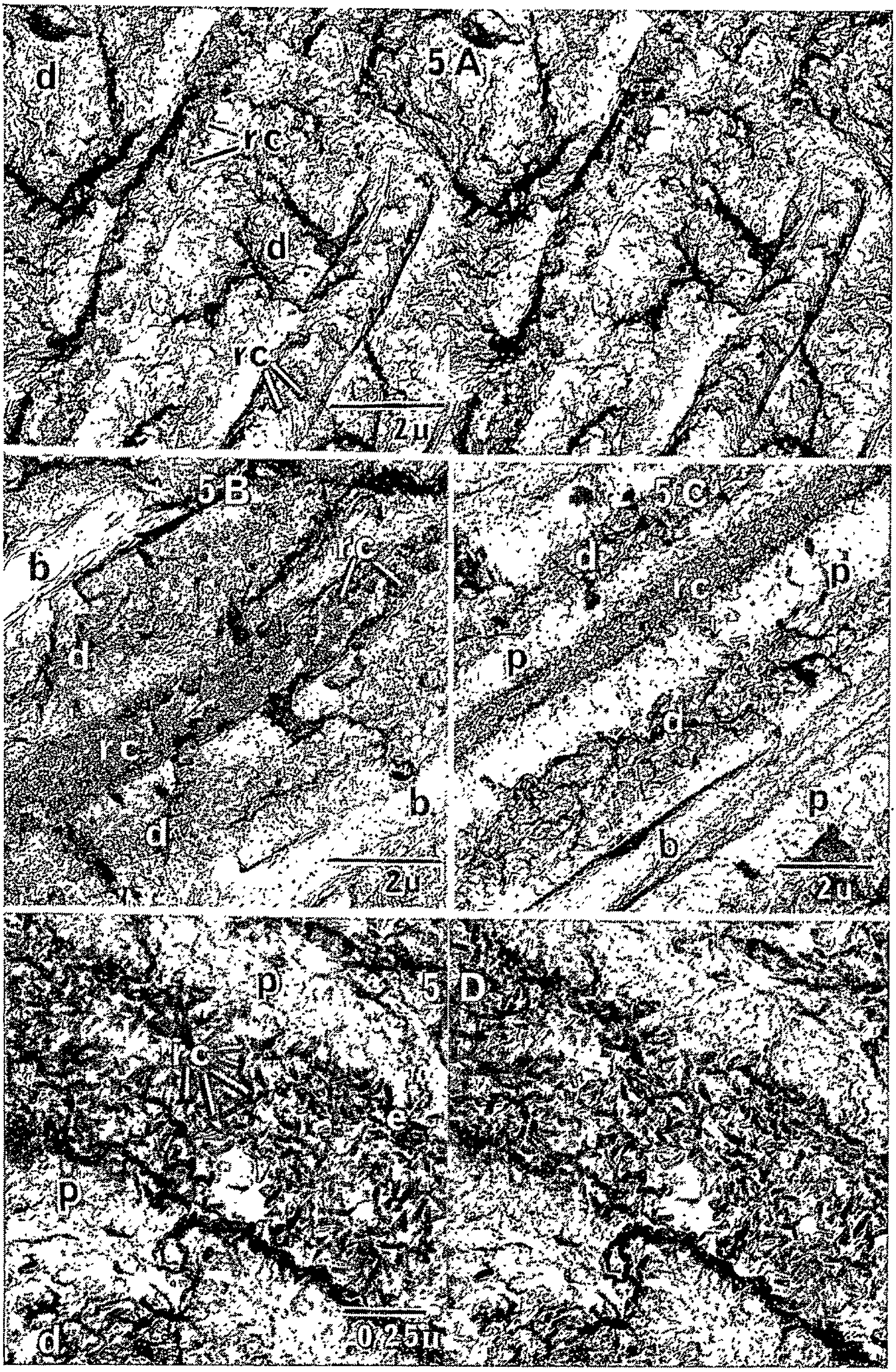
4D
d
1µ

Transmission electron micrographs (Figs. 5A and 5D are stereo-pairs) of carbon replica of longitudinally fractured, human, carious, permanent dentine.

Figs. 5A, 5B, 5C and 5D show various stages in the accumulation of rod-like crystals from small dendritic clusters (Figs. 5A and 5B) to the occlusion of the tubule (Figs. 5C and 5D). This third crystal type is characteristic of carious dentine. The crystals are obviously many times larger than the peritubular dentine crystallites, yet smaller than the rhombohedric crystals shown in Plate 4, and are tentatively identified with octacalcium phosphate.


5A
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2μ
5B
b
rc
d
rc
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b
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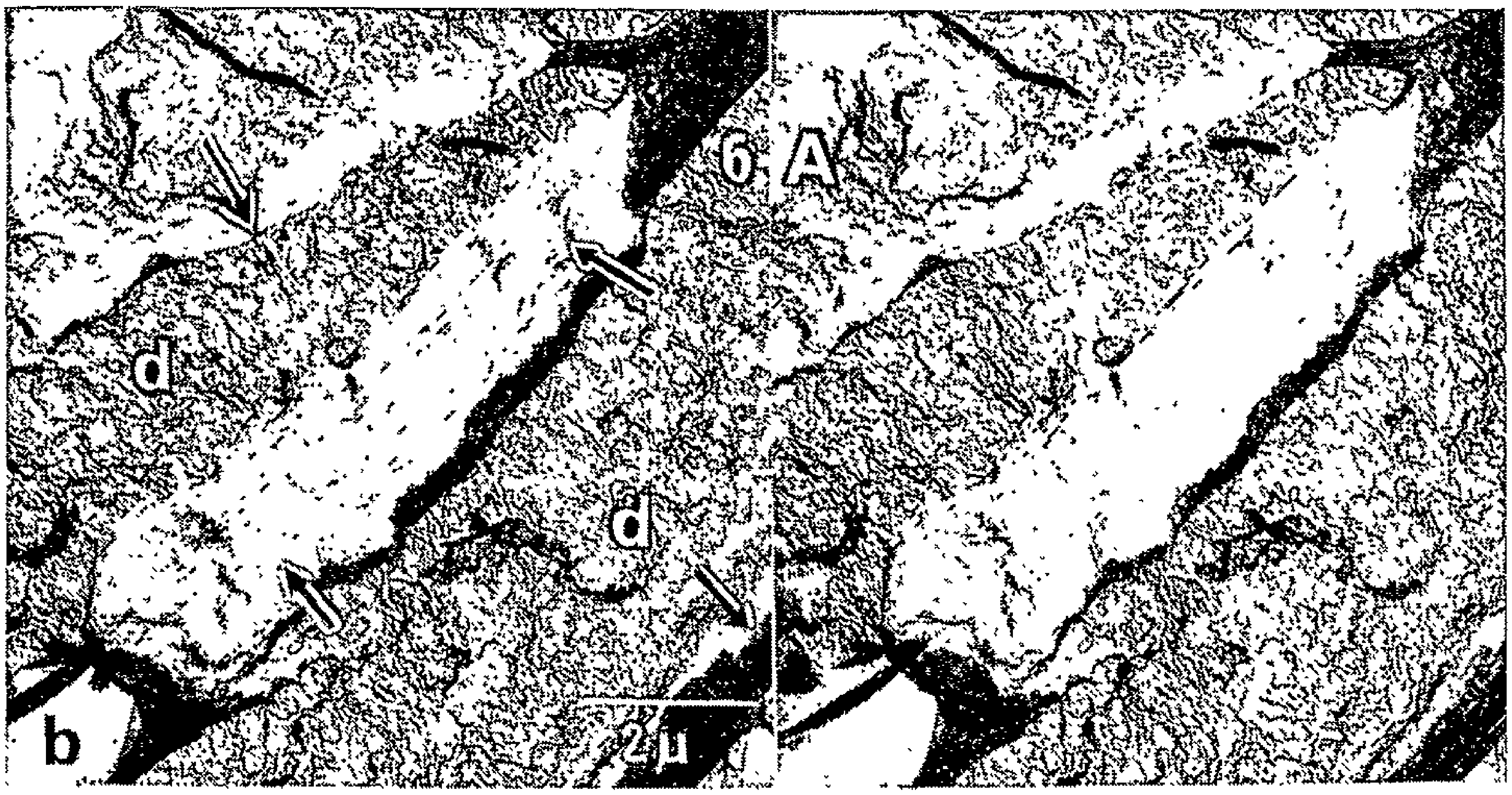
5C
d
rc
p
p
d
p
b
2μ
p
5D
rc
e
p
d
0.25μ

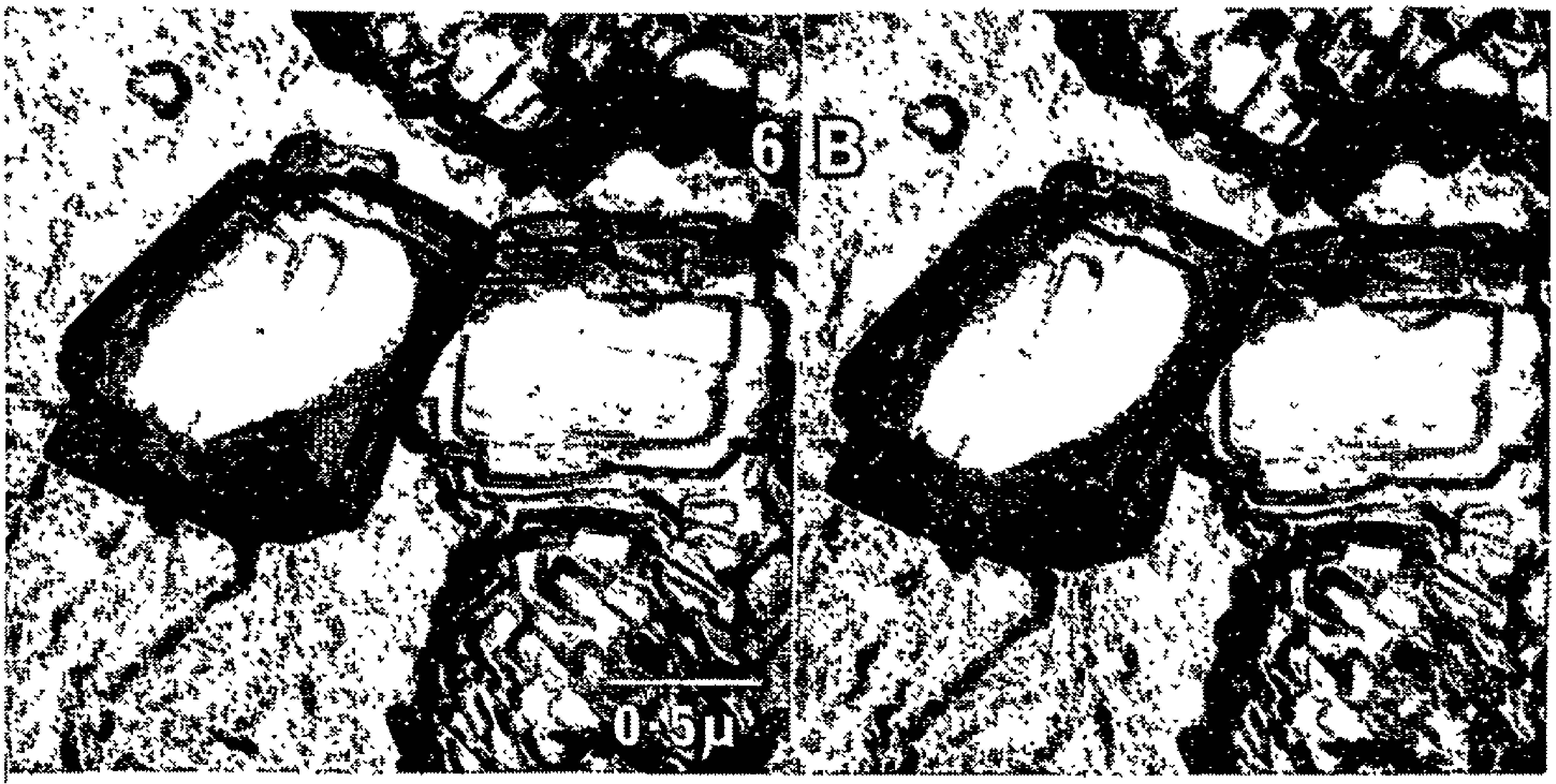
Stereo-pair transmission electron micrographs of fractured dentine.

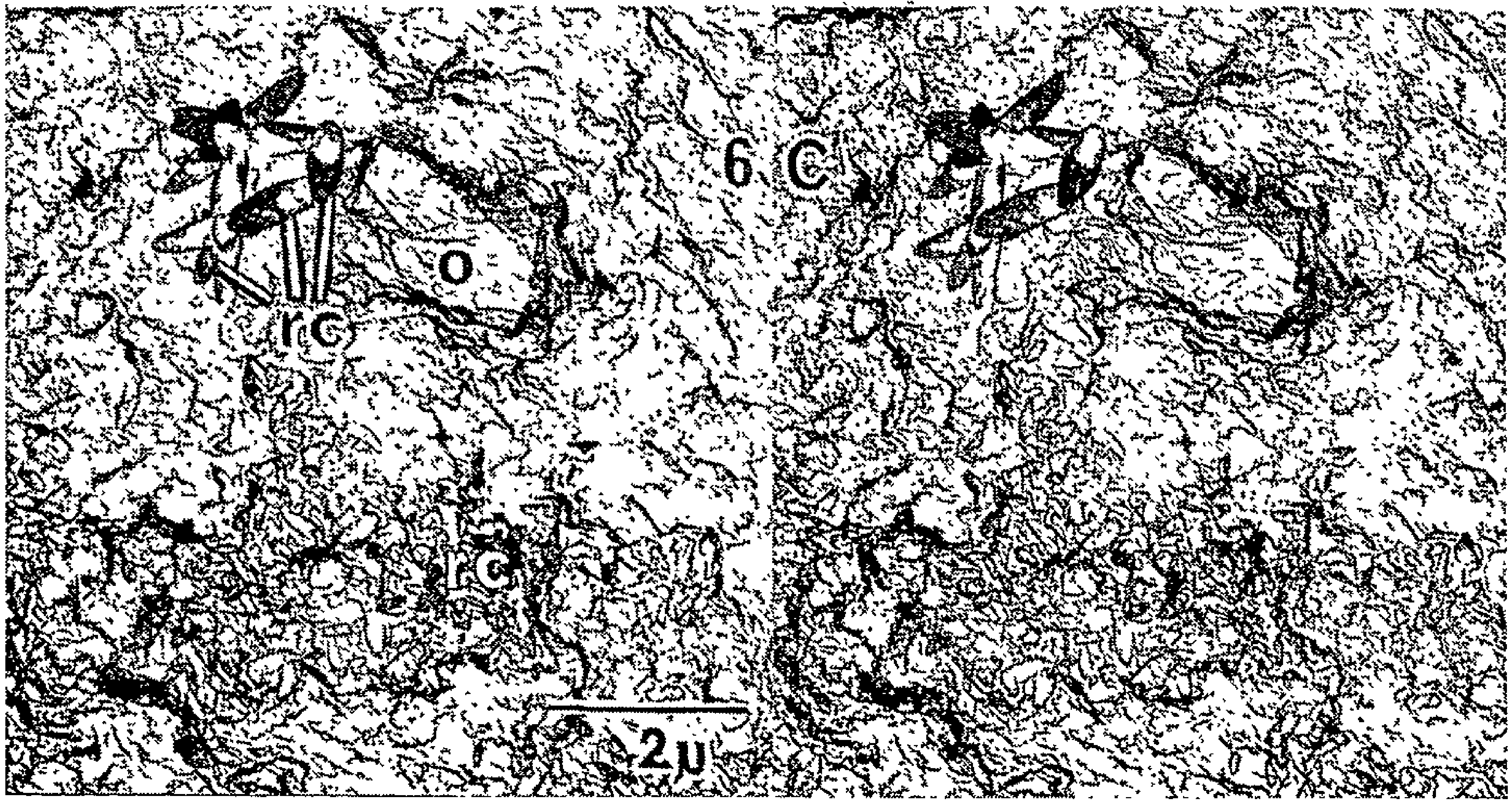
Fig. 6A: Transversely fractured manatee dentine exposing intratubular collagen (at arrow) normally bound about the odontoblastic process and which has come to lie over part of the exposed intertubular dentine as a result of the fracturing process.

Fig. 6B: Remineralisation crystals at the border-projection between two Howship's lacunae in resorbing human deciduous dentine. Such stacks of well-formed rod-shaped crystals are quite characteristic of this region. Their morphology is suggestive of their being octacalcium phosphate (see NEWESELY, 1965).

Fig. 6C: These "artefact" crystals were formed on the surface of the mineralising front of dolphin dentine exposed by extracting the organic matrix with hot 1:2 diaminoethane. It is probable that these rods (which have rounded or tapered ends indicative of a rapid crystallisation process terminated whilst in progress) were formed by the crystallisation from solution of dissolved $Ca^{++}$ and $PO_4^{---}$ ions during the procedure of drying down from water after washing out the organic solvent.


6 A


6 B


6 C

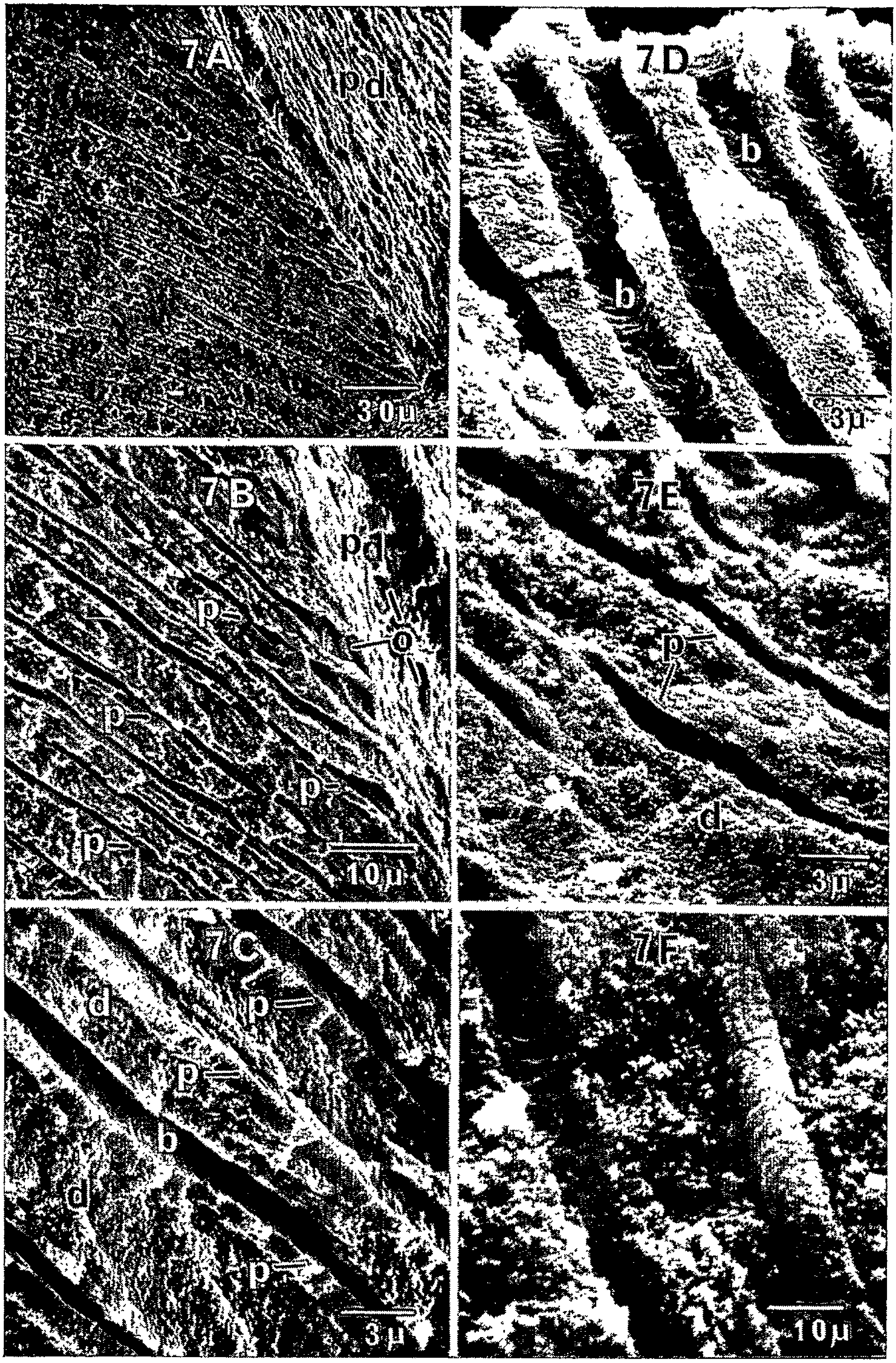
Scanning electron micrographs of fractured dentine.

Figs. 7A, 7B and 7C: Calf deciduous molar developing dentine surface, cuspal surface is to the top of the picture. Note the eccentric location of the peritubular dentine zones to the side of the tubules making an obtuse angle with the predentine surface (pd). The peritubular dentine forms very close to the developing surface in this species.

Fig. 7D: Anorganic human premolar, developing surface of dentine to the top of the picture. Note that the pattern of the mineralised (collagen fibres of the) intertubular matrix can be clearly seen in the tubule walls.

Fig. 7E: From the same group of dentinal tubules as in Fig. 7D at a level midway towards the enamel-dentine junction. The intertubular (fibrillar) pattern is now obscured by the deposition of an even layer of peritubular dentine around the circumference of the tubules.

Fig. 7F: Anorganic rat-tailed opossum dentine. The tubules in this region were completely occluded with " peritubular dentine." In the tubule on the left, the rod of peritubular dentine has fractured out of its bed of intertubular dentine (in which the fibrillar pattern of the collagen matrix is exposed to view). The negative imprint of the fibrillar pattern can be seen in the rod of peritubular dentine retained in the tubule on the right. Extraction with 1:2 diamino-ethane seems to predispose towards this type of fracture where the intertubular and the peritubular dentine separate from each other.


7A
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7C
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b
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3μ
7D
b
b
3μ
7E
p
d
3μ
7F
10μ

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# Surface Electron Microscopy of Caries Crystals in Grossly Carious Teeth:

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University College, London.

A variety of crystalline forms occur in the enamel and dentin of teeth that have lesions undoubtedly due to caries. These crystals differ in form from those normally regarded as constituting the sound tissue and are presumably the result of remineralisation. Formalin-fixed human teeth, deciduous and permanent, were subjected to 1:2 diamino-ethane at a temperature of 116° C. for 14 days and subsequently washed in alcohol. Specimens were then broken open to expose surfaces of enamel and dentin in the region of the carious cavity. These surfaces were either replicated in carbon and examined in a transmission electron microscope, or given a conducting coat of carbon and gold and examined directly in a scanning electron microscope. Stereo-pair images were recorded in each instance. Specimens fixed in acetone and not subjected to 1:2 diamino-ethane before replication were examined as controls. Two definite crystal types were found in carious enamel; a flattened rhombohedral (monoclinic) crystal with two large rhomboidal faces, and a prismatic crystal with a hexagonal cross section and typically, flat end-faces. Also found on carious enamel surfaces were bodies strongly resembling bacterial forms and with crystal faces apparent at their surfaces. In carious dentin, three different morphological types of crystalline inclusion were found in tubule lumens in addition both to the rhombohedral crystals usually described and to the accumulation of peritubular (intertubular) dentin. The most abundant crystalline species found as a contaminant was identified by X-ray diffraction as B-tricalcium phosphate.

19

Virchows Arch. Abt. A Path. Anat. 344, 196—212 (1968)

# Some Preliminary Observations on Caries ("Remineralization") Crystals in Enamel and Dentine by Surface Electron Microscopy

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Department of Anatomy, University College London

Received February 15, 1968

# *Beobachtungen zur Remineralisation von Zahnhartsubstanzkristallen, Oberflächen-Elektronenmikroskopie*

*Zusammenfassung.* Beschreibung verschiedener Kristallmodelle in cariösen Zähnen als Ausdruck eines Remineralisationsprozesses. Es können 2 Kristalltypen im cariösen Schmelz nachgewiesen werden: a) ein flach rhomboider Kristall mit 2 rhomboiden Breitseiten und b) ein prismatischer Kristall mit charakteristischem hexagonalem Querschnitt und typisch abgeflachten Enden. An der Schmelzoberfläche cariöser Zähne werden ferner bakterienähnliche Körper gefunden mit kristallähnlichen Oberflächen. In den Dentinröhren cariöser Zähne finden sich zusätzlich 3 Typen kristalliner Einschlüsse. Der größte Anteil kristalliner Verunreinigungen kann mit der Röntgenstrahlbrechung als $\beta$-Tricalciumphosphat identifiziert werden. Es ist wahrscheinlich, daß dieser Verbindung die rhombischen Kristalliten entsprechen.

*Summary.* The aim of this paper is to demonstrate a variety of crystalline forms that occur in the enamel and dentine of teeth with lesions undoubtedly due to caries and which are presumably the result of a "remineralization" process. Two definite crystal types were found in carious enamel; a flattened rhombohedral (monoclinic) crystal with two large rhomboidal faces, and a prismatic crystal with a hexagonal cross-section and typically flat end-faces. Also found on carious enamel surfaces were bodies strongly resembling bacterial forms and with crystal faces apparent at their surfaces. In carious dentine, three different morphological types of crystalline inclusion were found in tubule lumens in addition both to the rhombohedral crystals usually described and to the accumulation of peritubular (intratubular) dentine. The most abundant crystalline species found as a contaminant was identified as $\beta$-tricalcium phosphate by X-ray diffraction, and we presume this to correspond to the rhombohedral crystallites.

## Introduction

There have been a number of reports of the occurrence in both carious enamel and dentine of crystals which are larger than, and of a different form to, those generally regarded as constituting the sound tissue (for example, HELMCKE, 1955, 1962; LENZ, 1955; TORELL, 1957; HÖHLING, 1961a, 1961b, 1966; TAKUMA and KURAHASHI, 1962; HERTING, 1966). Other workers have described structures similar to these "caries crystals" in teeth apparently unaffected by caries, although affected by processes such as masticatory wear, erosion, or osteoclastic resorption (see for example, SOGNNAES, 1963; KATTERBACH et al., 1965; BOYDE and LESTER, 1967). Although caries crystals, as generally conceived, represent the "crystallization by-products" of the caries process, they have received scant attention in their own right. It seemed important therefore, to examine human teeth with lesions undoubtedly due to caries to see if some basis could be established for comparison of the morphology of the caries crystals with those of apparently similar crystals occurring in other situations.

## Materials and Methods

25 deciduous and 25 permanent human teeth with gross caries were fixed upon extraction in neutral formol saline and stored in 70% ethanol. 6 specimens were fixed and stored in acetone to eliminate the possibility of some demineralization occurring as a result of formic acid formation in the formalin solution. The carious cavity in each of these teeth involved extensive loss of enamel and dentine and the pulp chamber was "exposed" in most instances. Excess food debris (if present) was removed from the carious cavity with a suitable hand instrument. The specimens were placed in glass extraction thimbles in a Soxhlet condenser and subjected to 1:2-diamino-ethane ($NH_2 \cdot CH_2 \cdot CH_2 \cdot NH_2$ and commonly known as "ethylene diamine") at a temperature of 116° C, the organic solvent being distilled through this apparatus continually for a period of at least 14 days. At the end of this period, 3 changes of absolute ethanol were distilled through in place of the diamino-ethane in order to wash the specimens, which were then stored in a desiccator until required. Specimens were broken open (by closing the jaws of a small vice in which they were held) so as to expose surfaces of enamel and dentine adjacent to the lesion. These prepared surfaces were coated prior to examination by one of two methods as described below.

The specimens for scanning electron microscopy (Cambridge Instrument Co. *Stereoscan*, operated at 10 kV; 100 seconds recording time) were given an electrically conducting coat of ca. 200° A of carbon and then ca. 300° A of gold, which were evaporated *in vacuo* onto the specimens whilst they were rotating and facing at an angle of approximately 45° to the evaporation sources.

Some details of the method for preparing material for examination via the replica method for transmission electron microscopy have been given by Boyde (1967). However, the method used in the present study differs in that the specimens were extracted with 1:2-diamino-ethane before the carbon replica coating was applied. Thus, to release the replica it was only necessary to dissolve the resulting substrate in N/10 HCl. Some preselection of areas of interest was exercised by destroying the carbon replica coat on the tooth in the region which we did not wish to examine. The stereo-pair transmission electron micrographs were prepared using the standard tilt cartridge for the Siemens *Elmiskop I* (operated at 80 kV; 100 μ condenser aperture). The tilt device was calibrated by the method described by Boyde and Willis (1966). The methods of stereophotogrammetric analysis applied to the resulting images have been detailed previously (Boyde, 1967).

We would emphasize that the images as reproduced here will be of little value and may be confusing *unless* examined stereoscopically when they will be easily interpreted for what they are, namely, an indication of the three-dimensional shape of the surface in question. The easiest method of viewing these paired images stereoscopically is by means of a simple stereo-viewer, which consists of two lenses held at their focal distance from the images. The distance between the lenses should correspond to the inter-ocular width of the observer. The use of these lenses relaxes the focus of the eyes to infinity when the viewing axes of the eyes also become parallel. With some practice, it is possible to separate the functions of focus and of convergence of the eyes so that the pairs may be seen stereoscopically without the aid of a viewer.

## Observations

*Enamel.* The carbon replica method provides a clear illustration of three morphologically distinct types of "caries crystal" within carious enamel. These crystals are found both within the bulk of the enamel surrounding the cavity, where surfaces were exposed for study by deliberate fracture, and on the surface of the enamel forming the walls of the caries cavity itself.

One type of caries crystal is typically flattened or tabular, with its two largest faces (up to 0.75 μ across) rhomboidal in outline (Fig. 1 and Helmcke, 1955, 1960, 1962; Torell, 1957; Höhling, 1961 b). These crystals occur typically in extensive clusters over the surface of recognizable well-ordered enamel, there being an abrupt transition from the one to the other. The individual caries crystal elements,

Fig. 1

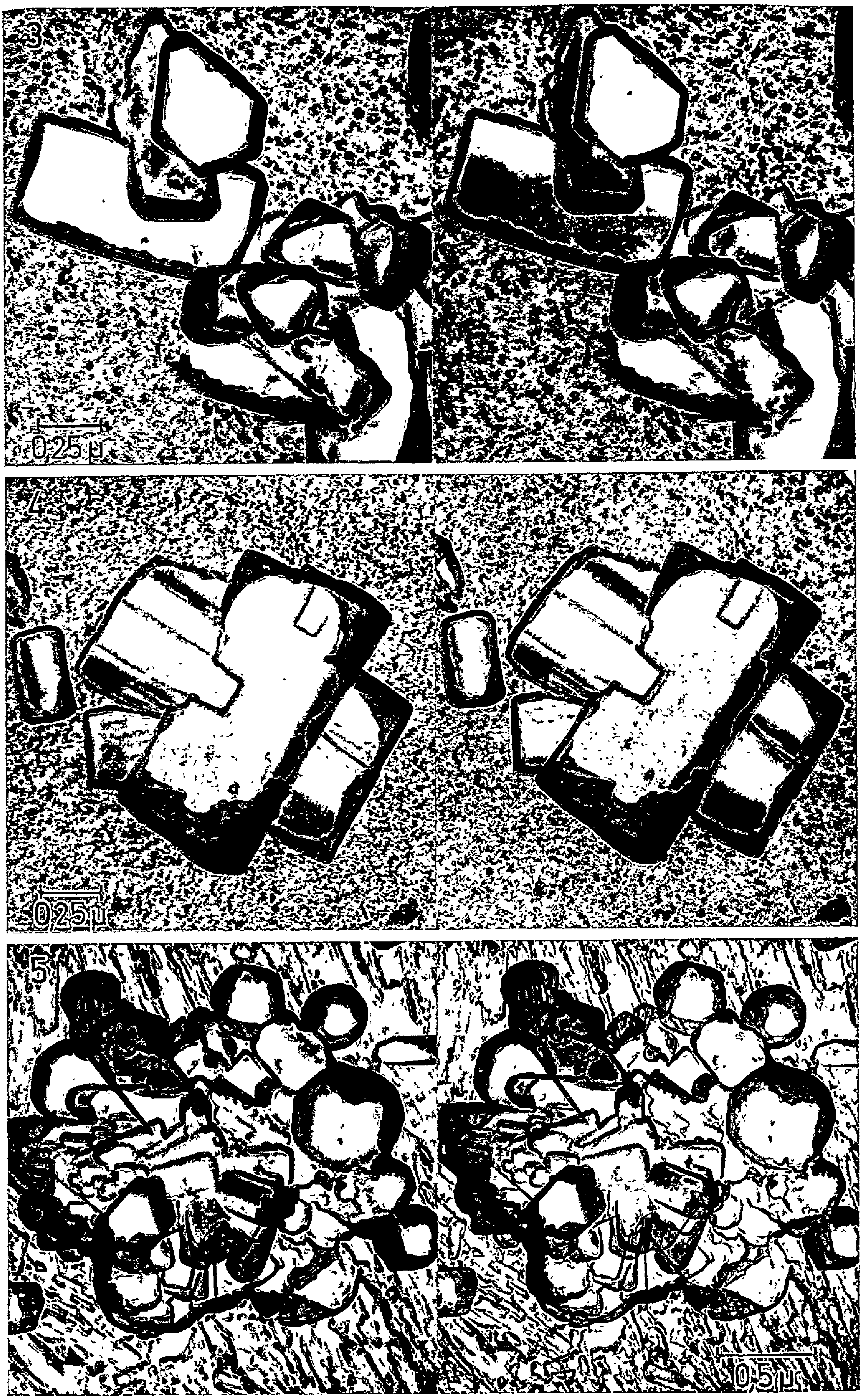
Fig. 2. Stereo-pair transmission electron micrograph of carbon replica of fractured carious enamel surface. Part of Fig. 1 to show three-dimensional form of the caries crystals

when exposed for replication, contribute to a characteristic, intricate and repetitive three-dimensional step-like pattern (Fig. 2). Preliminary measurements of the face angles of the large faces, obtained both directly from the images and indirectly via the *Stereosketch* (see Boyde, 1967) suggest a variation inconsistent with a single crystal moiety. This aspect must be studied further however, for we are unable as yet to distinguish with certainty between faces or facets of these crystals which are truly growth faces and those which are a result of fracturing during our preparation of the material.

The second type of caries crystal in the enamel is prismatic in form and hexagonal in cross-section (the 6 sides of the flat end-face are not usually equi-dimensional but the face angles between them are consistently 120°) (Figs. 3, 4). These crystals do not form extensive clusters as do the flattened "rhombohedral" type described above, but occur singly or in small groups (Fig. 5). The diameter of these crystals varies from ca. 1.5 μ down to a dimension approaching that of the typical hydroxyapatite crystallite of "sound" enamel (Fig. 6). Although the end-faces of these hexagonal crystals are characteristically flat, there are isolated examples of pointed terminations: the angle formed at the point was measured as 82°, the measurement being made directly from photographs in which the body of the crystal could be determined to lie in the horizontal plane.

Small, less regular crystals which cannot be easily identified with either of the two crystal types described above comprise a third group and are found constituting *en masse* what appear to be bacterial forms (Figs. 7, 8). In their external

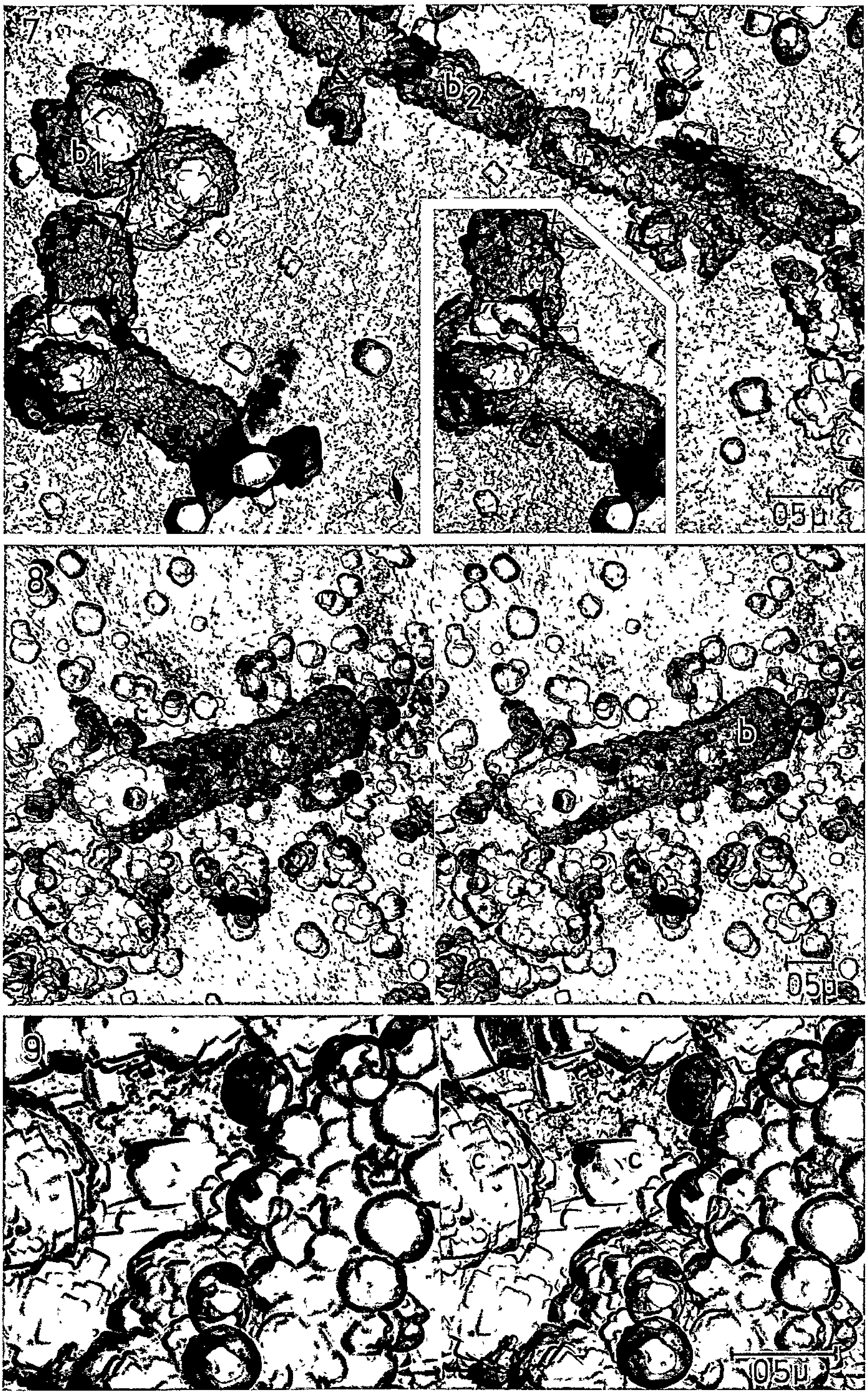
Fig. 1. Transmission electron micrograph of carbon replica of fractured carious enamel surface showing a large cluster of "rhombohedral" caries crystals and, at top left, the closely packed, elongated crystallites typical of "sound" enamel. *c* caries crystal; *b* bodies strongly resembling bacteria; *id* intertubular dentine; *pd* peritubular dentine; *t* dentinal tubule; *en* "sound" enamel



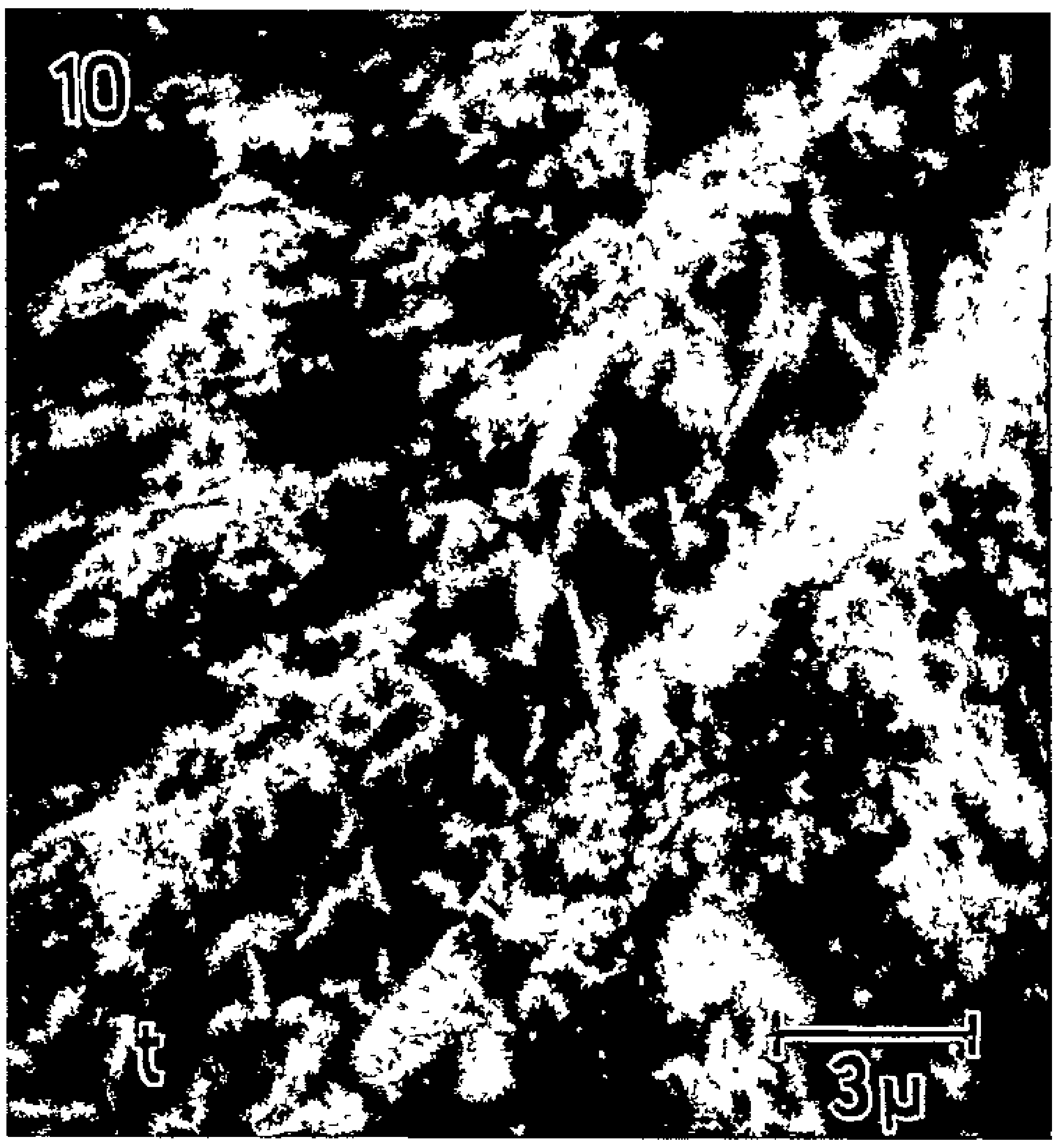
Figs. 3—5. Relatively high magnification stereo-pair transmission electron micrographs of carbon replica of carious enamel surfaces. Note the small clusters and the form of the hexagonally-based caries crystals. Fig. 5 also shows (at far right) smooth-surfaced bodies resembling coccal bacteria

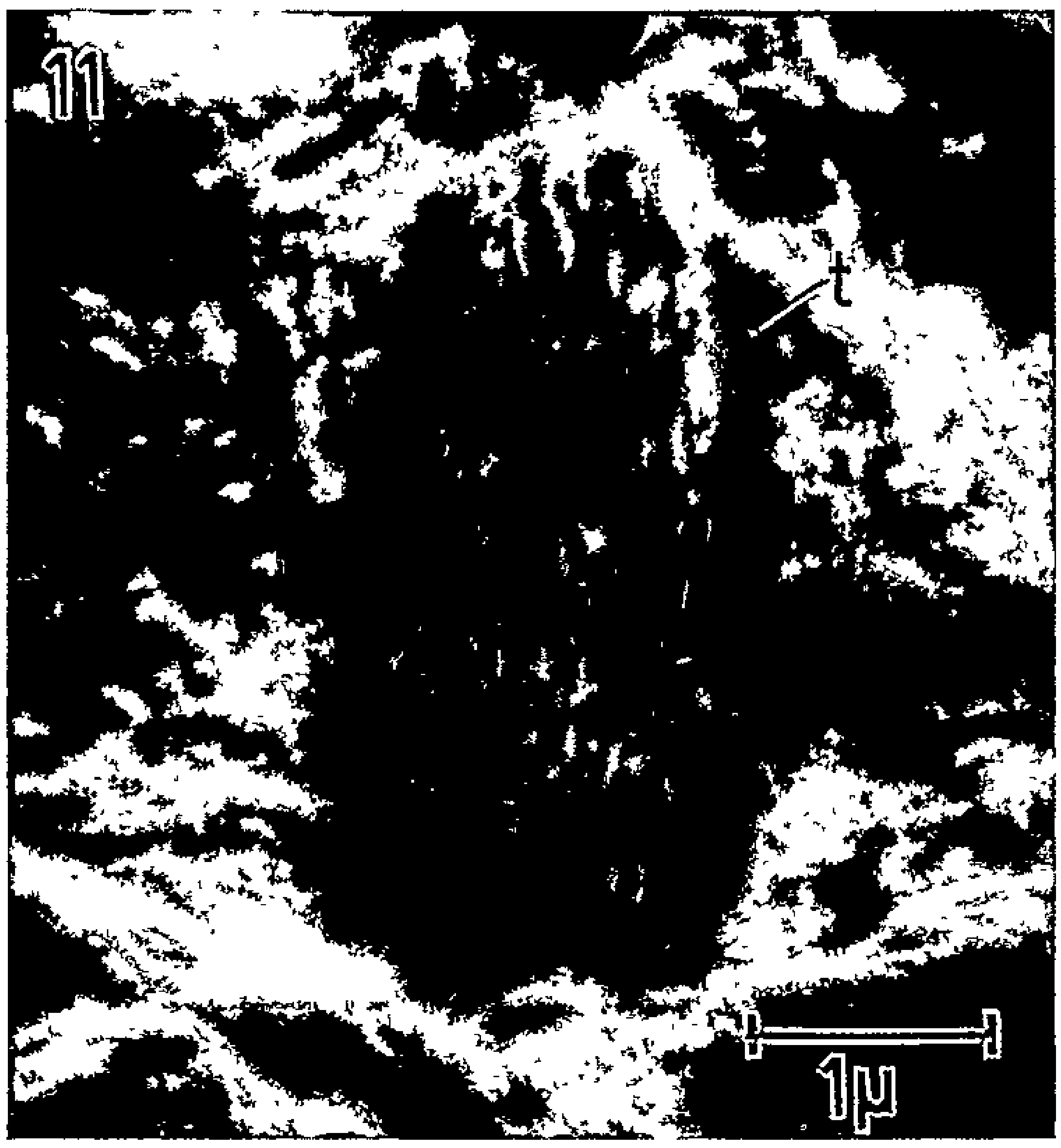
Fig. 6. Transmission electron micrograph of carbon replica of carious enamel surface. Small rod-like crystals of the hexagonal system which (at top left and bottom right) are aggregated into masses similar in size to the bacterial forms of Figs. 7 and 8

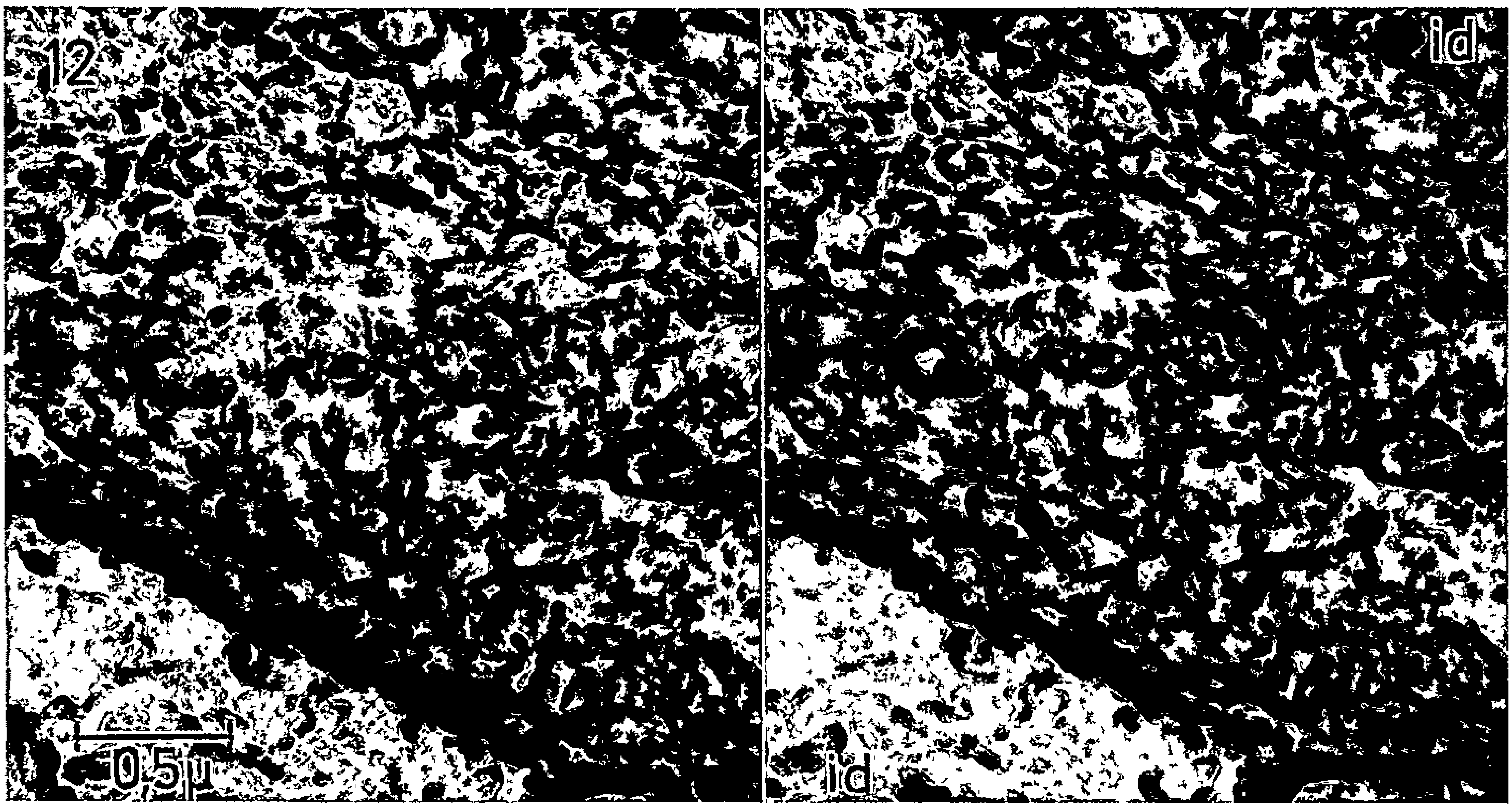
morphology, these bodies resemble coccal, and chain-forming, bacillus-like bacteria. We cannot determine, from the present results alone, whether these bacteria-like bodies are mineralized solid or whether the crystal faces represent an external coat only. Interpretation of these structures in these terms is complicated by the fact that over the same enamel surfaces, well-circumscribed bundles of crystals may be found in close proximity to smooth, rounded bodies with no visible crystalline component at all (Fig. 9 and cf. Fig. 6).



Figs. 7—9. Transmission electron micrographs of carbon replica of carious enamel surfaces







Figs. 10 and 11. Scanning electron micrographs of deliberately fractured surfaces of dentine adjacent to the caries cavity

Fig. 10. Thin, plate-like crystals in the tubule lumen

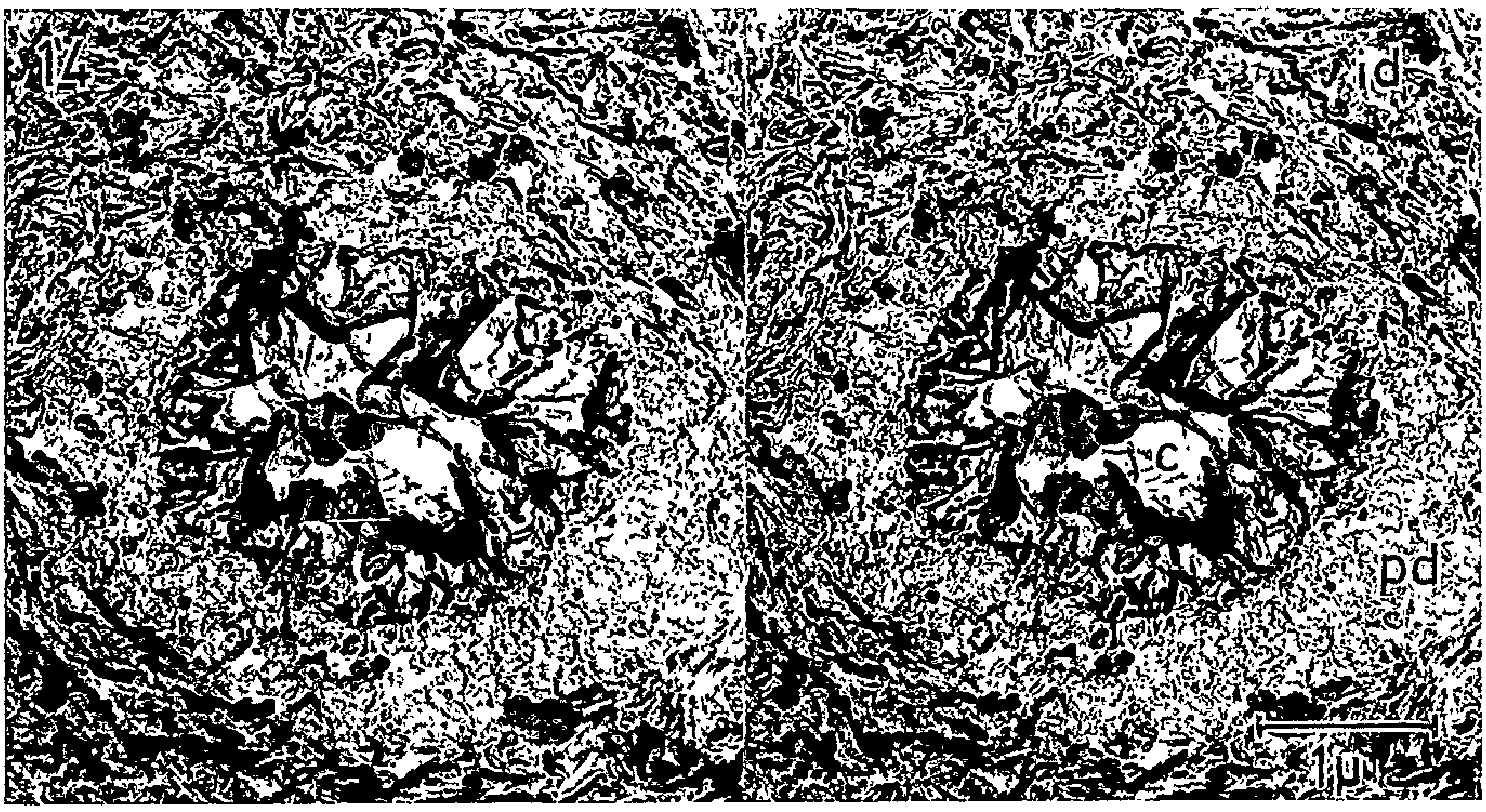
Fig. 11. Small, rod-like crystals forming a delicate meshwork and occluding the tubule lumen

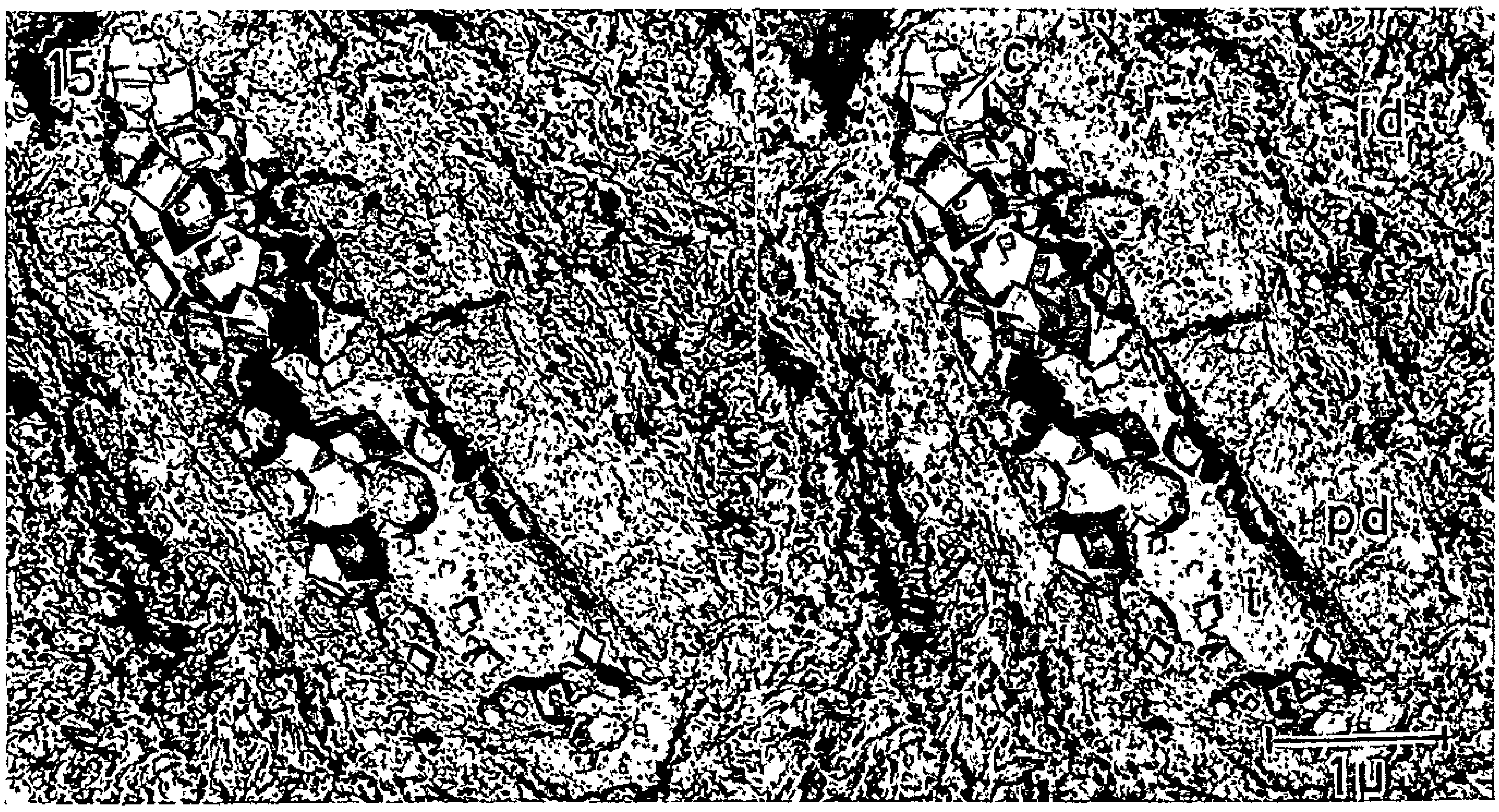
Fig. 12. Stereo-pair transmission electron micrograph of carbon replica of deliberately fractured surfaces of dentine adjacent to the caries cavity showing small irregular rod-like crystals projecting into the lumen of the tubule

Fig. 7. Bodies resembling coccal ($b_1$) and bacillus-like ($b_2$) bacterial forms and with a surface pattern made up of small, individual, crystals. *Inset:* forms stereo-pair with adjacent part of figure

Fig. 8. Stereo-pair of bacillus-like bacterial form. Note the similarity of its surface pattern to the faces of the scattered crystals surrounding

Fig. 9. Stereo-pair showing smooth surfaced coccal-like forms and caries crystals, the latter occurring both singly and in well-circumscribed bundles





Figs. 13—15