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INTERPRETING THE REFLECTED-LIGHT APPEARANCE OF ENAMEL IN THE DOG

K. S. LESTER*

Department of Histology and Embryology, University of Sydney
and Department of Anatomy, King's College, London, England

Summary—Thin ground polished sections of dental enamel were examined by transmitted polarized light and under light of known incidence using a universal rotating stage. The polarized light was used to assess the inclination of particular groups of parallel rods to the section surface. By the incident light, determination was made of the three-dimensional range of positions that these rod groups could assume relative to the incident light source and remain reflective.

Explanation is offered of a number of patterns of behaviour or sequences of appearances of the bands of Schreger which have been described elsewhere as occurring with horizontal rotation of specimens through 360° relative to a fixed source of incident light. Thus, explanation is found for the maximal demarcation and width of bands when the incident beam is aligned with the longitudinal axes of the rods, for the "splitting" of light bands accompanying mutual band reversal, for the similarity of band appearance when the specimen is in the two positions where the incident beam is perpendicular to the longitudinal axes of the rods and for the lack of band reversal in some specimens.

This information together with controlled incident light examination of specimens affords a useful means of assessing the sub-surface orientation of enamel rods.

INTRODUCTION

IT HAS been shown that enamel rods appear maximally bright when they are orientated to incident light so as to satisfy the laws of reflection; this light-optical characteristic of enamel rods has been related to the appearance of the bands of Schreger (LESTER, 1965a). The aim of the present study was to map out in three dimensions the range of positions that a group of relatively parallel enamel rods of known orientation could assume relative to a fixed source of incident light and continue to reflect that light to the observer. It was hoped that this information might help to explain the varying appearances of the bands of Schreger occurring upon rotation of the specimen and that these varying appearances might in turn be of use in predicting the sub-surface orientation of enamel rods in a specimen where this was unknown.

MATERIALS AND METHODS

Thin (ca. 12 μ), plano-parallel, bucco-lingual sections of dog (*Canis familiaris*) enamel were ground and polished essentially after the method of FREMLIN, MATHIESON and HARDWICK (1961). The sections were mounted in a Leitz four-axis universal

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*Address for correspondence: Department of Anatomy, New York Medical College, New York, N.Y. 10029, U.S.A.

rotating stage using hemispheres and mountant of refractive index 1·647. Defined areas of groups of parallel rods in the middle third of the lateral plate of enamel were examined by transmitted polarized light in order to determine for each area the mean longitudinal crystallite axis and then to assess its inclination to the surface of the specimen. The method of examination is described elsewhere (LESTER, 1965a, 1965b). The same areas were then examined under a fixed incident light source—a very thin beam angled at 45° to the horizontal (Fig. 1). The range of specimen positions in which each area remained reflective was assessed within four rotations of the stage. Two of these involved rotation of the specimen about horizontal axis A_4 (effectively, a change in incident angulation of the illumination) with the mean longitudinal axis of the area firstly in the 0° and then in the 180° rotation position (Fig. 1). The remaining rotations were about horizontal axis A_2 with the mean longitudinal axis successively in the 90° and the 270° rotation positions (Fig. 1). The range of inclinations of the specimen stage (and therefore of the specimen surface) within which the area concerned remained reflective were noted in each case.

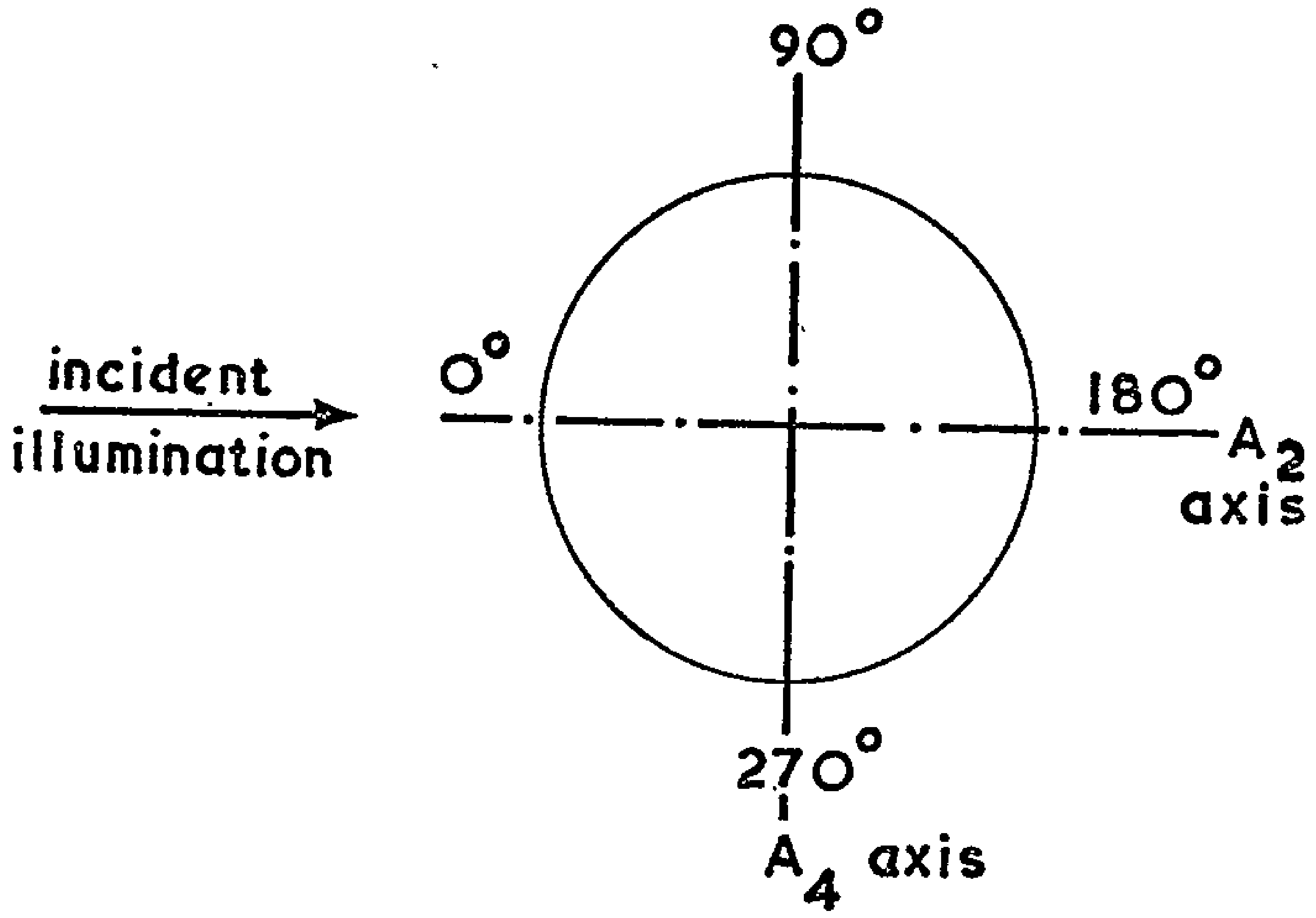

FIG. 1. Plan of rotation positions showing relation to light source and to axes of rotation.

RESULTS

By an interpretation of the polarized light data similar to that reported previously (LESTER, 1965a, 1965b), it was possible to assess and to represent diagrammatically the orientation of the mean longitudinal axis of the crystallites in the areas considered in terms of angulation to the specimen surface. The mean longitudinal axis of the crystallites in dog enamel is closely coincident with the longitudinal axis of the enamel rod, both in a vertical bucco-lingual or labio-lingual plane and in a transverse mesio-distal plane (CAPE and KITCHIN, 1930). In human enamel, on the other hand, it is common for the axes to agree only in the transverse mesio-distal plane (POOLE and

BROOKS, 1961; GLAS, 1962). It follows that, in dog enamel, definition of the mean longitudinal crystallite axis affords definition also of rod longitudinal axis. With this information for each of the areas examined by polarized light, individual consideration was then given to each of the rotations observed under incident light.

Within the four rotations considered, there was a rotational arc of some 35° within which enamel rods could lie and exhibit some degree of reflection. That is to say, with change in the incident angulation of the illumination (change in altitude), enamel rods remained reflective within a range of nearly 20° on either side of the position of maximal reflection. Because an enamel specimen is most commonly examined for the Schreger band appearance when horizontal, a diagram was prepared to show, with respect to a 45° angled incident light beam, the angles which a rod running to the section surface (in this case the horizontal) from a given direction could make and still be expected to exhibit some degree of reflection (Fig. 2). As indicated, these angles were found to be between 5° and 40° at the 0° rotation position and between 0° and 20° at the 90° and 270° rotation positions. The rods as represented in Fig. 2 are assumed to arrive at the specimen surface from the same direction as the incident light (from the right in Figs. 2 and 3) when the specimen is in the 0° rotation position. At the 180° rotation position, there was no possibility of a rod which passed to the section surface in the direction considered reflecting light to the observer. It should be stressed, however, that within any one rotation the change in brightness or reflectivity is a very gradual one. Figures indicating the limits of the range of rod inclinations at which reflection occurs (Fig. 2) could therefore more correctly be said to represent the approximate centre of the final arc of rotation within which change occurred from a reflective to a non-reflective appearance and vice versa. Although rod reflection has been considered at the 0°, 90°, 180° and 270° rotation positions only, (because of the difficulties involved in the two-dimensional representation of the intermediate positions), it was apparent that the inclinations to the horizontal at which the

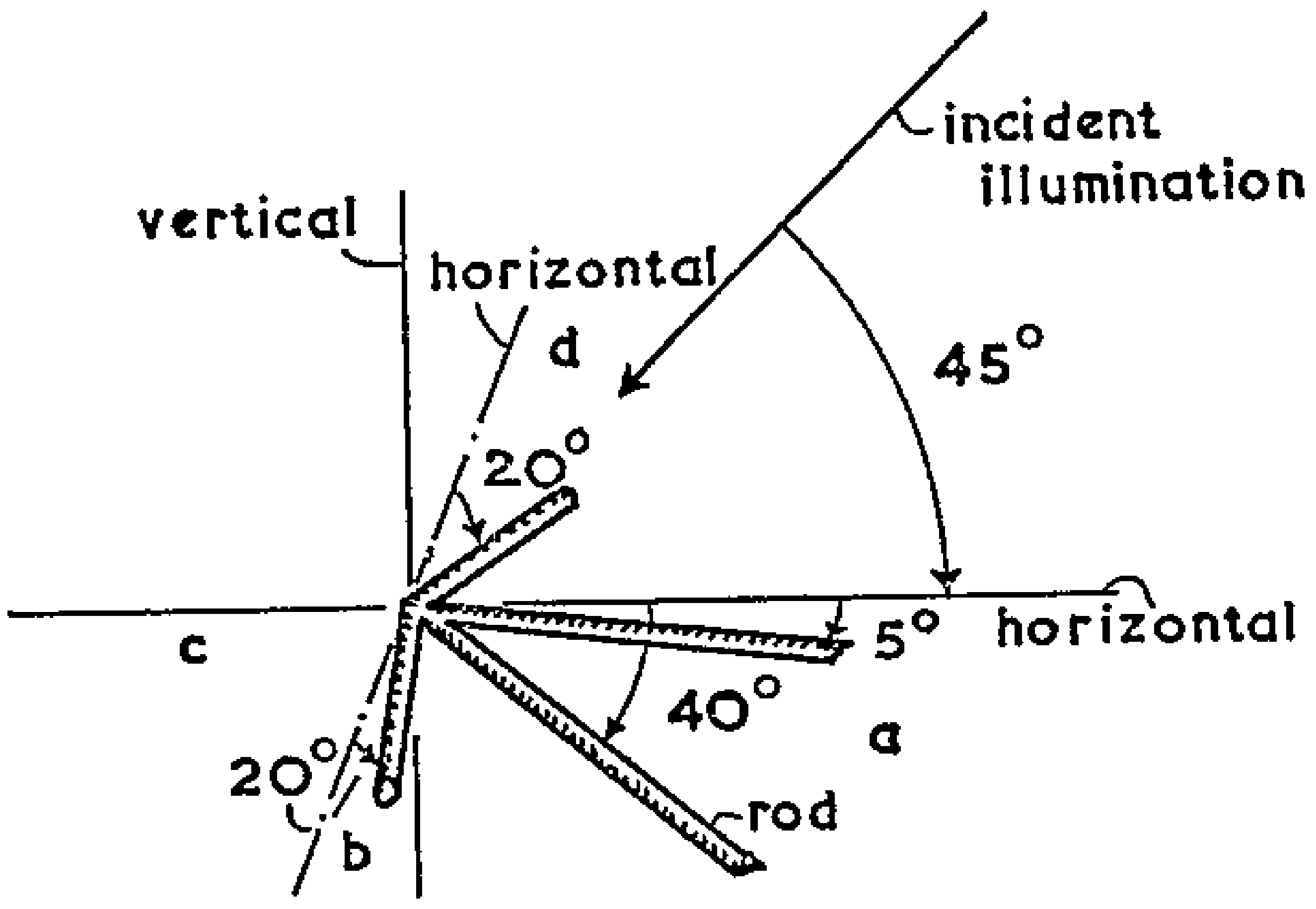

FIG. 2. Diagram relevant to the rotation through 360° of a horizontal specimen, indicating the arcs of rotation within which enamel rods appear reflective. The rods are assumed to arrive at the specimen surface from the same direction as the incident light (in Figs. 2 and 3 from the right hand side) when the specimen is in the 0° rotation position (at a).
b—90°, c—180° and d—270° rotation positions.

rods lost their brightness constituted a gradual transition from, for example, 40° at the 0° rotation position to 20° at the 90° and the 270° positions.

Figure 3 indicates the orientation of the plane (as determined theoretically and confirmed experimentally) within which the longitudinal axis of enamel rods should lie in order to exhibit maximal brightness (reflection). The diagram pertains to the particular conditions of illumination used in this investigation and to the range of specimen manipulation made possible by the goniometer stage. The enamel rod is assumed to pass through the centre point of the sphere at which the incident light is directed.

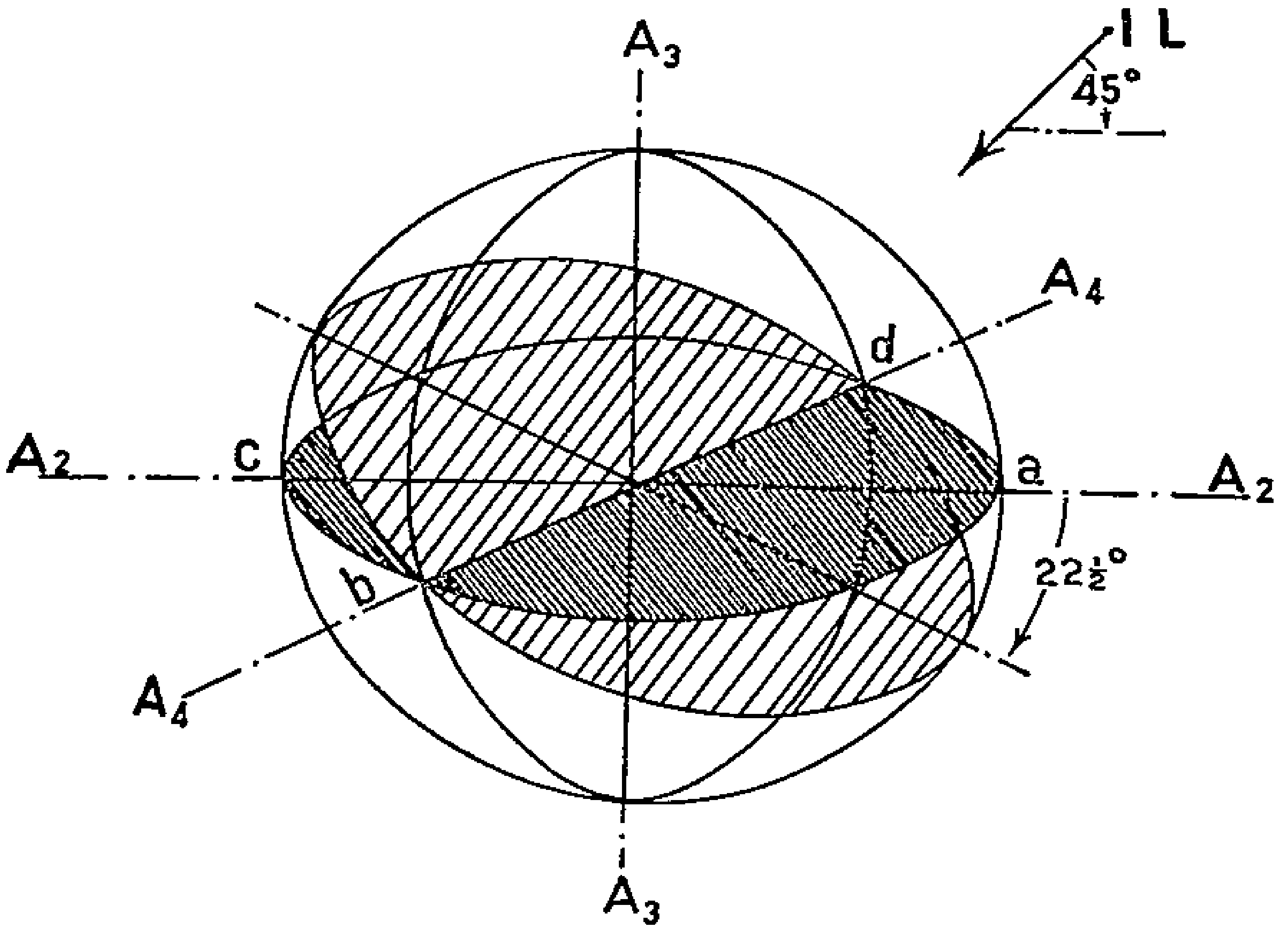

FIG. 3. Diagram relevant to the specimen movement possible with the universal rotating stage. The plane within which the longitudinal axis of enamel rods must lie to exhibit maximal reflection is indicated by the open hatching and is shown to be at 22·5° to the horizontal (indicated by the close hatching).
a—0°, b—90°, c—180° and d—270° rotation positions.

DISCUSSION

The results may be utilized both to explain and to predict the appearance of the bands of Schreger where a specimen is examined with light of predetermined incidence. It may be seen from Fig. 2 that rods angled at between 5° and 40° to the section surface would exhibit reversal of their reflective nature with rotation of a horizontal specimen through 180° relative to a fixed light source (azimuthal rotation). It follows that for a whole band of Schreger to exhibit reversal upon azimuthal rotation, the enamel rods constituting that band must be inclined to the section surface at angles between 5° and 40°. It follows further that for mutual reversal of Schreger bands to occur on azimuthal rotation, all the enamel rods involved must be inclined to the section surface at angles between 5° and 40°. These rods would be divisible into adjacent rod groups (each constituting a reversible band of Schreger), the rods of adjacent groups arriving at the section surface from opposite directions. This configuration of enamel rods is a pre-requisite for complete reversal of the bands of Schreger and it accounts for two points noted during the sequence of band appearances which accompanies reversal (LESTER, 1965c).

Firstly, maximal demarcation and width of bands would be expected at the 0° and the 180° rotation positions (DE BOER and STIEBELING, 1958; KLEES, 1964) where the incident beam was by definition aligned parallel with the longitudinal axes of the rods because it is there that the greatest range (5°–40°) is found within which rods can lie and appear reflective to some degree (Fig. 2). Thus, it is where the incident beam is aligned with the longitudinal axes of the rods that the greatest range of inclinations (or the greatest potential) exists for rods to exhibit reversal and it is, therefore, at these positions that maximal band reversal and demarcation will occur.

Secondly, some explanation may be found for the apparent splitting of the "light" (reflective) bands which occurs at the 90° and the 270° rotation positions (that is where the incident beam is aligned perpendicular to the longitudinal axes of the rods) during mutual band reversal (HOLLANDER, *et al.*, 1935; LESTER, 1965c). At these two positions of the specimen, reflection is exhibited by rods inclined to the section surface at angles between 0° and 20° regardless of the direction from which the rods arrive at the section surface (Fig. 2). This means, in effect, that for each group of similarly directioned rods arriving at the section surface and previously demarcated as either a "light" or a "dark" band of Schreger, the potential exists for two basically different appearances. As stated above, the maximal rod inclination to the section surface would be 40° if mutual band reversal were to occur. This maximal inclination (or deviation) has been shown to occur at the centre of a diazone (after PICKERILL, 1913) or the mid-region of a "rod group" whilst the rods on either side of the mid-region gradually alter their inclination until, at the border of the rod group, the rods run near-parallel to the section surface (see, for example, LESTER, 1965b). It follows that, at the 90° and 270° rotation positions, groups of similarly directioned rods previously appearing as a single "light" band of Schreger would exhibit a central dark area (the rods involved being inclined to the section surface at angles greater than 20°) and two bordering "light" band segments (those rods involved being inclined to the section surface at angles less than 20°). Such an appearance would, for a rod group previously appearing as a "light" band at the 0° or the 180° position, constitute a "split" light band. Taking the specimen overall, there would be a doubling of the total number of bands of Schreger.

The appearance of any one area during rotation of a specimen through 360° about an axis perpendicular to the section surface has previously been found to be essentially the same at the 90° and the 270° rotation positions, even though the axis of rotation may have been inclined to the vertical. Because of the manner in which the rotating stage was set up, however, and it would certainly be the case for a purely horizontal specimen rotation, the same rods are inclined equally to the horizontal in both these positions. This equal inclination of rods to the horizontal where their longitudinal axis is transverse to the light beam would obviously account for the similarity in appearance at these two positions, for it has been shown that at these positions the direction of the rod to the section surface does not influence reflection and that the range of inclinations at which the rods will reflect is the same (Figs. 2 and 3).

Some explanation can also be found for those rotation sequences in which no band reversal takes place in certain regions of the enamel, these areas remaining either "dark" or "light" throughout the entire rotation. In the former instance, the rods in the region concerned would lie at some inclination to the section surface (in this case the horizontal) greater than 40° and (see Figs. 2 and 3) would remain non-reflective throughout the entire rotation. In the latter instance, however, the rods in the region concerned would lie near horizontal (within 5° either side) and so could remain reflective, to some degree at least, throughout the entire rotation. There seems little point in attempting to relate an appearance by transmitted light of zones of transversely sectioned rods (diazones) and zones of longitudinally sectioned rods (parazones) which are virtually unalterable by azimuthal rotation of the specimen, to an appearance by reflected light (dark and light bands of Schreger), which is potentially reversible. Nevertheless, descriptions of a "dark diazone" and a "light parazone" are not uncommon (PICKERILL, 1913; CHURCHILL, 1935; WIDDOWSON, 1952; STAZ, 1946; ERASQUIN, 1955). Although such descriptions are unjustifiable, some explanation of their probable origin can be found in the present results. Under the experimental conditions described, rods inclined to the section surface at an angle greater than 40° would not reflect at any point during a horizontal rotation of the specimen through 360°. By transmitted light, however, the more obliquely angled of these rods would constitute a diazone. On the other hand, rods inclined to the section surface at an angle of between 5° and 20° would retain some reflective appearance during the major part of such a rotation. By transmitted light, however, these same rods would constitute a parazone.

It is obvious that the actual assessment of reflection will not depend entirely upon the reflective source but will be determined in part by the numerical aperture of the optical system. For the experiments described above, the value for the numerical aperture of the objective was 0·30, which is quite low for its listed magnification (×32). A relatively long working distance is necessitated, however, by the presence of the upper hemisphere of the universal rotating stage.

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Résumé—Des coupes, par usure, polies de l'émail dentaire sont étudiées en lumière polarisée transmise et en lumière transmise à angle d'incidence connu, en utilisant un dispositif universel de rotation. La lumière polarisée permet d'évaluer l'inclinaison de certains groupes de prismes parallèles à la surface de la coupe. La lumière incidente permet d'évaluer la variation tri-dimensionelle de positions que ces groupes de prismes peuvent présenter par rapport à la lumière incidente, tout en restant en réflexion.

Une série de comportement ou d'aspect des bandes de Schreger, décrite antérieurement après rotation horizontale de 360° par rapport à la source fixe incidente est ainsi expliquée. Elle permet de rendre compte, en outre, de la différenciation maximale et de l'épaisseur des bandes, lorsque le faisceau incident est orienté par rapport aux axes longitudinaux des prismes. De même elle permet d'expliquer: (1) le "fractionnement" de bandes lumineuses, accompagnant l'inversion mutuelle des bandes, (2) l'identité d'apparence lorsque l'échantillon est situé dans les deux positions, où le faisceau incident est perpendiculaire à l'axe longitudinal des prismes et (3) l'absence d'inversion des bandes dans certains specimens.

Ces techniques permettent de définir l'orientation des prismes en sub-surface.

Zusammenfassung—Dünne, polierte Schmelzschliffe wurden im durchfallenden polarisierten Licht und im Licht mit bekanntem Einfallwinkel unter Verwendung eines rotierenden Universalhalters untersucht. Polarisiertes Licht wurde benutzt, um die Neigung bestimmter Gruppen paralleler Prismen zur Schnittoberfläche zu bestimmen. Mit Hilfe einfallenden Lichts wurde die Anordnung dieser Prismenbündel im dreidimensionalen Bereich und in Beziehung zur Lichtquelle und ihrer Reflektionsfähigkeit bestimmt.

Über die Anzahl von Erscheinungsbildern oder -folgen der Schreger-Streifen wird eine Erklärung gegeben, nachdem die Schreger-Streifen an anderer Stelle nach horizontaler Rotation der Untersuchungsobjekte um 360° im Verhältnis zur fixierten Lichtquelle beschrieben worden waren. Dadurch wurde eine Erklärung für die maximale Demarkation und Breite der Bänder gefunden, wenn das einfallende Lichtbündel in der Richtung der Längsachsen der Prismen gerichtet ist. Weiterhin kann die scharfe Begrenzung der Lichtbänder erklärt werden wie auch die Ähnlichkeit des Erscheinungsbildes, wenn sich das Objekt in den zwei Positionen befindet, bei denen der Lichtstrahl senkrecht zu den Längsachsen einfällt. Schließlich erklärt sich auch das Fehlen der Bandumkehrung bei einigen Objekten.

Diese Techniken erlauben es, die Richtung der Schmelzprismen unter der Oberfläche zu definieren.

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Carbon Replica Study of Developing Dentin:

K.S. Lester and A. Boyde, King's College
and the London Hospital Medical College, London.

The nature and morphology of the surface resulting after pulp is removed from the developing dentin was studied in a number of different species by direct one-stage carbon replication. The evaporated carbon layer that was to constitute the replica was separated from the tooth by extracting the organic material with hot diamino-ethane, washing with water in place of the organic solvent, and dissolving the remaining inorganic material in dilute HCl. Pieces of replica were collected on copper grids, and stereopair electron micrographs were prepared at 40kV. Contour maps were drawn and profiles were constructed with the aid of a simple photogrammetric instrument. The study revealed details of the three-dimensional shape of the predentinal surface and the orientation of the collagen fibrils at this surface.

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Electron Microscopy of Predentinal Surfaces

K. S. LESTER[1] [2] and A. BOYDE[1]

Anatomy Departments, The London Hospital Medical College[1], London E. 1.
and King's College[2], London W.C. 2, Great Britain

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The nature and morphology of the surface resulting when the pulp is stripped away from the developing dentine was studied in a number of different mammalian species. The surface was examined both by a one-stage carbon replica technique and directly in a scanning electron microscope. In both instances, stereo-pair electron micrographs were prepared by tilting the specimen between exposures. In the case of the replica film, stereo-photogrammetric techniques were applied so that the third dimension could be appreciated and reconstructed. Collagen fibrils were evident at most of the replicated surfaces and appeared always to be orientated parallel with the developing front. Within this orientation, the fibrils formed an extensive network and, especially in manatee predentine, were orientated circumferentially about the odontoblastic tubule openings.

La nature et la morphologie de la surface dentinaire, en voie de développement, sont étudiées dans un certain nombre de mammifères, après ablation de la pulpe. Cette surface est examinée à l'aide d'une technique de réplique en carbone et directement sous un microscope électronique "scanning". Dans les deux cas, des micrographies électroniques stéréoscopiques sont réalisées en inclinant le specimen d'une photo à l'autre. Pour les répliques, des techniques stéréo-photométriques sont appliquées afin d'apprécier et de reconstituer la troisième dimension. Des fibrilles collagènes sont visibles au niveau de la plupart des surfaces étudiées. Ces fibrilles sont toujours orientées parallèlement au front de développement. Les fibrilles constituent un réseau étendu dans le cadre de cette orientation majeure et principalement dans la prédentine du morse, où elles sont orientées circulairement autour des ouvertures des canalicules dentinaires.

Die Eigenschaft und Morphologie der Oberfläche nach Entfernung des Marks vom in der Entwicklung befindlichen Dentin wurde in einer Reihe von verschiedenen Säugetieren untersucht. Die Oberfläche wurde einer einstufigen Kohlereplikatechnik und direkter Raster-Elektronenmikroskopie unterworfen. In beiden Fällen wurden stereoskopische Elektronenmikrographien angefertigt, indem die Präparate zwischen zwei Belichtungen geneigt wurden. Beim Replikafilm wurden auch stereogrammetrische Methoden angewandt, um die dritte Dimension sichtbar und rekonstruierbar zu machen. In den meisten replizierten Oberflächen konnten Kollagenfibrillen gesehen werden, die immer parallel zu der sich entwickelnden Front erschienen. Innerhalb dieser Orientierung bildeten die Fibrillen ein ausgedehntes Netzwerk; insbesondere umgaben die Fibrillen in Manateepredentin die Öffnungen der odontoblastischen Kanälchen.

Introduction

Many electron microscope replica studies of the calcified dental tissues have been made since the original work of GEROULD (1944, 1945) and of RICHARDS and THOMASSEN (1944). However, the only studies of the predentinal surface seem to be those of HELMCKE and JAHN (1952a, 1952b) and HELMCKE (1953). These workers used a two-stage technique in which the plastic replica film stripped from the specimen surface was shadowed with tungsten oxide and coated with silicon monoxide by evaporation in vacuo. The original replica was then dissolved.

For Reprints: Dr. A. BOYDE, Anatomy Department, The London Hospital Medical College, London E. 1, Great Britain.

Further investigation of the surface both by a one-stage carbon replica technique, and directly in a scanning electron microscope, seemed justified on several grounds.

The replicas examined by Helmcke and Jahn were in part pseudo-replicas; that is to say they were contaminated with material withdrawn from the specimen when the replica was stripped from it.

Significant distortion of surface detail caused by inelastic deformation of plastic replica may occur during the stripping process. The one-stage carbon replica technique, on the other hand, has the advantages that the surface to be replicated is dissolved away from the replica, leaving the latter relatively undisturbed; and that the layer of carbon constituting the replica is quite rigid and fractures rather than bends.

While no information concerning the preparation of the predentinal surface for replication was given by Helmcke and Jahn (1952a, 1952b), Helmcke (1953) did mention that the unfixed pulp and odontoblastic layer were easily removed from the predentinal surface. This has not been our experience. It is possible that in Helmcke's preparations there was incomplete removal of cellular material prior to replication and this is supported by his description of extracted odontoblastic cell membranes attached to the replicas.

Materials and Methods

1. Carbon Replication

The predentinal surfaces of a number of different mammalian species (human, monkey, dog, calf, pig, dolphin and manatee) were prepared and examined. The predentinal surface was exposed for replication by stripping the pulp away from it after the tooth had been immersed in 10% neutral formol saline for at least 48 h, the latter step having been found to greatly facilitate the removal of the cellular material.

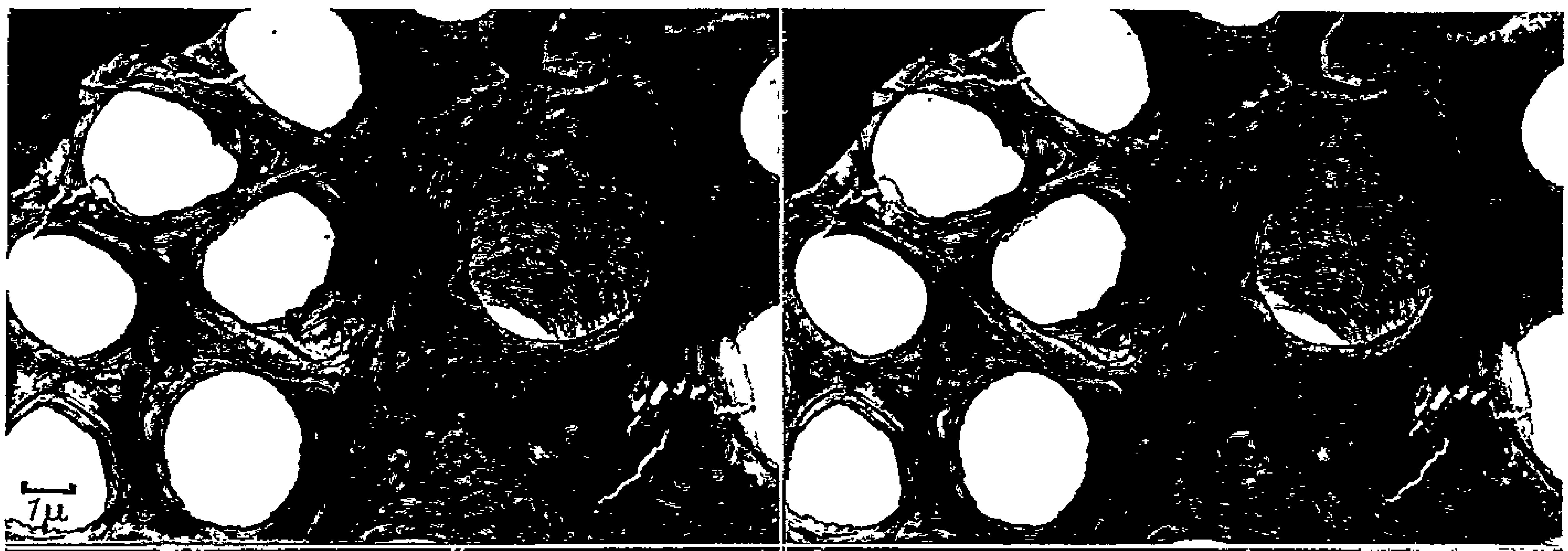
The replica technique has been described by Boyde (1967). The area for replication was selected from material dried *in vacuo*. A layer of carbon was evaporated onto the specimen whilst rotating and facing the carbon arc at an angle of 45°: this ensured the complete coverage of even very rough surfaces. The aim of the replica removal technique was to leave the carbon layer mechanically undisturbed, by dissolving the substrate (in this case the tooth) away from it, rather than by stripping the replica from the substrate as in "conventional" replica procedures. The specimen (covered with the evaporated carbon layer) was placed in a Soxhlet apparatus and the organic material extracted with 1:2 diamino-ethane over a period of at least 24 h. With the specimen still in place, careful washing was then carried out by refluxing water through in place of the organic solvent. Finally, the remaining inorganic component was dissolved in N HCl. Small pieces of carbon film were then picked up on copper grids and examined in a Siemens Elmiskop I at 40 kV.

2. Stereophotogrammetry

In order to appreciate and reconstruct the three dimensional nature of the replica film, stereo-pair electron micrographs were prepared. These were examined with a mirror stereoscope to obtain a subjective impression of the three-dimensional morphology of the surface. Contour maps were prepared, and profiles through the surface were reconstructed as part of a closer, quantitative analysis using a Hilger and Watts stereometer (Martin, 1966).

3. Modelling Via the Stereosketch

The three-dimensional optical model resulting from the fusion of the stereo-pair electron micrographs was itself reproduced with the aid of a Hilger and Watts "Stereosketch". This



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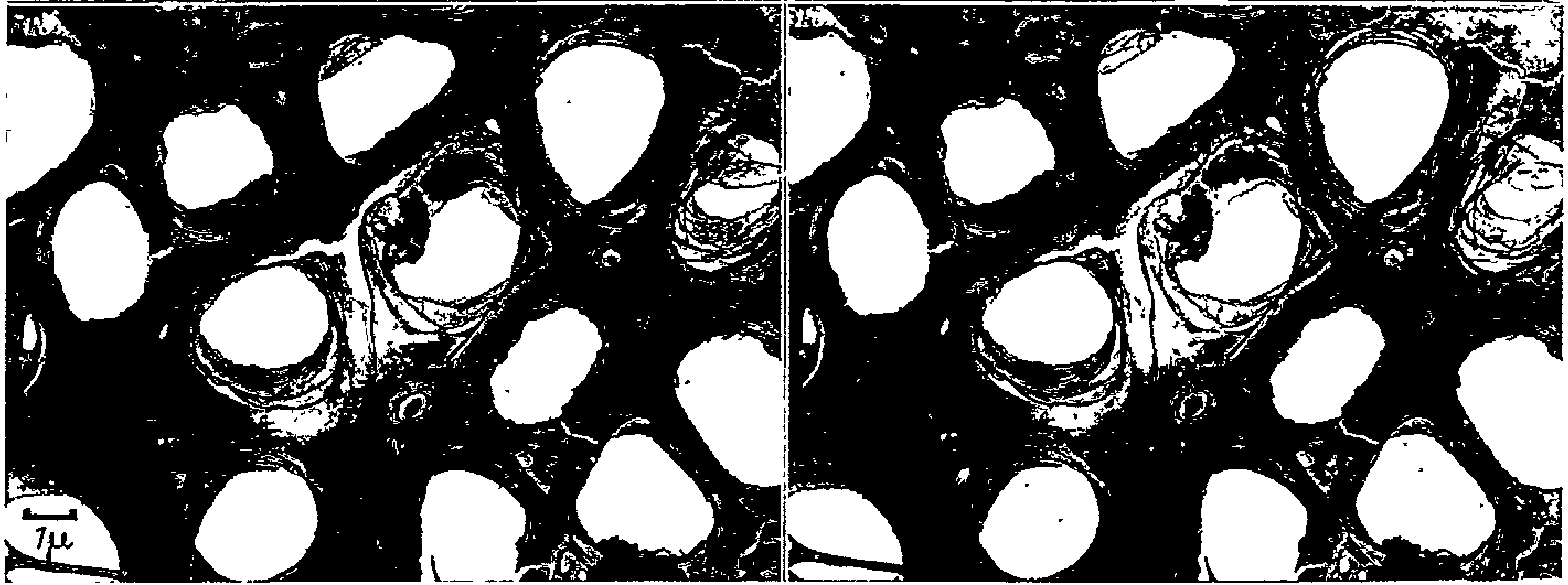

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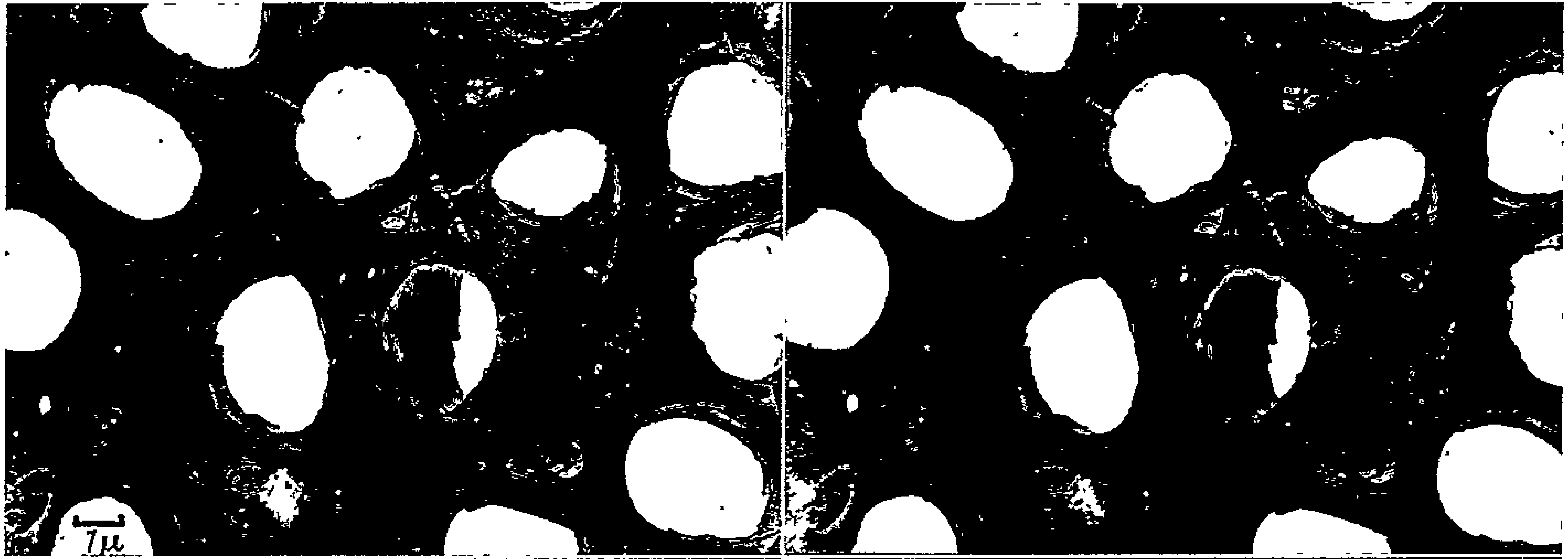

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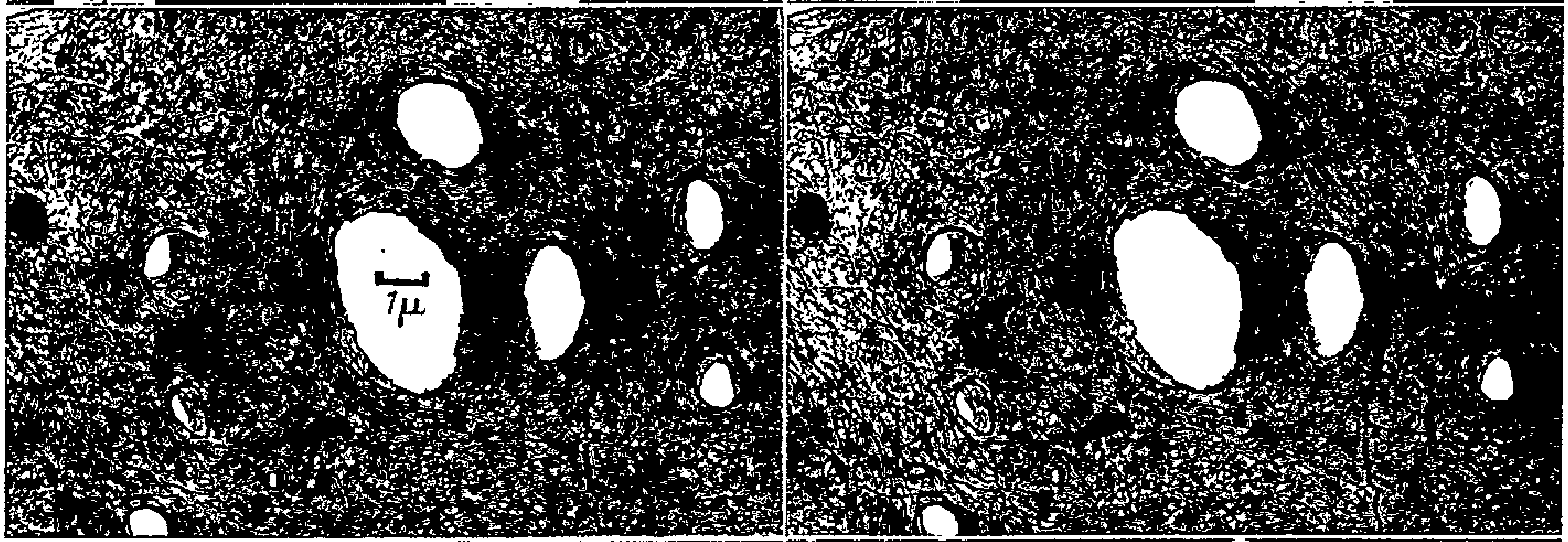

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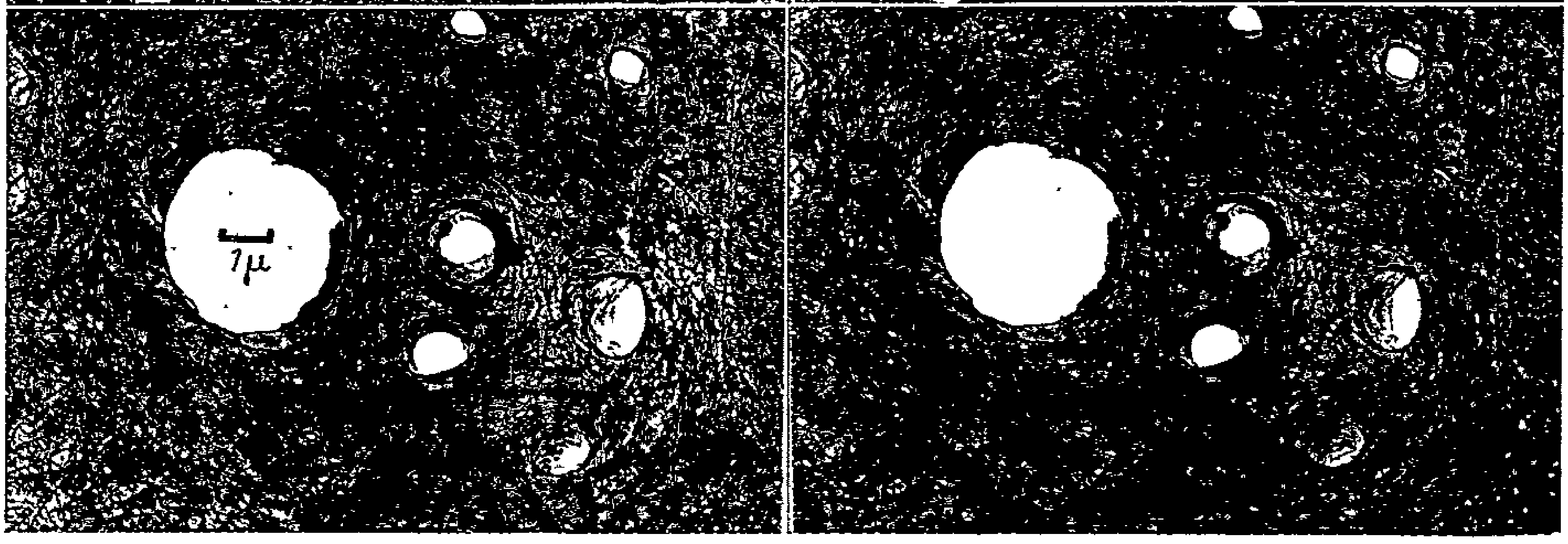

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Figs. 1—5. Legends see p. 47

device is essentially a mirror stereoscope in which the first pair of mirrors are only semi-reflecting, thus permitting a working surface to be seen at the same level as the stereoscopic image apparently hanging in space. After some practice, a plastic material can be moulded to fit this image.

4. *Scanning Electron Microscopy*

Predentinal surfaces exposed as above were examined directly in a scanning electron microscope (Cambridge Instrument Company "Stereoscan"). Some surfaces were covered with a conducting layer of gold (ca. 400 Å thick) and examined using accelerating voltages in the range 2—20 kV. Other surfaces were left uncovered and examined using accelerating voltages in the range 1—2 kV. Again, stereo-pair images were recorded by tilting the specimen between exposures.

Results

At all the predentinal surfaces examined, the circular openings leading to the dentinal tubules were clearly evident. Each large opening in the developing front led to a rapidly tapering funnel-shaped depression, from the base of which one or more parallel-sided dentinal tubules continued. There was little regularity in the distribution of these openings however, and variations existed with regard to both the number and the size over very limited areas of the predentinal surface (Figs. 1, 4, 5, 6). In manatee predentine there was a greater variation in the size of tubule openings than in the human material (cf. Figs. 1, 8 with Figs. 4, 5). Also, much larger areas of predentinal surface devoid of tubule openings existed in manatee material.

The stereopsis afforded by the method of examination allowed some assessment to be made of the angle at which the tubules leave the surface, and while in the majority of instances, the neighbouring tubules appeared to leave the surface running largely parallel to each other, adjacent tubules could occasionally be seen passing away from the surface in entirely different directions (Fig. 3).

The concept of the predentinal surface as a smooth surface from which evenly tapering, regularly spaced depressions lead away to more parallel-sided dentinal tubules, as given by Helmcke (1953) (and probably generally accepted), needs to be modified in two ways as a result of the studies. Firstly,the predentinal surface is irregular on a scale of tens of microns — the overall texture of the "wall of the pulp chamber" is not smooth. There are large depressed areas of considerable extent (see Figs. 10, 11). Secondly, the predentinal surface is rough on a scale of

Figs. 1—9. Stereo-pair electron micrographs of carbon replicas of the surface of predentine. Tilt angle $= 8^0\,40'$. The two images are mounted "orthoscopically" so that the appearance of the resulting fused image is as if the surface were viewed from the pulpal aspect

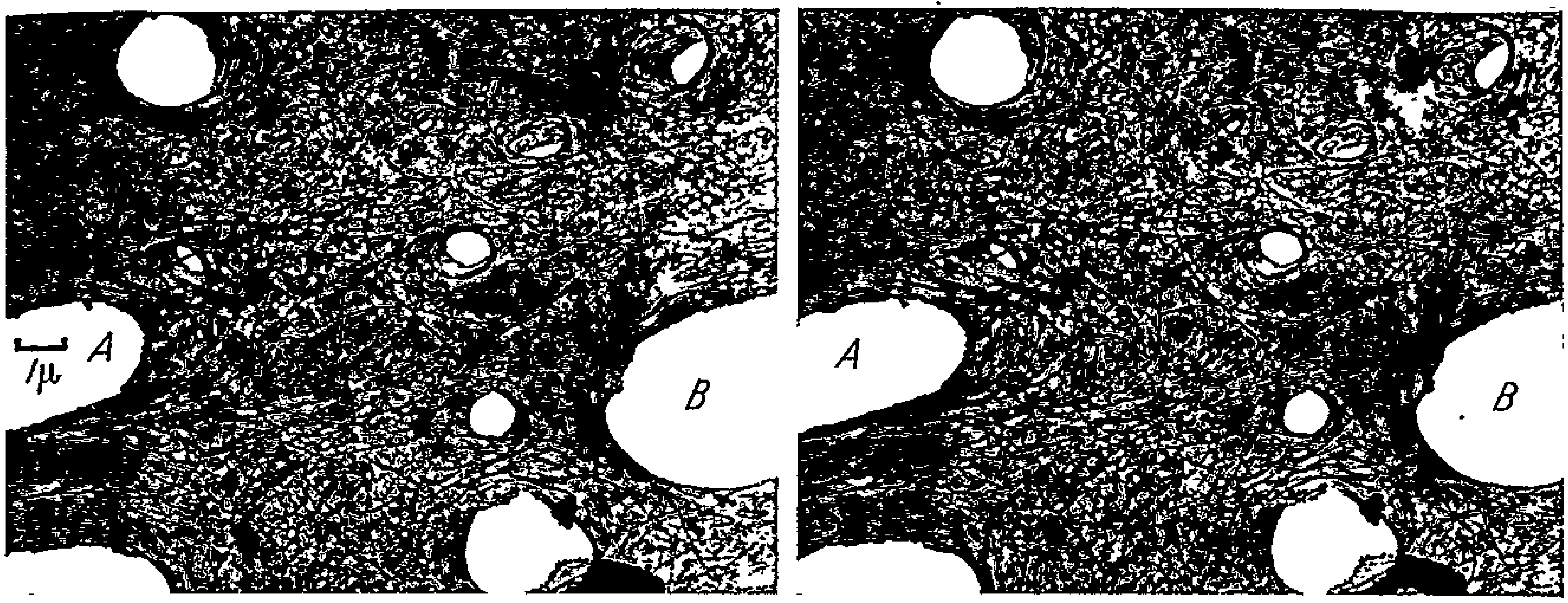
Fig. 1. Human deciduous predentine showing arrangement of collagen fibrils over the surface and the distribution of the tubule openings

Fig. 2. Human deciduous predentine showing a marked ridging of the surface between tubule openings

Fig. 3. Human deciduous predentine. Two adjacent tubules pass away from the surface in different directions

Fig. 4. Manatee predentine. Note the difference in size and distribution of the tubule openings and the circumferential orientation of fibrils about these openings

Fig. 5. Manatee predentine. The five most central tubule openings lie in the same major depression in the developing surface. There is a circumferential arrangement of collagen fibrils about them



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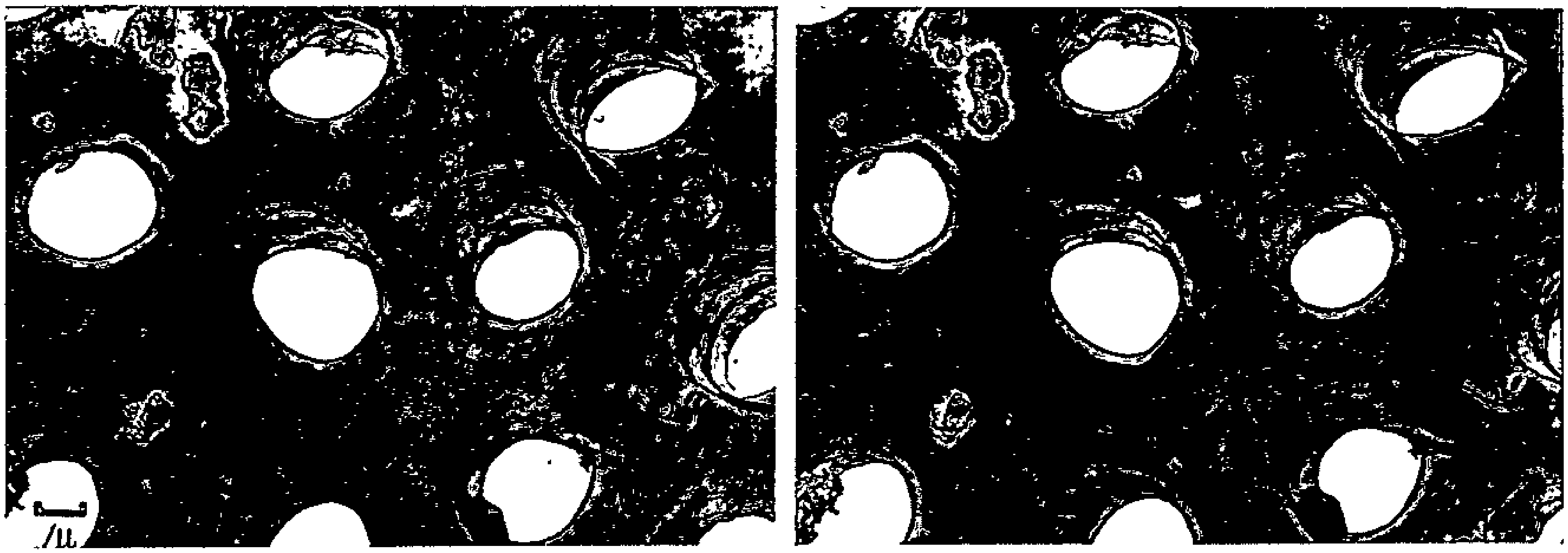

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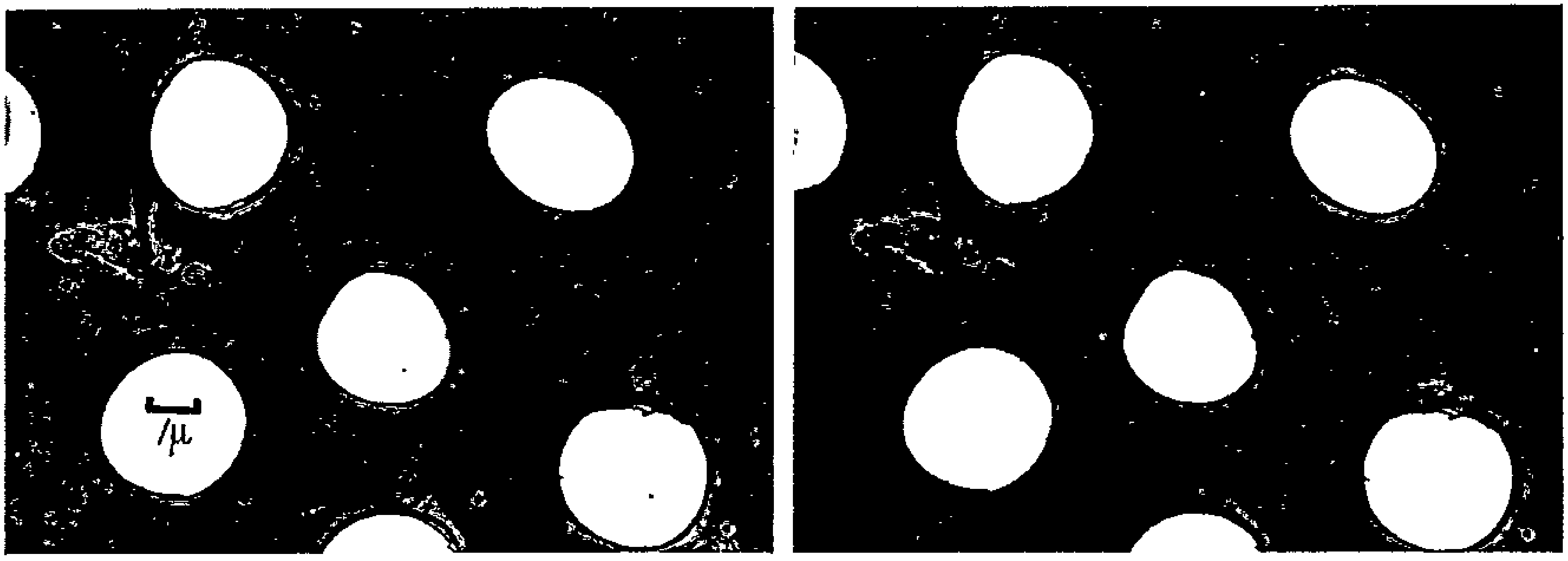

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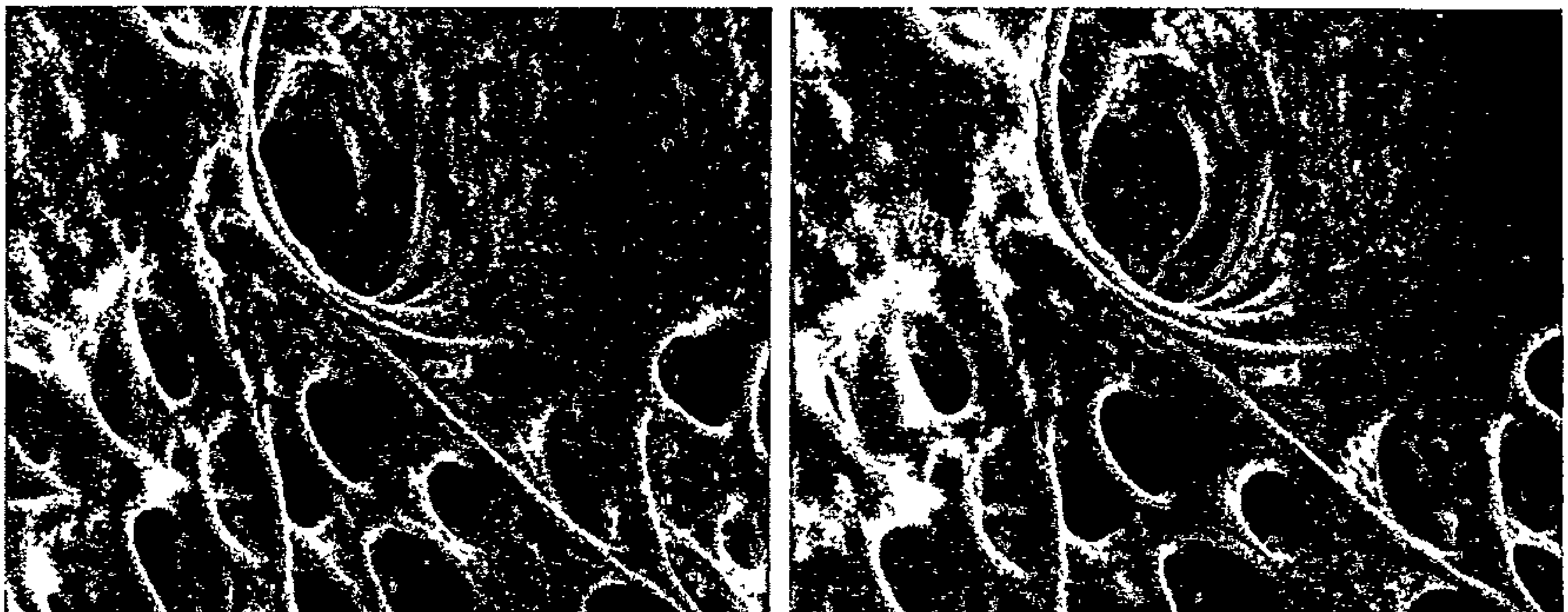

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Figs. 6—10. Legends see p. 49

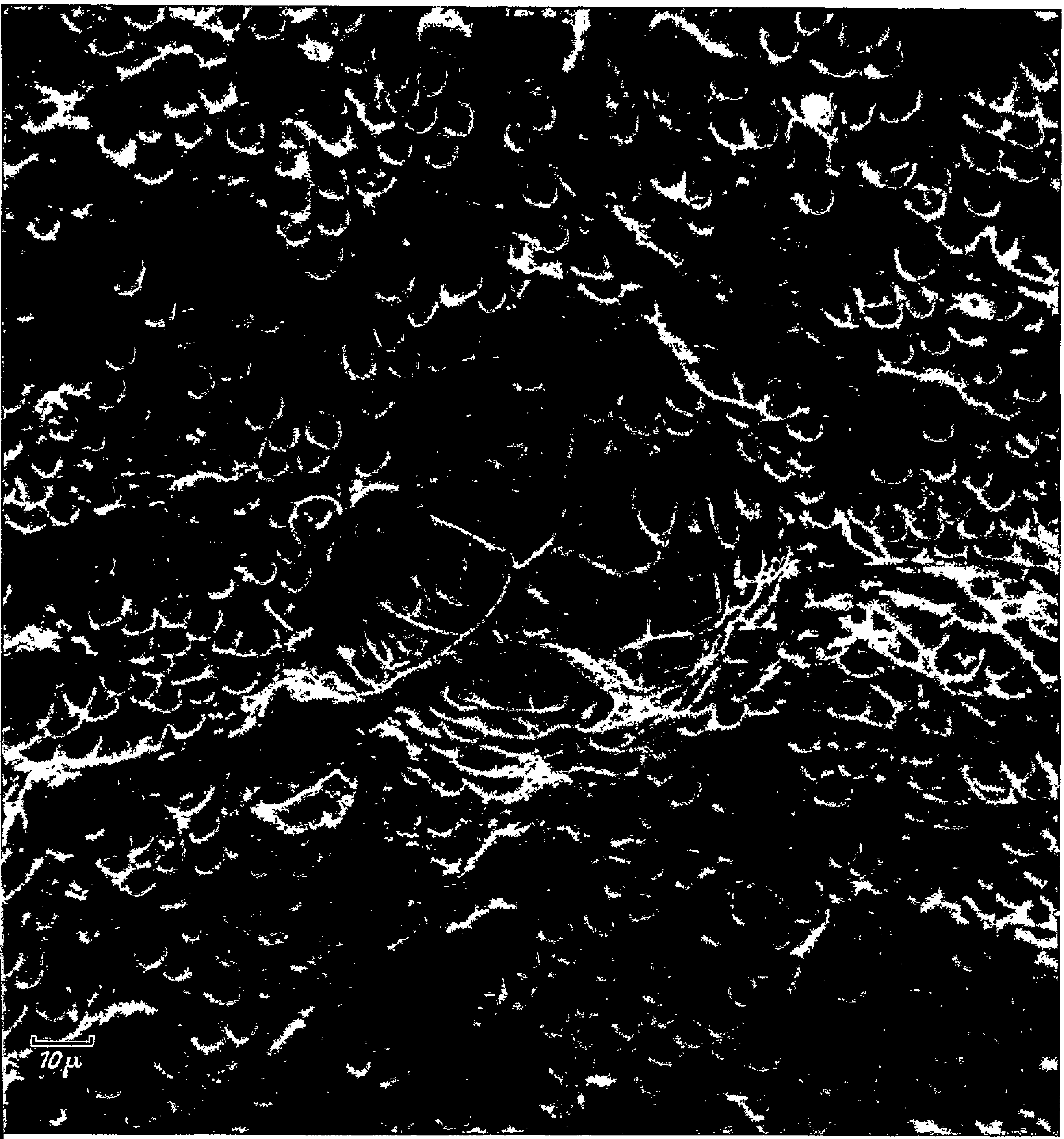


Fig. 11. Scanning electron micrograph of the surface of human permanent predentine. Note the irregularity of the surface contour and the uneven distribution of the tubule openings

Fig. 6. Manatee predentine. Note the groove joining two tubule openings (*A* and *B*)

Fig. 7. Human permanent predentine showing a slight convexity of the surface between tubule openings

Fig. 8. Human permanent predentine. Note the relative flatness of the surface between tubule openings

Fig. 9. Human permanent predentine. Note a shallow groove lies beside a ridge joining two tubule openings (*A* and *B*)

Fig. 10. Stereo-pair scanning electron micrographs of the surface of human permanent predentine. Tilt angle 10°. Because of the nature of the image formation in the scanning electron microscope, a stronger psychological impression of depth results from the fusion of the stereo-pairs than in the case of the transmission microscope

Fig. 12

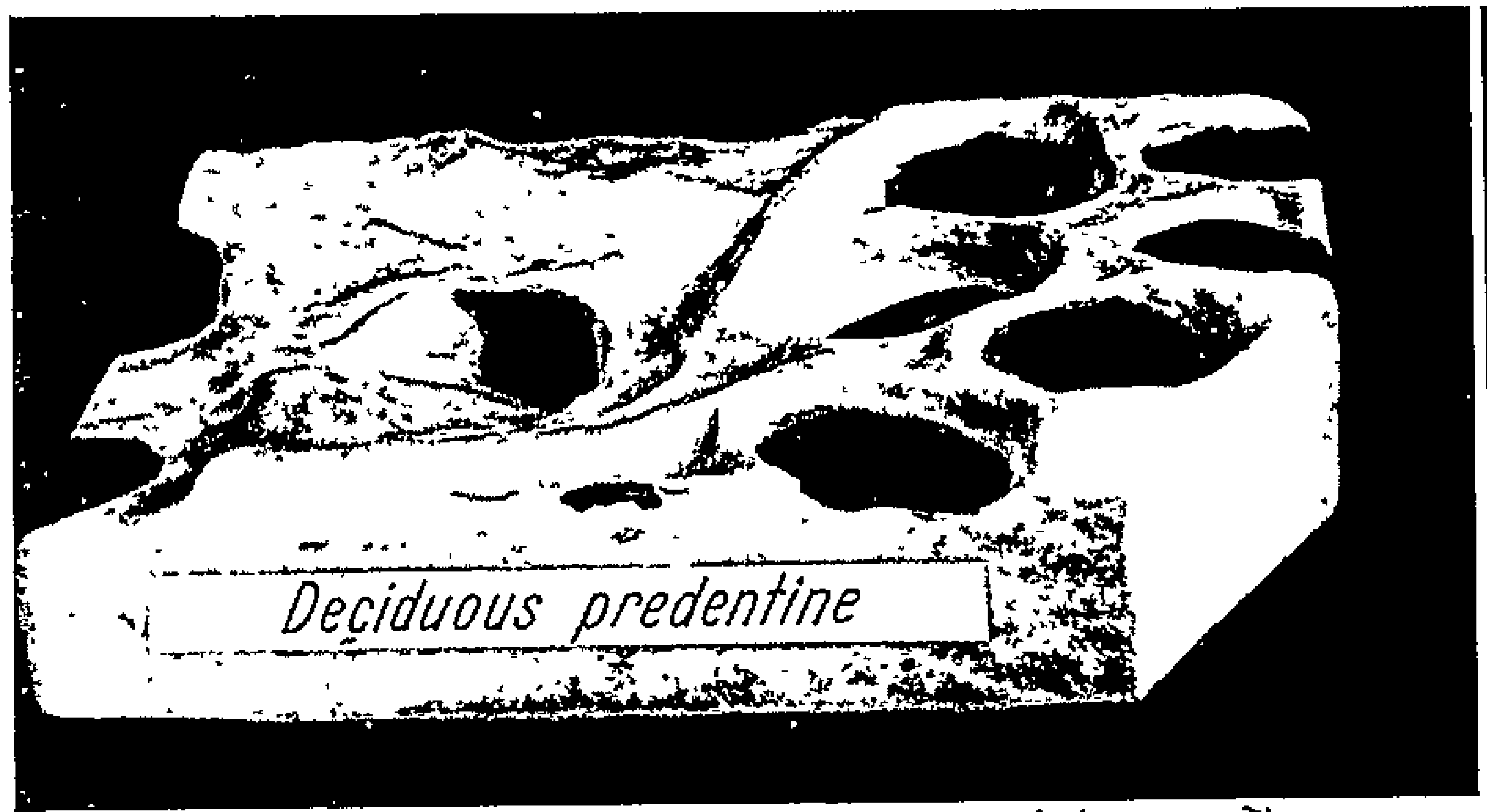
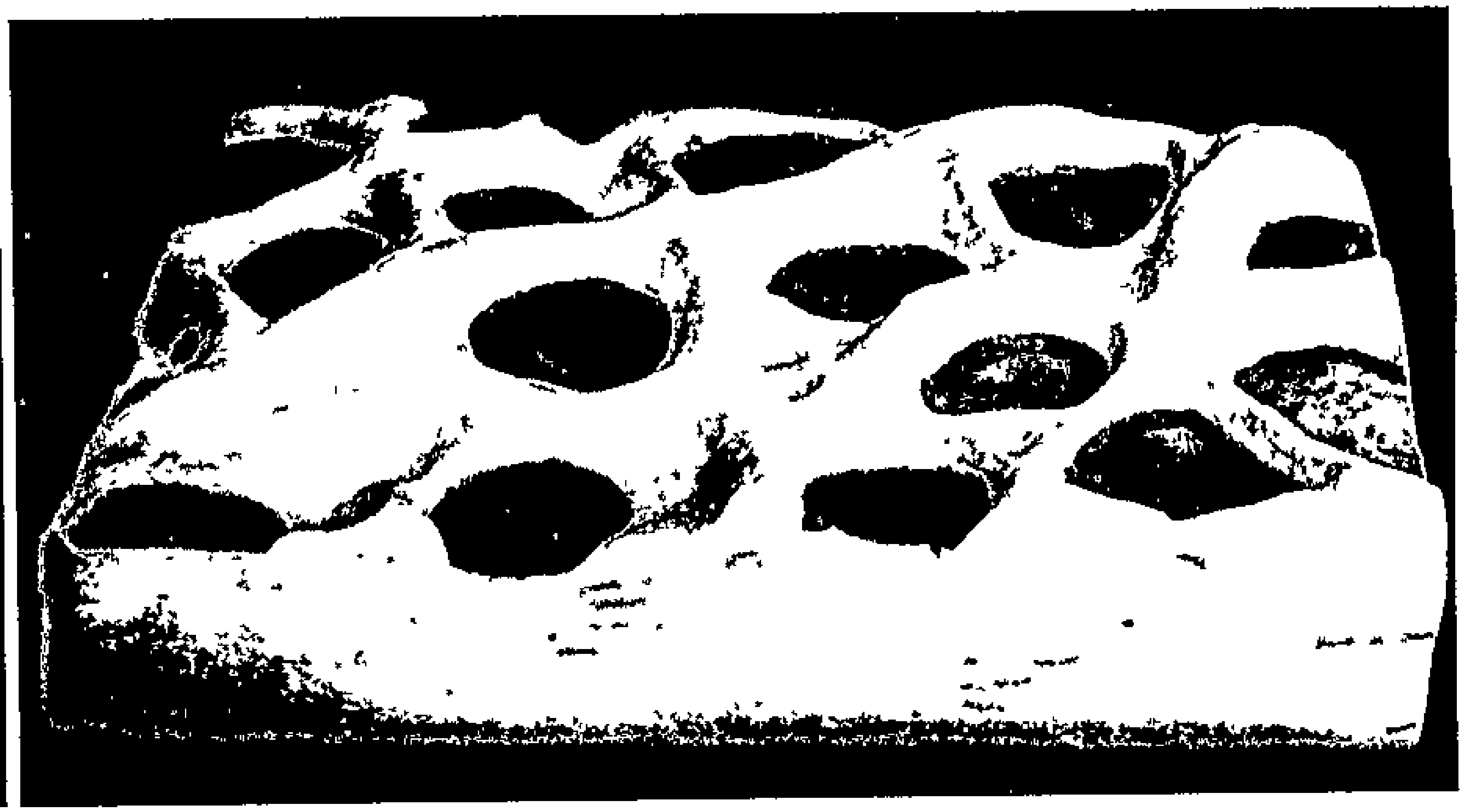
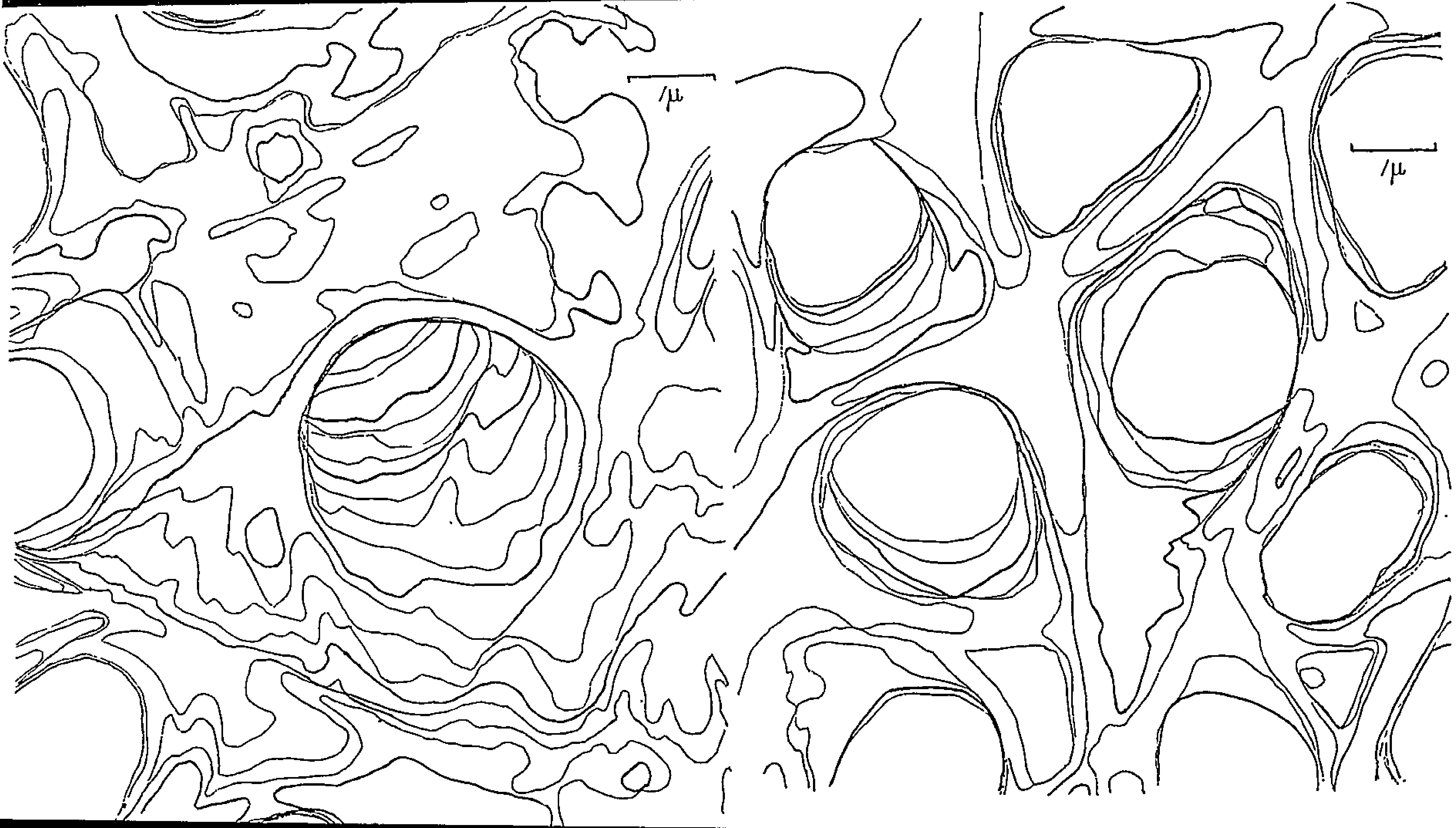


Fig. 13



Figs. 12 and 13. Plaster models prepared with the aid of the "Stereosketch" from the same stereo-pairs as in Figs. 1 and 2 respectively. The tubule openings have been blacked out



Figs. 14 and 15. Contour maps prepared from the same stereo-pairs as in Figs. 1 and 2 respectively (the images are transposed). The contour lines (0.1 μ apart) demonstrate in a quantitative way the ridging between the tubule microns — a profile drawn through the surface from one tubule to another is not necessarily smooth, but is more often irregular or "bumpy", perhaps associated with the presence of large bundles of collagen fibrils at that point.

The contour of the surface also varied from species to species. Thus, whereas in the human material examined the profile between two tubule openings varied from a marked ridging (Figs. 1, 2, 12, 13, 14, 15) through a more gentle convexity (Fig. 7) to a relatively flat surface (Fig. 8), the manatee predentine characteristically exhibited a relatively flat surface contour with little ridging between the major tubule openings (Fig. 4).

At most of the replicated predentinal surfaces, collagen fibrils with typical cross-striations could be observed embedded in an amorphous, slightly granular material (Figs. 1, 6). The amount of this replicated ground substance visible at the surface was variable but occasionally tended to preclude the fibrils from view (Figs. 7, 8).

There was relatively little variation in the extent to which the collagen fibrils lay unmasked at the predentinal surface within the material from any one species, but there was a considerable variation in this unmasking between the human and manatee material on the one hand, and the other species examined on the other hand. For the latter cases, preliminary work has shown that some enzyme digestion may be necessary to unmask the collagen fibrils to a similar degree.

As far as could be determined, the collagen fibrils, whether situated within, about or between the tubule openings, were always oriented parallel to the developing surface. Within this orientation the fibrils formed an extensive network, many of them aggregated into bundles of varying diameter. Both bundles and individual fibrils often changed their course over the surface (Fig. 1). Apart from this general network, the fibrils were preferentially oriented circumferentially about many of the tubule openings (Figs. 4, 5). The diameter of the fibrils ranged from 500—700 Å.

Cellulose acetate and formvar replicas were also prepared because carbon replication of the wall of the pulp chamber resulted in the reproduction of only a very limited length of the dentinal tubules themselves. Although a greater length of tubule wall was represented in these replicas, they were markedly inferior to the carbon replicas with regard to the resolution obtainable.

Discussion

The large water content of predentine means that some distortion of the surface due to shrinkage during drying must be regarded as inevitable. However, the predentine neither cracks nor separates from the underlying dentine, and this is taken to indicate that any shrinkage must occur in a direction normal to the overall plane of the surface. Hence, any distortion would tend to produce a flattening of the profile between adjacent tubules. This distortion would be relieved by the punctuation of the surface as a whole by the openings of the tubules, and it might be said, therefore, that an enlargement of the diameter of the tubules took place during drying.

The overall impression gained from a study of the predentinal surfaces to date is one of irregularity in the distribution, number and size of the tubule openings and in the contour of the developing surface. The irregularity in the distribution of tubule openings at the predentinal surface correlates with the existence of the "tubule deficient" and "tubule-free" zones described by KRAMER (1951).

The appearance of more than one tubule opening within the same major depression in the developing surface strongly suggests that one odontoblast occupied the depression in life and gave origin to a number of odontoblastic processes, each entering one of the tubule openings within that depression. Such an arrangement of odontoblast and processes would result, in mature dentine, in the appearance of multiple lateral branchings of odontoblastic processes as figured and photographed by HANAZAWA (1917) and WALKHOFF (1923). For a lateral branch to establish its separate identity with respect to the major tubule at a given point within the finished tissue, the lateral branch must extend over the developing surface prior to its becoming embedded in the predentine.

Except at the enamel-dentine junction, intercommunicating lateral branches are uncommon. Some slight evidence of grooves joining two tubule openings at the predentinal surface may be seen in Figs. 6 and 9, although this has been encountered very rarely.

In the preparations examined there were no structures which could be readily identified with the "corkscrew fibres of von KORFF". While it is generally agreed that KORFF's fibres are intimately associated with the formation of mantle dentine, there is some difference of opinion as to their later role. While KORFF's fibres are thought by some to persist unchanged throughout the formation of circumpulpal dentine (ORBAN, 1929; BEVELANDER, 1941; NYLEN and SCOTT, 1958), SYMONS (1956) has described a reduction in their diameter. NOBLE, CARMICHAEL and RANKINE (1962), on the other hand, have reported that KORFF's fibres are difficult to detect during the later stages of dentine formation. The apparent absence of demonstrable KORFF's fibres in the present study is a difficulty which may well be resolved by future careful separation and localization of areas to be examined. It is possible however, that this problem in interpretation reflects the difficulties inherent in satisfactorily exposing an uncalcified surface for examination.

The orientation of collagen fibrils in mature circumpulpal intertubular dentine is said to be largely perpendicular to the dentinal tubules (VON EBNER, 1891; KRAMER, 1951; MATSUMIYA and TAKUMA, 1954; SHROFF, WILLIAMSON and BERTAUD, 1954; SCOTT, 1953; FRANK, 1965). It is clear that this predominant transverse or oblique orientation of fibrils with respect to the odontoblastic process in mature dentine would arise as the collagen fibrils — observed in the present study to lie parallel with the developing front — became embedded in predentinal substance as the developing front advanced. Not all dentinal collagen is oriented in this manner however, since collagen fibrils aligned parallel with the odontoblastic tubules have been described in other than the first formed dentine (WEIDENREICH, 1925; BEVELANDER, 1941; JOHANSEN and PARKS, 1962; FRANK, 1965).

The fact that some collagen fibrils are orientated longitudinally with respect to the dentinal tubules and therefore (in very general terms) not parallel to the pulpal surface might be difficult to correlate with the observed parallelism of the fibrils to the developing front at first sight. There is no such difficulty, however, if we regard the walls of the dentinal tubules as extensions of the developing front. The flatter the developing front between tubule openings, the greater will be the proportion of fibrils in a transverse direction. Where the predentinal surface is contoured rather than flat, there is likely to result a three-dimensional "trellis-like" arrangement of fibrils as described by ROUILLER, HÜBER and RUTISHAUSER (1952), SCOTT (1955) and JOHANSEN and PARKS (1962). The successive layers of fibrils lining the sides of the depressions and ridges of the developing surface become ever more nearly parallel with the tubule wall.

The circumferential orientation of the collagen fibrils seen immediately around the openings of the dentinal tubules agrees with the observations made on fully formed dentine by FRANK (1952), SCOTT (1953, 1955) and SHROFF *et al.* (1956) on replicas of polished and acid-etched dentine surfaces and on ultra-thin sections. JOHANSEN and PARKS (1962) found no evidence of this circumferential orientation in human dentine. In the present study it was found to be more evident in the manatee than in the human material (the two species studied in the greatest detail).

The observed fibril diameter of 500—700 Å agrees more with the 600—700 Å recorded by JOHANSEN and PARKS (1962) than the 350—500 Å of NYLEN and SCOTT (1960).

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Electron Microscopy of Resorbing Surfaces of Dental Hard Tissues

A. Boyde and K. S. Lester*
Anatomy Department, University College London, London W.C. 1

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* Our thanks are due to Mr. A.D.G. Stewart for permission to publish Fig. 5, which was prepared by him. The Cambridge Instrument Company Stereoscan scanning electron microscope was provided by the (U. K.) Science Research Council.

Summary. The resorbing surfaces of exfoliated or extracted human deciduous molar teeth were studied directly in the scanning electron microscope and indirectly by a single stage carbon replica technique for the transmission electron microscope. Some specimens of resorbing bone were also examined. Some of the material was examined after a simple washing process and some after removal of the organic matrix with hot 1: 2 diamino ethane.

The typical crossbanding of collagen could be seen on resorbing cement and dentine surfaces. This is taken as an indication that demineralisation is the first step in resorption. The very highly mineralised peritubular dentine remained proud of the resorbing surfaces thus indicating that its mineral component is in some way selectively protected.

Enamel prism "sheaths" were also found to be selectively resistant to resorption and this is assumed to be related to the protection of the mineral component in these regions by their higher and/or different organic content. No prism sheaths were found next to the enamel-dentine junction and there was only a slight step down from the enamel to the dentine.

Large "remineralization" crystals were found located at the borders between adjacent Howship's lacunae.

The natural resorbing surfaces were compared with surfaces subjected to purely physical erosion by 5 keV argon ion beam bombardment (Boyde and Stewart, 1962).

Introduction

There have been conflicting reports in the literature on ultrastructural studies of bone resorption, as to whether the organic matrix or the mineral component is removed first. Most authors seem to be in agreement that bone crystals may be found lying free from the resorbing bone surface (Scott and Pease, 1956) and particularly within the osteoclasts (Cameron and Robinson, 1958; Gonzales and Karnovsky, 1961; Dudley and Spiro, 1961; Hancox and Boothroyd, 1961, 1964). Only the last authors cited have also reported the regular occurrence of demineralised collagen fibres at the resorbing bone surface. Sognnaes, Albright and Frank (1961) described denuded fibrillar material in dentinal resorption sites but thought that the removal of organic and inorganic material was virtually simultaneous.

The present report concerns studies of the morphology of dental hard tissue surfaces undergoing resorption. Enamel, dentine and cement were selected for study both because they have been largely neglected in the past from the point of view of electron microscopic examination and because resorbing deciduous teeth yield large areas of readily identifiable resorbing surface.

Materials and Methods

Enamel and dentine were studied in exfoliated human deciduous molars selected for obvious naked eye resorption of the crown region. Thus resorption was proceeding from the "pulpal aspect" and at right angles or at an oblique angle to the direction of the dentinal tubules and the enamel prisms.

Cementum resorption was studied in the roots of extracted human deciduous molars where obvious resorption bays could be identified. One example of pathological resorption in the very thick cementum of a sperm whale tooth was also studied.

Bone resorption was studied in a piece of inter-radicular alveloar bone attached to an exfoliated human deciduous molar.

All the material used in these studies was fixed in 10% neutral formol saline, and stored in 70% alcohol until used. Cellular debris was removed as far as was possible by the use of a gentle stream of tap water. Some of the specimens were rendered anorganic by prolonged extraction with 1:2 diamino ethane in a Soxhlet apparatus: these were then washed by distilling over absolute ethanol in place of the 1:2 diamino ethane, and dried direct from the alcohol. The untreated specimens were dried down from water or alcohol under vacuum.

The specimens for scanning electron microscopy were glued to aluminium stubs and given conducting coatings of ca. 200 Å carbon and then ca. 300 Å gold in the vacuum evaporator. The surfaces were arranged to face at approximately 45° to the evaporation sources, and rotated during evaporation in order to ensure good coverage. Carbon was used because it has been found to produce a stable surface more resistant to cracking and "island charging", and gold because it is a better secondary electron emitter.

The single stage carbon replica technique for transmission electron microscopy used in these studies has been described by Boyde (1967). Briefly, the ca. 200 Å thick evaporated carbon layer which is to constitute the replica is removed by the complete destruction of the original specimen in hot 1:2 diamino ethane followed by dilute hydrochloric acid.

The morphology of the natural resorption surfaces observed here has been compared with that of polished surfaces of enamel and dentine subjected to physical erosion by bombardment with 5 keV argon ion beams in a scanning electron microscope. The experimental details have been published by Boyde and Stewart (1962).

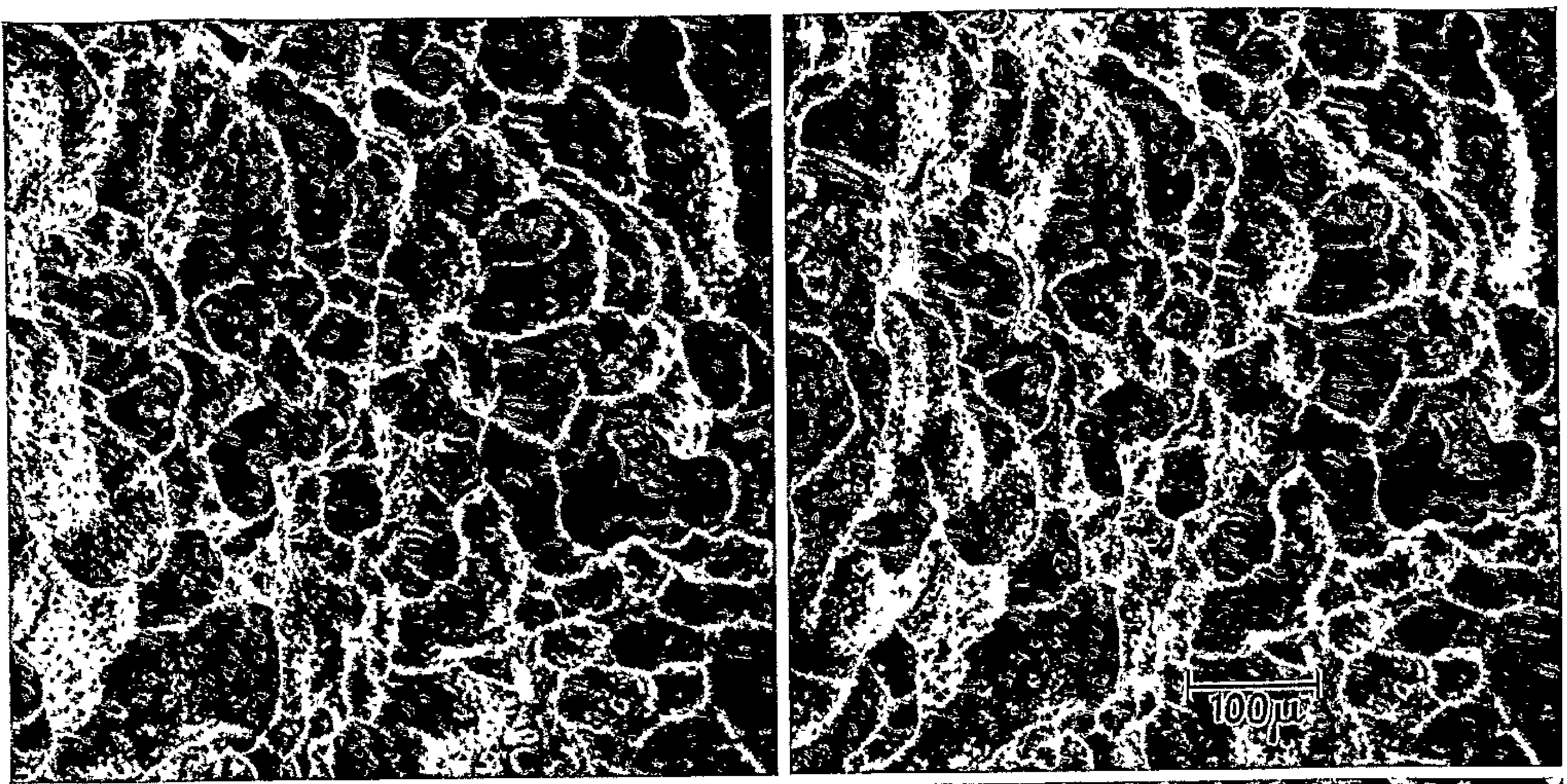
Results

General features of the morphology of resorbing calcified tissue surfaces were easily identified and very clearly displayed in the scanning electron micrographs (Figs. 1—4, 6—10). The great depth of focus of the scanning electron microscope gives it the (so far unique) ability to form coherent images of such very deeply embayed surfaces as those presented by Howship's lacunae. The stereo-pair images of carbon replicas prepared with the transmission electron microscope (Figs. 11, 12) showed a considerably better resolution (ca. 20—50 Å) than the scanning electron micrographs (200—500 Å).

Dentine

An impression of the extent of the undermining that was found to occur during dentine resorption can be gained from Fig. 1. It is apparent that significant amounts of dentine might be freed as irregular particles where two or more Howship's lacunae meet beneath the surface.

The peritubular dentine was found to project above the level of the surrounding intertubular dentine, (Figs. 2, 3, 4) completely sclerosed tubules being prominent (Fig. 4). A zone immediately surrounding the peritubular dentine was frequently observed to be eroded deeper than the surrounding intertubular dentine (Fig. 3). The prominence of the peritubular dentine projections at the resorption surface was slightly increased in the 1:2 diamino ethane extracted material.



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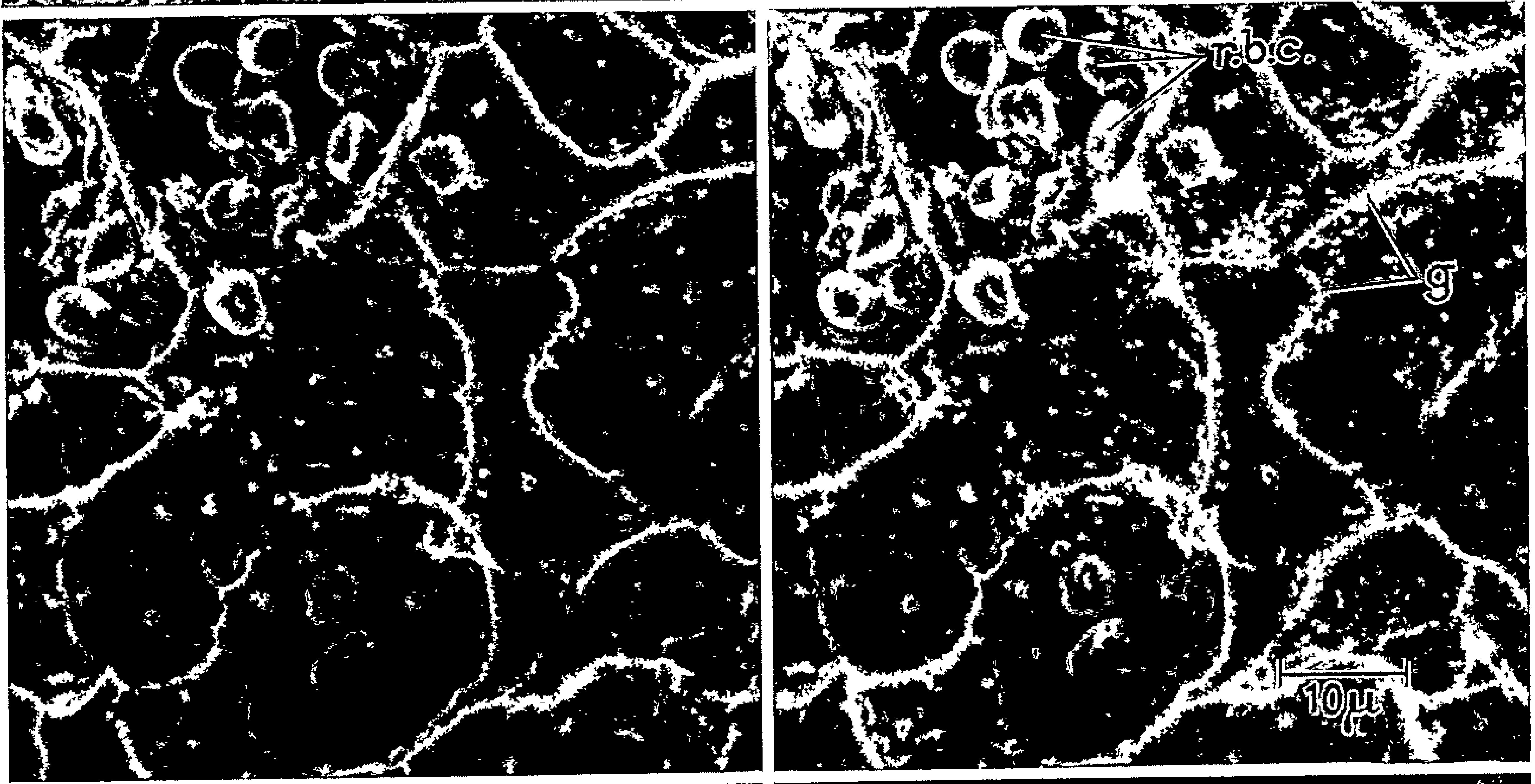

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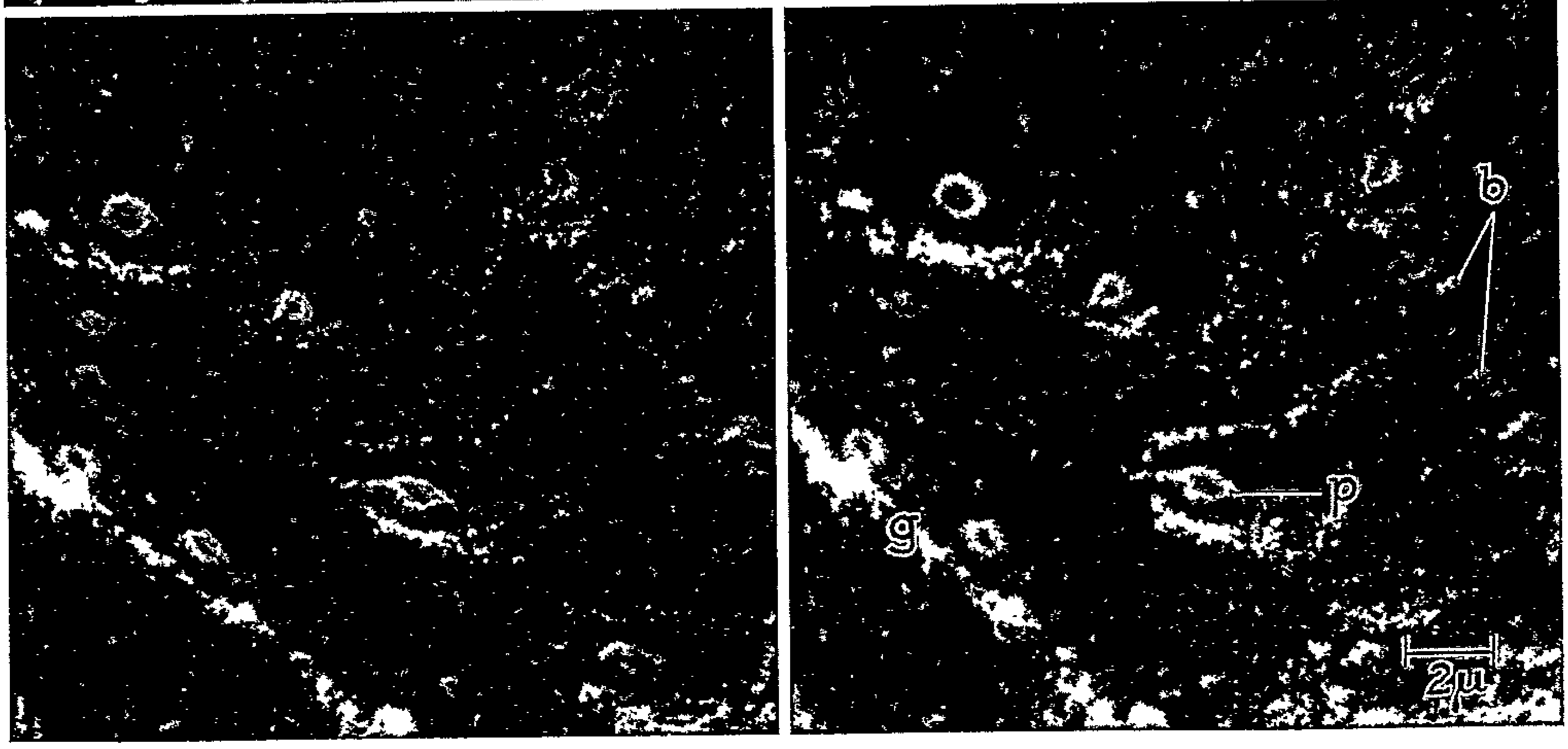

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Figs. 1—3 (for Legends see p. 541)

The replica pictures of the resorbing dentine (and cement) showed the surface to consist of naked, unmasked collagen (Fig. 11). We consider this to indicate that the collagen was demineralized, since our own unpublished findings have shown that the ca. 640 Å cross banding cannot be seen in replicas of mineralized collagenous matrices.

Accumulations of large, regularly shaped crystals were found at the peripheries of the Howship's lacunae in the anorganic material. The peritubular dentine zones in the anorganic material were found to consist of uniform, closely packed particles approximately 250 Å in diameter, corresponding to the description of the mineral component in these regions recently provided by Boyde and Lester (1967).

Enamel

The Howship's lacunae in enamel were generally of the same diameter as those in dentine, but the individual bays did not appear to undermine the overall resorption front to the same extent (Figs. 5, 6, 7, 8).

There was no great difference between the rates of resorption of enamel and dentine, judging from the appearances seen where a single Howship's lacuna covered both sides of the enamel-dentine junction. Figs. 7 and 8 show that although there is a small step down from the enamel to the dentine (indicating that dentine is resorbed a little more rapidly than enamel), its extent does not approach that of the step that forms owing to the much more rapid erosion of dentine under ion beam bombardment (see Boyde and Stewart, 1962).

In both the anorganic (Fig. 8) and the untreated material (Fig. 9) the "prism sheath" regions were remarkably prominent from the resorbing surface, indicating that they were more resistant to resorption than the "prism body proper" and "interprismatic" regions of the enamel. Many different configurations of the prism sheaths were seen, according to various directions of attack with respect to the prism direction.

Figs. 1—3. Scanning electron micrographs of resorbing human deciduous molar coronal dentine

Fig. 1. (Anorganic). Note the deep Howship's lacunae undermining the surface in some places

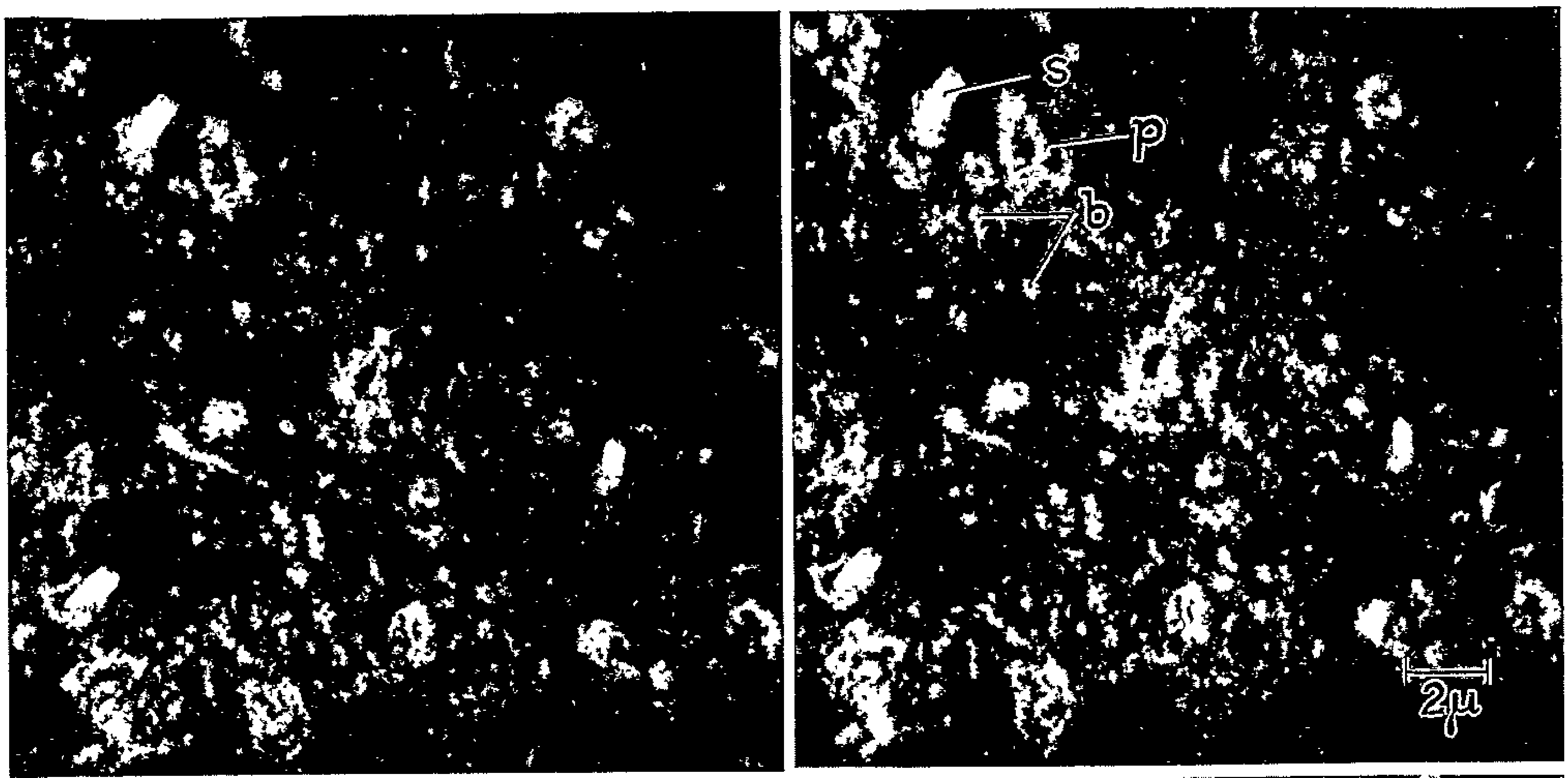
Fig. 2. The edges of the Howship's lacunae are very clearly defined. Several red blood corpuscles have remained adherent even after washing

Fig. 3. (Anorganic). Note that the peritubular dentine zones protrude from the surface and are surrounded by "moats"

The stereo-pair scanning electron micrographs were taken with a tilt-angle of 10° on a Cambridge Instrument Co. *Stereoscan* operating at 10 kV. The stereo-pair transmission electron micrographs were taken with a tilt-angle of 8° 40′ on a Siemens *Elmiskop I* operating at 60 kV. The resorbing surfaces shown in Figs. 1, 3, 4, 6, 7, 8, 10 and 12 were subjected to 1:2 diamino ethane extraction (anorganic).

List of abbreviations:

b	peritubular dentine of branch tubule	*j*	enamel-dentine junction
cs	cementum surface	*p*	peritubular dentine of major tubule
d	dentine	*ps*	enamel prism sheath
e	enamel	*rbc*	red blood cell
g	edge of Howship's lacuna	*s*	peritubular dentine of sclerosed tubule



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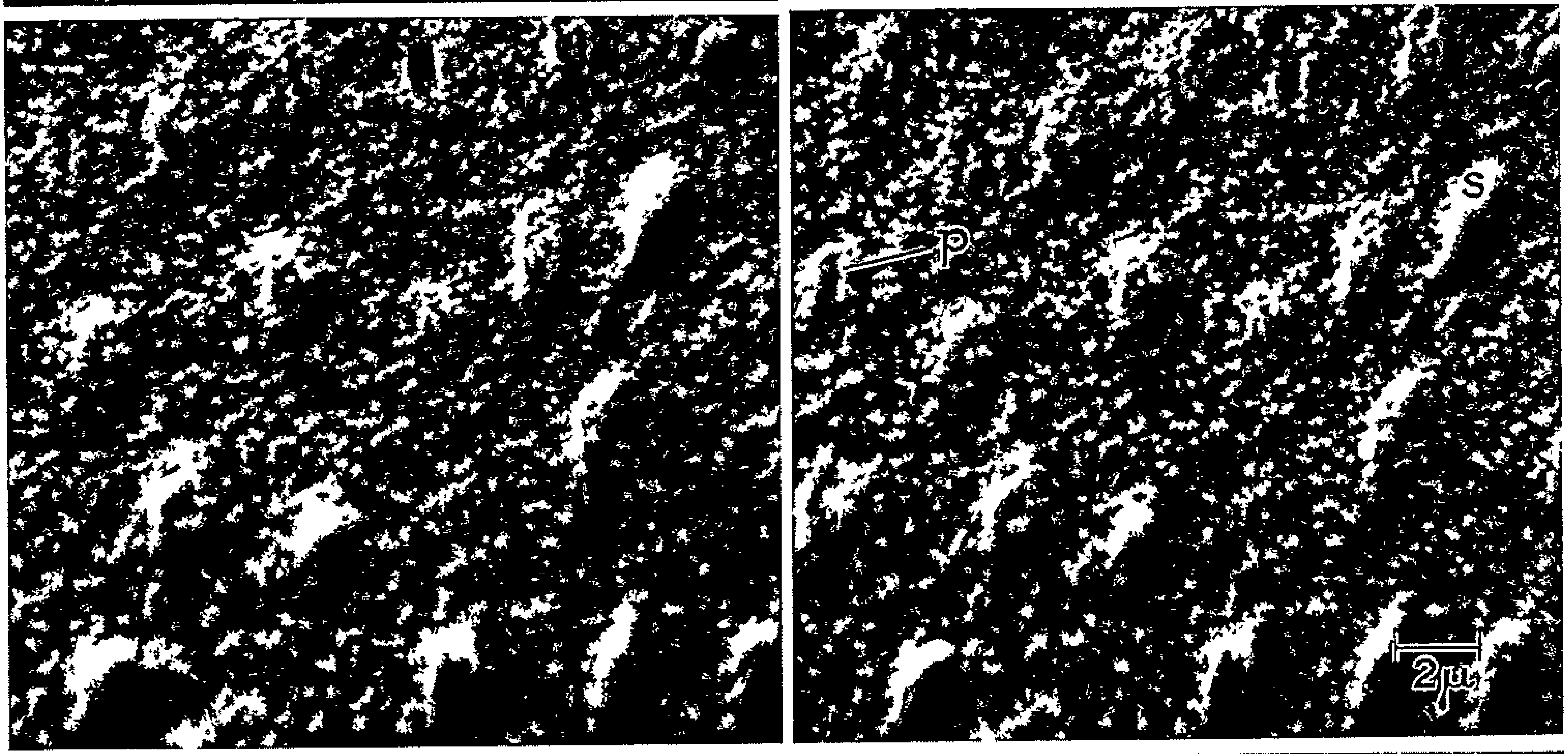

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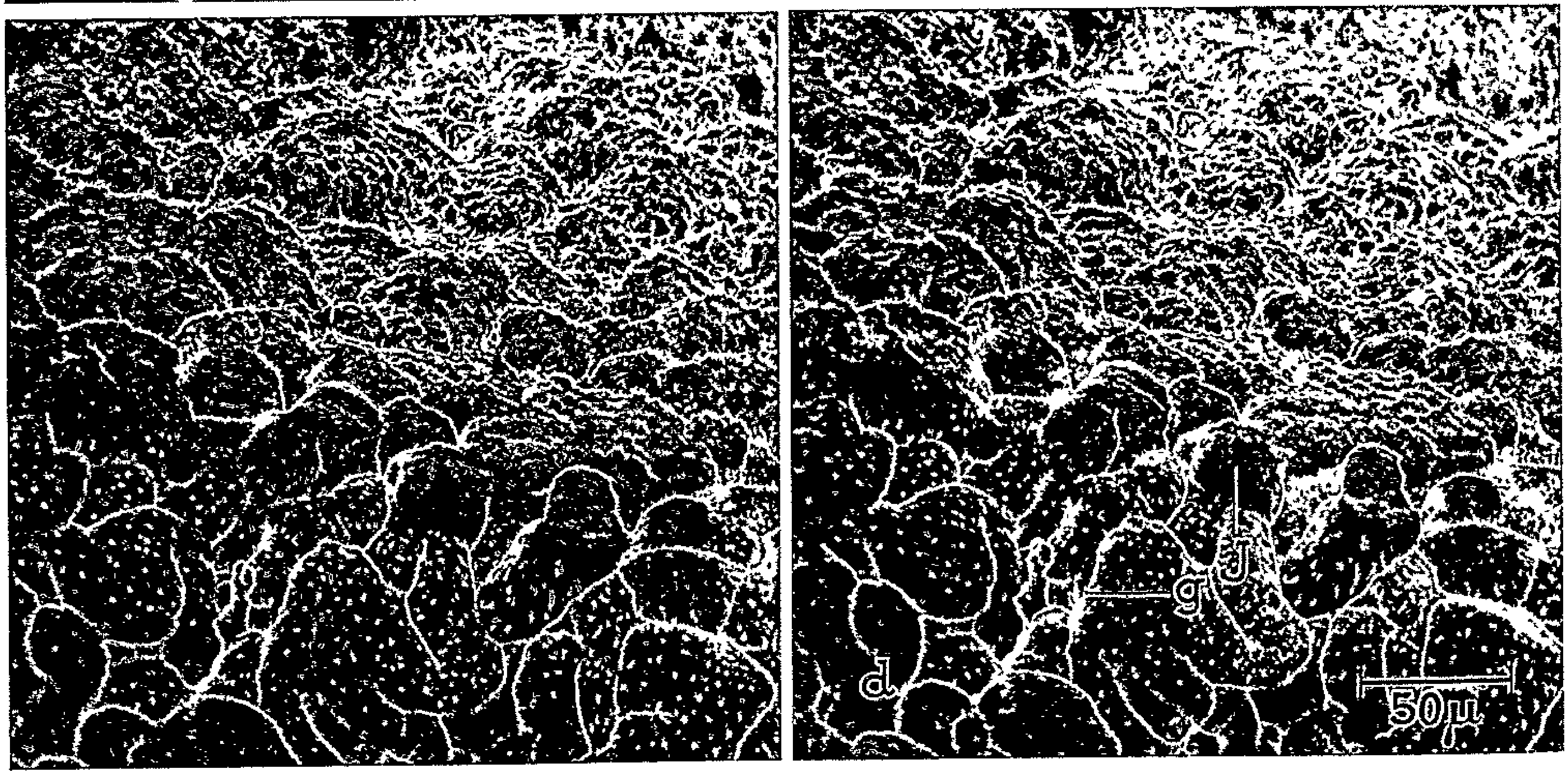

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Figs. 4—6 (for Legends see p. 543)

The innermost layer of enamel at the enamel-dentine junction, on the other hand, was uniformly attacked. Although the individual crystallites could be clearly resolved, no selectively resistant regions corresponding to the prism sheaths found more peripherally could be seen. This corresponds with the observations of BOYDE (1964) that there is no subdivision into prisms in this first formed layer of enamel.

Cementum

The most remarkable general feature of the resorption bays in the cementum was that their edges were very sharp (Fig. 10). A normal cementum surface showing the ends of Sharpey fibres projecting from the surface would suddenly give way to a large cavity formed by numerous, fused Howship's lacunae: these often gave an impression of considerable undermining, similar to that seen in the dentine.

The resorbing surface was interrupted by a series of minute projections, which from their size and distribution have been tentatively identified as sclerosed cementocyte canaliculi (cf. pericanalicular bone, MJÖR, 1962, 1963). In some instances where the resorption might have penetrated into the dentine, these projections might have corresponded to the "peritubular" dentine of sclerosed fine terminal branches of the dentinal tubules. The appearances presented by resorbing alveolar bone were in every way similar to those of cementum.

Discussion

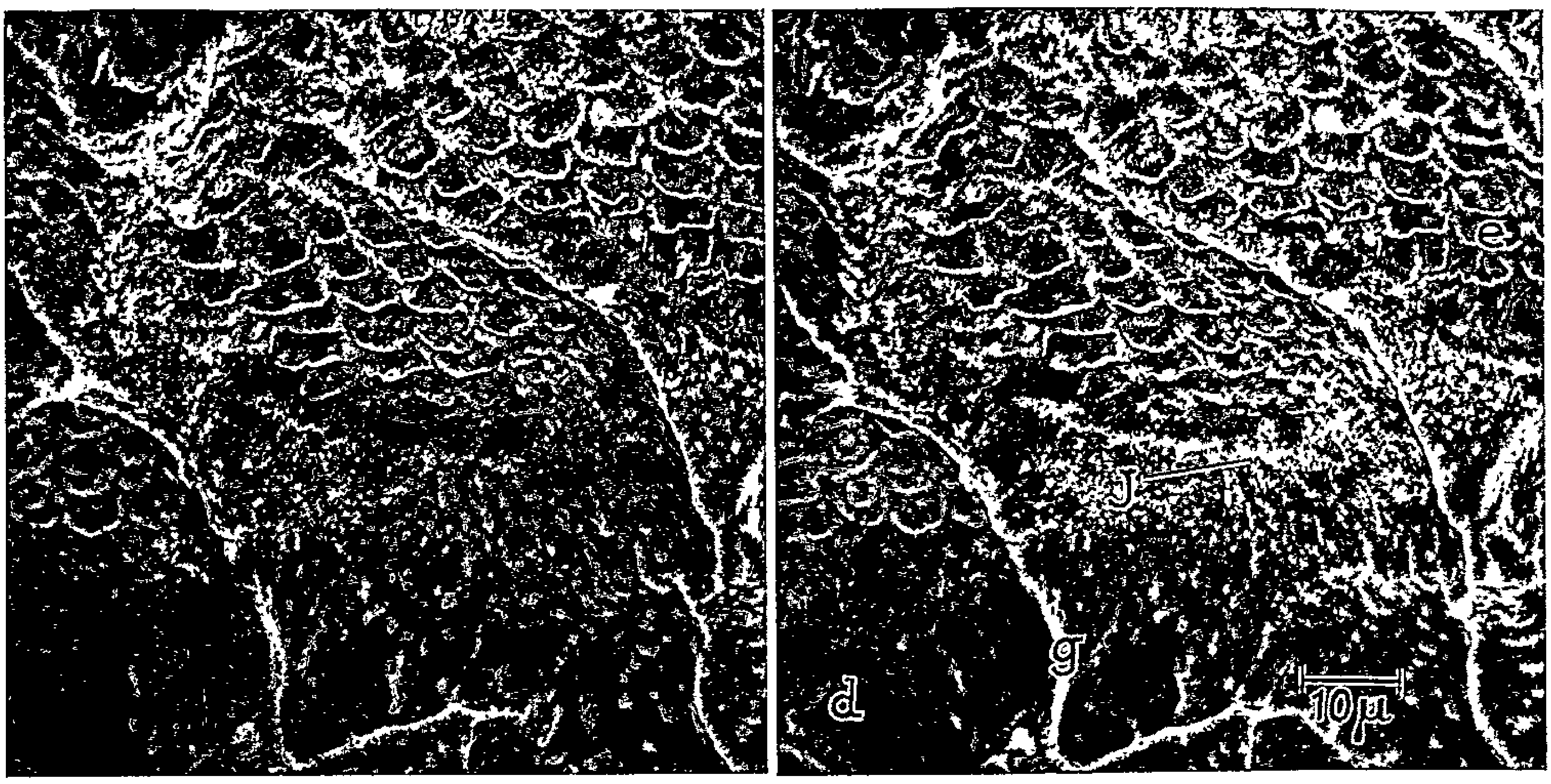
Perhaps the most important point to emerge from the present studies has been that peritubular dentine is more resistant to osteoclastic resorption than the surrounding intertubular dentine. It is well established that peritubular dentine is radically different from intertubular dentine. It is more highly mineralised (MILLER, 1954; BOYDE, SWITSUR and FEARNHEAD, 1961), the mineral component itself has a distinctive morphology (BOYDE and LESTER, 1967) and it has a matrix characterised by the predominance of sulphated acid mucopolysaccharides (SYMONS, 1961) and the absence of collagen (LESTER and BOYDE, in press). There are therefore several ways in which the greater resistance to resorption of peritubular dentine could be explained. Perhaps the simplest explanation is that it is due to the greater density of the peritubular dentine or in other words to a simple physical mass removal effect. In this respect, ion beam erosion of dentine provides an analogous system, and indeed the degree of "etching" of surfaces treated in this way (Fig. 5 and BOYDE and STEWART, 1962) is very similar to that found in resorption surfaces. However, the available evidence suggests that demineralisation is the first stage of resorption, which would lead one to expect the prior or selective

Figs. 4—6. Scanning electron micrographs

Fig. 4. (Anorganic) Resorbing human deciduous molar coronal dentine. Note the very prominent rods (s) corresponding to sclerosed dentinal tubules

Fig. 5. 5 keV argon ion eroded human dentine, showing the degree of prominence of the peritubular zones after purely physical etching

Fig. 6. (Anorganic). Resorbing human deciduous molar showing several Howship's lacunae overlying or crossing the enamel-dentine junction



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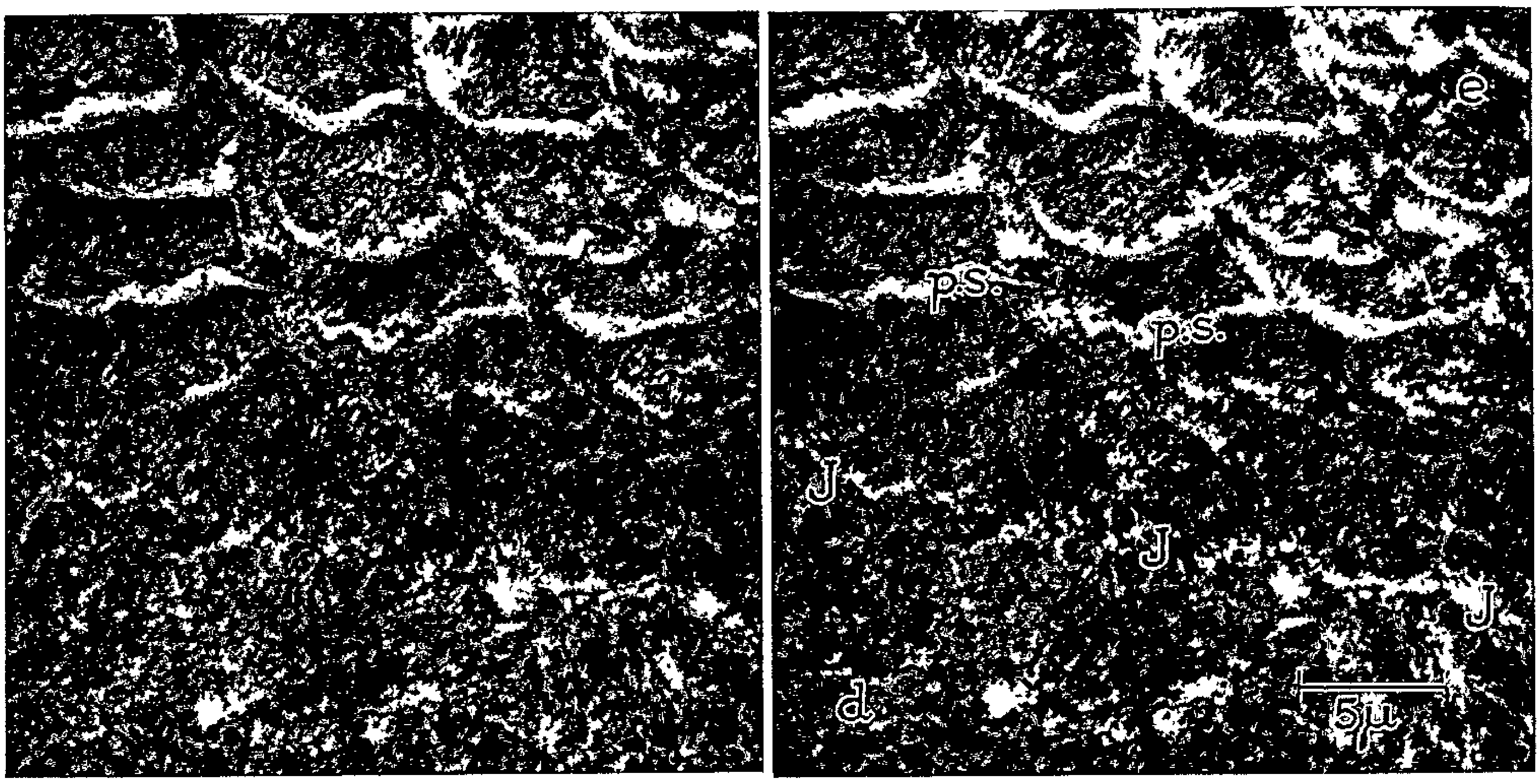

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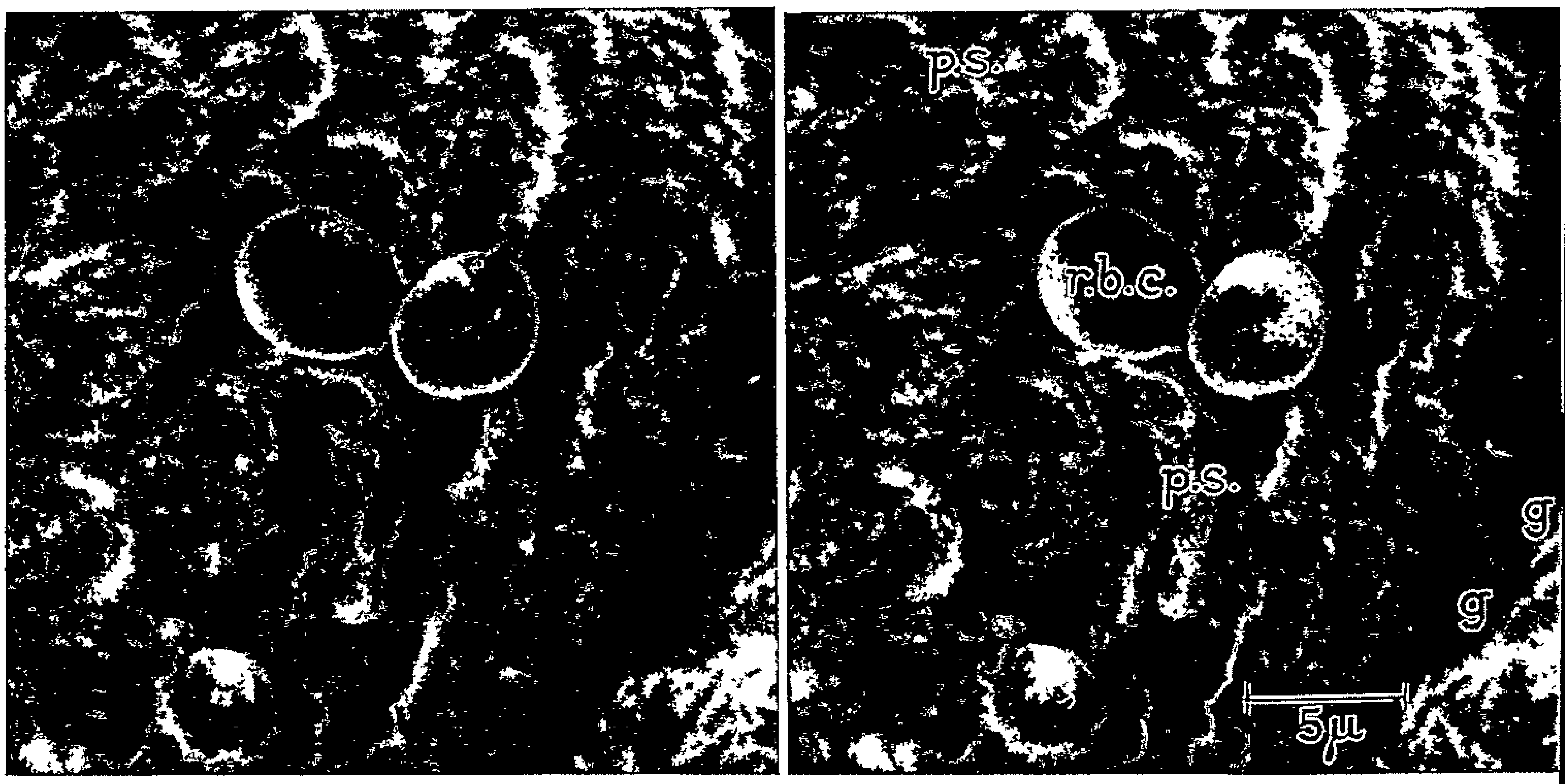

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Figs. 7—9 (for Legends see p. 545)

removal of the more highly mineralised *peritubular* dentine. Thus we have to suppose the existence of a factor leading to the relative insolubility of the peritubular dentine mineral: this factor could be due to a lesser solubility of the mineral species peculiar to this region and/or to a protective influence exerted by the mucopolysaccharide component of the matrix (cf. SOGNNAES, 1963). We are unable to choose between these two factors.

The observations that collagen demineralization is the first step in resorption and the mineral deposited in the non-collagenous portions is less soluble suggest an explanation for the origin of the loose "bone salt crystals" observed at the resorbing front of bone by SCOTT and PEASE (1956), CAMERON and ROBINSON (1958), GONZALES and KARNOVSKY (1961), DUDLEY and SPIRO (1961) and HANCOX and BOOTHROYD (1961, 1964). Small fragments of mineralized ground substance could become isolated and freed from the resorbing surface of intertubular dentine and intercanalicular bone regions. Peritubular dentine does not become detached from a resorbing surface because it is directly continuous with its own bulk in deeper layers (at least where the dentine tubules are approximately perpendicular to the resorbing front).

A close examination of the scanning electron micrographs of resorbing dentine reveals that there are several small projections of "peritubular dentine" apart from that lining the major dentinal tubules. We interpret these as representing side branches of the dentine tubules, which if our interpretation is correct, would indicate that these may also be "sclerosed" with "peritubular dentine". It is interesting to note that the small size of these branch tubules would preclude the possibility of reaching this conclusion from light microscopic and microradiographic techniques. We are only able to exclude the possibility that the branch dentinal tubules and the cement canaliculi were not filled with mineral derived from the resorbing part during the resorption of more superficial layers on the basis that their crystalline component resembles that found in the peritubular dentine (of major tubules) rather than the large "remineralisation crystals" we have described at the periphery of the Howship's lacunae.

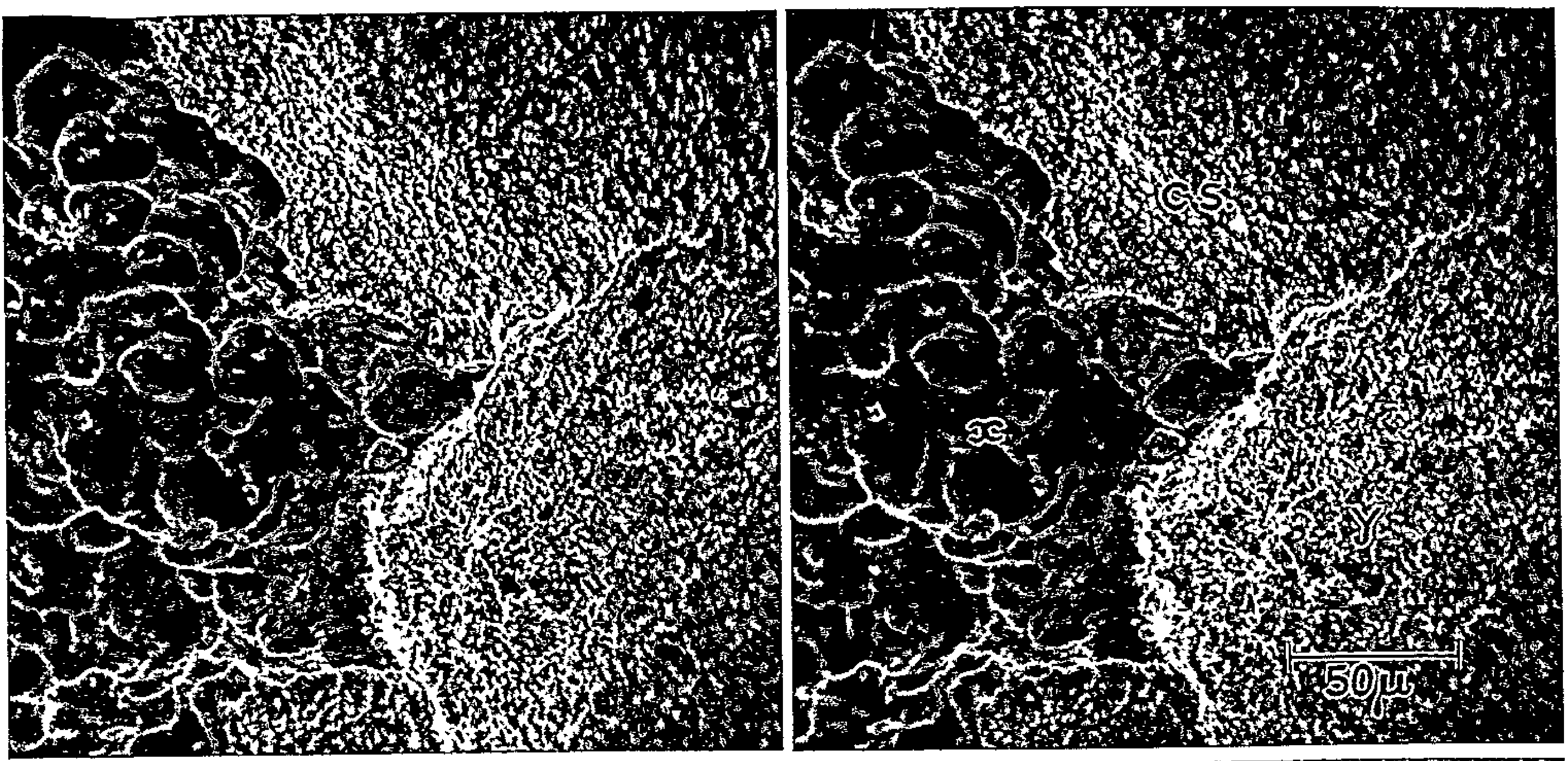
We are able to explain the appearance presented by resorbing enamel surfaces only by assuming that the organic matrix is 1. destroyed after the apatite crystals contained within it and 2. that it exerts a protective effect on those crystals in proportion to its concentration. Thus those regions (the prism boundaries) where the organic matrix was more concentrated remained more prominent from the resorbing surface, but when the organic material was destroyed by the 1:2 diaminoethane treatment, the same regions were found still to contain their inorganic component. We are not prepared to argue the question of whether the prism sheaths acquire their high organic content before mineralisation (e. g. GLIMCHER, DANIEL, TRAVIS and KAMHI, 1965), during mineralisation (BOYDE, 1964) or at a

Figs. 7—9. Scanning electron micrographs of resorbing human deciduous molar enamel

Fig. 7. (Anorganic) Enamel-dentine junction region showing parts of three Howship's lacunae straddling the junction

Fig. 8. (Anorganic) The enamel-dentine junction and adjacent enamel. Note the small difference in height (rate of resorption) of the enamel and dentine, the absence of "prisms" from the enamel nearest to the junction, and the prominent prism boundary regions (top)

Fig. 9. Note the prominent "prism sheaths"



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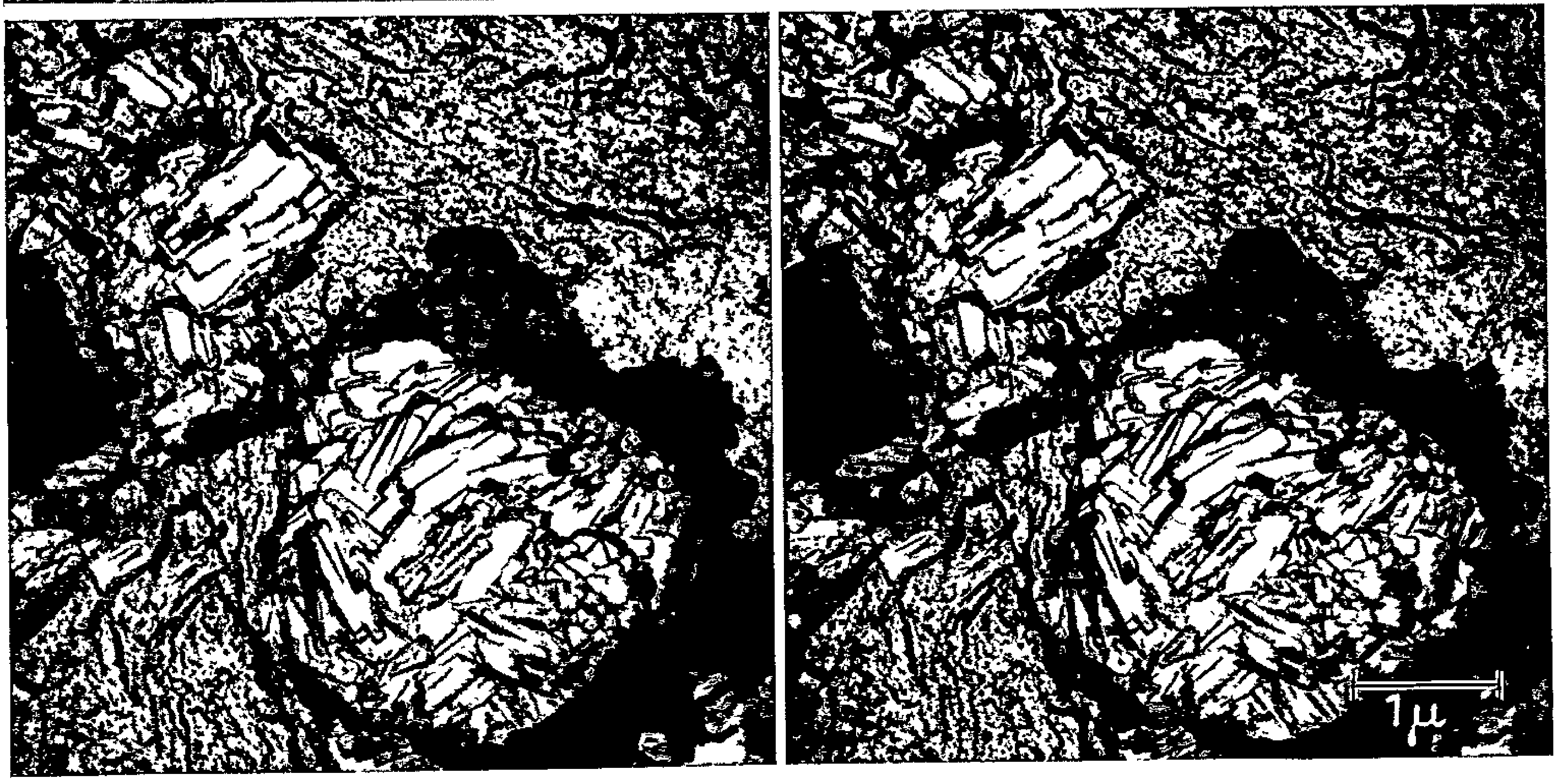

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Figs. 10—12 (for Legends see p. 547)

yet later stage and perhaps as a result of the remobilisation of matrix during the destruction of other parts of the tissue (Francis, Gray and Griebstein — personal communication). The fact remains that "osteoclasts" are able to recognise prism boundaries as less digestible, and, as these are merely regions at which there is a change in crystallite orientation (there has never been any significant evidence adduced for the existence of a different crystalline species in these regions), we therefore assume that there is a real difference in the nature and/or the concentration of the organic material of the "prism sheaths".

To return to the potential role of the dentinal ground substance in "protecting" its associated mineral from rapid dissolution, one might favour an alternative hypothesis on the basis of the apparently very small amount of ground substance present in the peritubular regions. However, in so far as we may allow ourselves to analogise with the situation in enamel, the fact that the less dense (more organic) prism sheath regions are more resistant to resorption favours the assumption of such a role.

The accumulation of large crystals at the borders of the Howship's lacunae presumably represent the result of a re-deposition in a different crystalline form of the mineral component removed at the resorbing front (cf. Sognnaes, 1963). They also indicate the existence of very different physiological states at the borders between adjacent osteoclasts and at the osteoclast-hard tissue matrix interface. Mineral solution occurring at the latter and precipitation at the former may be explained on the basis of radical local differences in $H+$ ion production. The site of formation of these crystals indicates that calcium and phosphate ions are liberated in high concentration into the extracellular tissue fluid environment during bone resorption, and this itself speaks strongly for the mediation of a low pH in mineral dissolution rather than the intervention of any chelating agents, which might be expected to remain bound to the ions they removed. Finally, the site and size of these "resorption crystals" leads us to comment upon those general aphorisms about biological mineralisation which would suggest that the nature of the "matrix" determines the crystalline species — we would rather believe that a matrix may be necessary for an initial nucleation, but that thereafter it only limits the size and shape to which the crystals grow. The resorption crystals described here apparently suffer from no real limit to their size and we assume they possess no matrix.

The demonstration of resorption crystals almost certainly depended upon our using the present carbon replica technique, since they would be too large to section for electron microscopy in the routine way. It is of interest to note in this connection that similar "remineralisation crystals" have been observed in several

Fig. 10. Scanning electron micrograph of surface of human deciduous molar cement (anorganic). An active resorption bay consisting of numerous fused Howship's lacunae is situated at centre left (*x*), a repairing bay at lower right (*y*), and the undamaged surface showing Sharpey fibres at centre, top and bottom

Fig. 11. Transmission electron micrograph of carbon replica of resorbing human deciduous molar dentine, showing typical collagen fibre cross banding

Fig. 12. Transmission electron micrograph of carbon replica of anorganic resorbing human deciduous molar dentine, showing an accumulation of large crystals at the border between two Howship's lacunae

electron microscopic studies of the caries process in teeth, but on these occasions the sectioning procedures were designed for very hard materials. Our observations on enamel and dentine using the carbon replica technique (Lester and Boyde, in press) indicate a far higher incidence of such "remineralisation crystals" than has been reported elsewhere.

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Dr. A. Boyde
Dept. of Anatomy and Embryology, University College London
London W. C. 1, Gower Street, England

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A Carbon Replica Study of Fractured Dentinal Surfaces:

K.S. Lester, King's College, London.

Direct, unshadowed, one-stage carbon replicas of surfaces presented by fracturing the unfixed dentine of a number of different mammalian species have been examined. Separation of replica from tooth substance was effected by firstly extracting the organic material by distilling diamino-ethane in a Soxhlet condenser and, after washing with water in place of the organic solvent, by dissolving the inorganic material in dilute HCl. Pieces of the separated film were picked up on copper grids and examined at 40kV in a Siemens' Elmiskop I. Stereo-pair electron micrographs were prepared and analysed subsequently with the aid of a Hilger and Watts stereometer. The coarse appearance of the intertubular matrix and the more homogeneous appearance of the peritubular matrix as described by previous workers is evident. No real indication of the structure of the organic or crystalline phases of the dentine is to be found in these regions, nor is there apparent any preferred line of cleavage with respect to the various structural features of the dentine.

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An Electron Microscope Study of Fractured Dentinal Surfaces

A. Boyde and K. S. Lester

Anatomy Departments, The London Hospital Medical College, Turner Street, Whitechapel,
London, E. 1 and King's College, London W.C. 2

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Unfixed fractured dentinal surfaces of a number of different mammalian species were examined both indirectly, by means of a single-stage replica technique, in a transmission electron microscope and directly in a scanning electron microscope. In both instances, so that the third dimension might be appreciated, stereo-pair electron micrographs were prepared by tilting the specimen between exposures. The structural features normally present in dentine — intertubular dentine, peritubular dentine, tubules and their branches — were clearly evident in all the species examined and details of their structure were observed. The appearance of intertubular dentine varied according to the presence or absence of a distinct fibrillar phase while evidence of such a component to the peritubular dentine was restricted to isolated instances. Various and distinct fibrillar conformations were found associated with the tubule walls, the inner aspect of which most often presented as a smooth surface. This surface was, in appearance, almost identical to the peritubular dentine and where the latter was present merged almost imperceptibly with it. Lateral branch tubules of various kinds were clearly demarcated, one very small variety appearing to remain confined to peritubular dentine.

Des surfaces dentinaires, non fixées, obtenues après clivage de dents, provenant de diverses espèces de mammifères, ont été étudiées indirectement sous le microscope électronique, à l'aide d'une technique de réplique, et directement sous un microscope électronique « scanning ». Dans les deux cas, pour d'apprécier la troisième dimension, des micrographics stéréoscopiaues sont réalisées en inclinant le specimen d'une photo à l'autre. Les structures de la dentine (dentine intercanaliculaire, dentine péricanaliculaire, canalicules et leurs ramifications) sont nettement visibles dans toutes les espèces étudiées. L'aspect de la dentine intercanaliculaire varie en fonction de la présence ou de l'absence d'une phase fibrillaire distincte, qui semble présente dans la dentine péricanaliculaire qu'en des endroits restreints. Des dispositifs fibrillaires variés et distincts sont visibles au niveau des parois canaliculaires, dont la surface interne présente le plus souvent un aspect lisse. Cette surface apparaît similaire à la dentine péricanaliculaire et semble en continuité avec elle. Des ramifications canalaires latérales de types variés sont nettement visibles; un type de ramifications, à trajet court, reste limité à la dentine péricanaliculaire.

Unfixierte Brüche von Zahnoberflächen verschiedener Mammalia-Arten wurden sowohl indirekt mit einstufiger Replicatechnik im Durchlicht-Elektronenmikroskop als auch direkt im Scanning-Elektronenmikroskop untersucht. In beiden Fällen wurden für die dreidimensionale Wahrnehmung elektronenmikroskopische Stereobildpaare durch Kippen des Objekts zwischen zwei Aufnahmen hergestellt. Die normalerweise im Dentin vorhandenen Strukturen — Intertubulardentin, Peritubulardentin, Kanälchen und ihre Verzweigungen — waren bei allen untersuchten Arten deutlich zu erkennen, wobei auch Struktureinzelheiten beobachtet wurden. Das Aussehen des Intertubulardentins war verschieden je nachdem, ob eine deutliche Fibrillenphase vorhanden war oder nicht, während eine solche Komponente im peritubulären Dentin auf einzelne Fälle beschränkt war. Unterschiedliche und gut ausgeprägte Fibrillenstrukturen waren an den Wänden der Kanälchen zu finden, die innen oft eine glatte Oberfläche

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For reprints: Dr. A. Boyde, Anatomy Department University College, Gower Street, London W.C. I. Great Britain.

zeigten. Diese Oberfläche war anscheinend nahezu identisch mit dem peritubulären Dentin und verschmolz bei dessen Auftreten unmerklich mit ihm. Laterale Kanälchenabzweigungen verschiedener Art waren deutlich markiert, wobei eine sehr geringe Zahl auf das peritubuläre Dentin beschränkt blieb.

Introduction

Since the original application to calcified dental tissues of replica techniques suited to electron microscopy (GEROULD, 1944, 1945; RICHARDS and THOMASSEN, 1944), this type of examination has been used extensively (reviews by SYRRIST and GUSTAFSON, 1951 and by SCOTT, 1953, 1955). Nearly all the replica studies as applied to dentinal surfaces have however, involved two- or three-stage techniques in which a plastic replica film is stripped from the intact specimen and either the original film, or a replica of it, subsequently metal-shadowed to provide a suitable medium for examination.

The replica technique used in the present study is a direct or one-stage technique, using evaporated carbon, in which no attempt is made to free the replica film from the specimen by manipulation but instead the mechanically undisturbed replica film is retrieved by gently dissolving the specimen away from it. Apart from the obvious advantage of minimal distortion, the resulting carbon replica film offers superior reproduction of detail compared to the plastic replica materials used in most two-stage procedures. Actual visualization of this detail is further enhanced by the preparation of stereo-pair electron micrographs.

The aim of this communication is to demonstrate the advantages of this combination of techniques by presenting the more common appearances resulting from its application to the fractured dentinal surfaces of a number of different mammalian species.

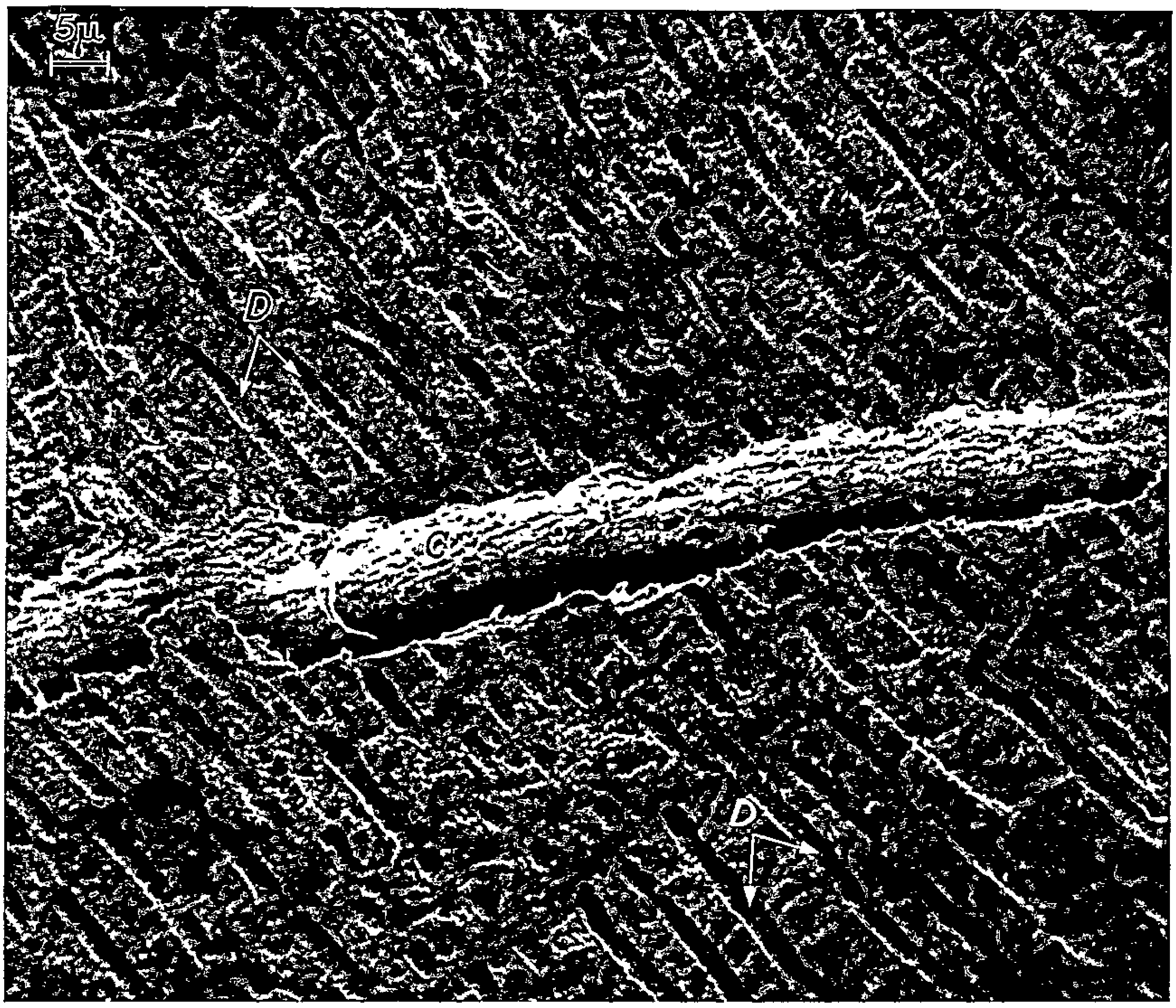
Materials and Methods

Sound unfixed teeth stored dry since extraction were fractured by closing the jaws of a vice in which they were held. The resulting fractured dentinal surfaces were replicated. Teeth from the following species were examined: human, deciduous and permanent teeth; monkey (Macaca mulatta), permanent; calf (Bos bovis), deciduous; dog (Canis familiaris), permanent; pig (Sus domesticus), deciduous; manatee (Trichecus manatus) and dolphin (Tursiops truncatus). The samples included both anterior and posterior teeth but no attempt was made to divide the samples with respect to type or age of tooth or to the site of dentinal fracture for the purposes of this preliminary investigation.

The method of carbon replication and of subsequent examination is described in detail by BOYDE (1967). Briefly, the area to be replicated is selected from the material after drying in vacuo. A layer of carbon is evaporated on to the area whilst the specimen, which faces the carbon arc at an angle of 45°, is rotating so that complete coverage of even very rough surfaces is ensured. The aim of the technique is to leave the carbon replica film mechanically undisturbed by dissolving the tooth away from it. To that end, the organic material is first extracted by placing the specimen (covered with the layer of evaporated carbon constituting the replica film) in a Soxhlet condenser and distilling through 1:2 diamino-ethane for a period of at least 24 hours. With the specimen still in place, careful washing is effected by distilling water through in place of the organic solvent. Finally, the remaining inorganic component is dissolved in NHCl. Small pieces of carbon film are then picked up on copper grids and examined in a Siemens Elmiskop I. Electron micrographs were taken at the lowest accelerating voltage available (40 kV.) in order to achieve the maximum image contrast.

Stereo-pair electron micrographs were prepared by tilting the specimen in the microscope between exposures so that the third dimension of the replica film might be appreciated. The stereo-pairs were subsequently examined with the aid of a mirror stereoscope.

It is obviously desirable to supplement a replica study with direct examination of the true surface. For this reason, fractured human dentinal surfaces (prepared as before from similar material) were covered with a conducting layer of gold and examined in a scanning electron microscope (Cambridge Instrument Company Stereoscan) using accelerating voltages in the range 2—20 kV. Other surfaces were left uncovered and examined using accelerating voltages in the range 1—2 kV. Again, stereo-pair images were recorded by tilting the specimen between exposures (Figs. 1 and 2).



Figs. 1—20. *Note on viewing stereo-pairs.* It cannot be too greatly emphasized that most of the information obtainable from the stereo-pair electron micrographs can only be gained if the pictures are viewed stereoscopically. A simple stereo-viewer consists of two identical lenses held at their focal distance (approx. 12 cms.) above the stereo-micrographs. The inter-ocular width should be adjustable in the range 5.5 to 7.5 cms. A cheap, collapsible pocket viewer is manufactured in the U.K. by C. F. Casella Ltd., Regent House, Britannia Walk, London, N. 1

Master Legend. *A* fibrillar network transversely orientated about tubule; *B* break in replica film; *C* crack; *D* dentinal tubule; *E* large collagen fibril bundles; *F* individual collagen fibrils lying along tubule wall; *G* granular material of tubule wall; *H* fibrillar intertubular dentine; *J* non-fibrillar intertubular dentine; *K* peritubular dentine; *L* lateral connecting branch tubule; *M* 'peritubular' lateral branch tubule

Fig. 1. Scanning electron micrograph of the surface of fractured human dentine. Bar = 5 microns

Results

The typical structural features of dentine, namely the intertubular dentine, the peritubular dentine, the tubules and their branches were clearly evident in all the species examined.

Intertubular Dentine. Two basically different appearances were presented by the intertubular dentine in each of the species examined. One was a coarsely granular, unevenly contoured appearance in which there was no indication at all of any fine structure which could be taken as representing either the organic or crystalline phases (Figs. 3, 9 and 10). The other type of appearance was one in which the fibrils (ca. 800 Å in diameter), which did not exhibit striations, could be seen standing out from the surface in all directions from their point of attachment (Figs. 6 and 7). Appearances intermediate between these two extremes were also observed (Fig. 8). In some areas of the dolphin material, wide tracts of relatively large diameter fibrils (ca. 0.12 μ) were situated in this fibrillar intertubular dentine arranged parallel to each other and to the longitudinal axis of the tubules (Fig. 16).

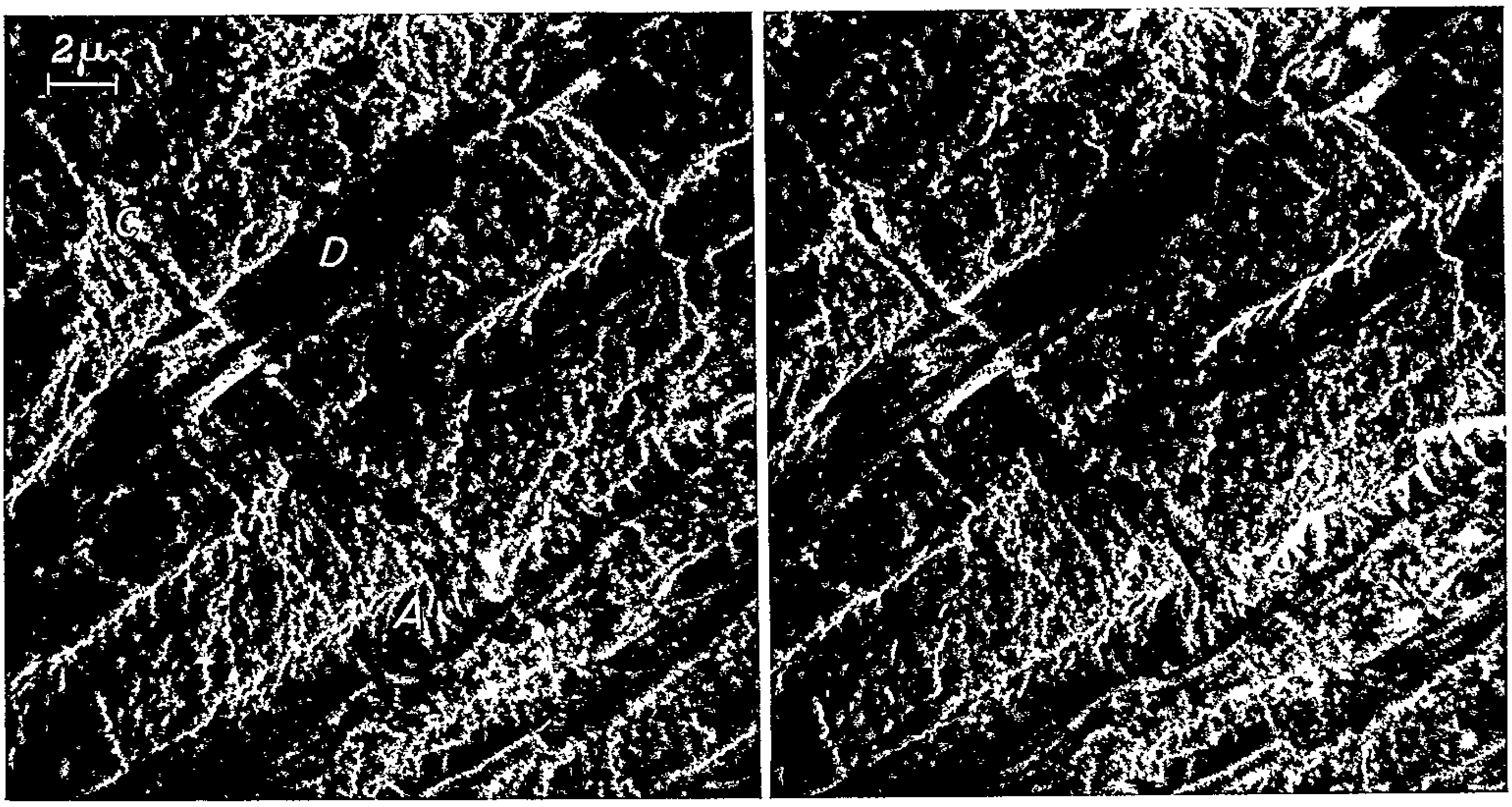


Fig. 2. Stereo-pair electron micrographs of the surface of fractured human permanent dentine. Tilt angle = 10°. Bar = 2 microns

Peritubular Dentine. Immediately about many dentinal tubules and separating their boundaries from the intertubular dentine was an area more homogenous in appearance and more evenly contoured than the intertubular dentine. This area of peritubular dentine was found to be associated with tubules both in the fibrillar and in the non-fibrillar types of intertubular dentine (cf. Figs. 7 and 11).

The distribution of the peritubular dentine was characterised by its irregularity, for in most instances it varied not only in amount about neighbouring tubules but also in the eveness of its distribution about any one tubule. Over limited areas of a fractured surface there was a preferred placement of this bulk of peritubular dentine with respect to the tubules (Figs. 9, 11 and 14).

Only in very few and isolated instances was there any evidence of a fibrillar component to the peritubular dentine. For example, in some manatee material, as a result of the peritubular dentine fracturing in two directions from a common edge (Figs. 12 and 13), fine fibrils (ca. 400 Å in diameter) were apparently plucked from the substance of the peritubular dentine during the fracturing process.

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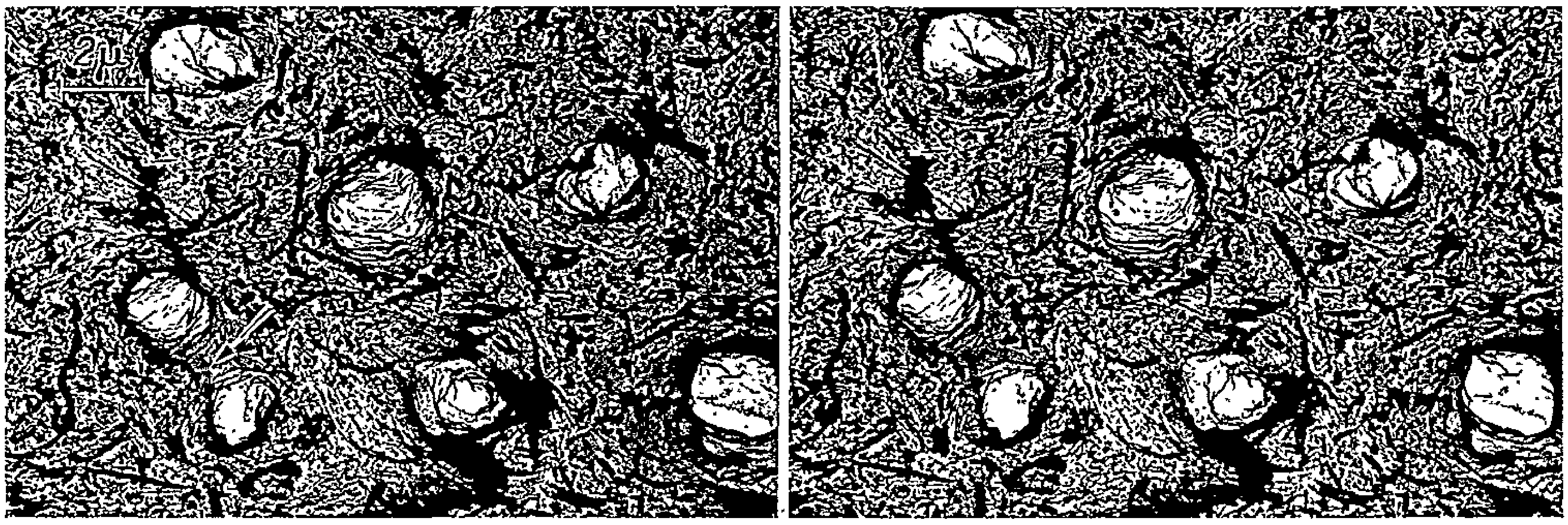

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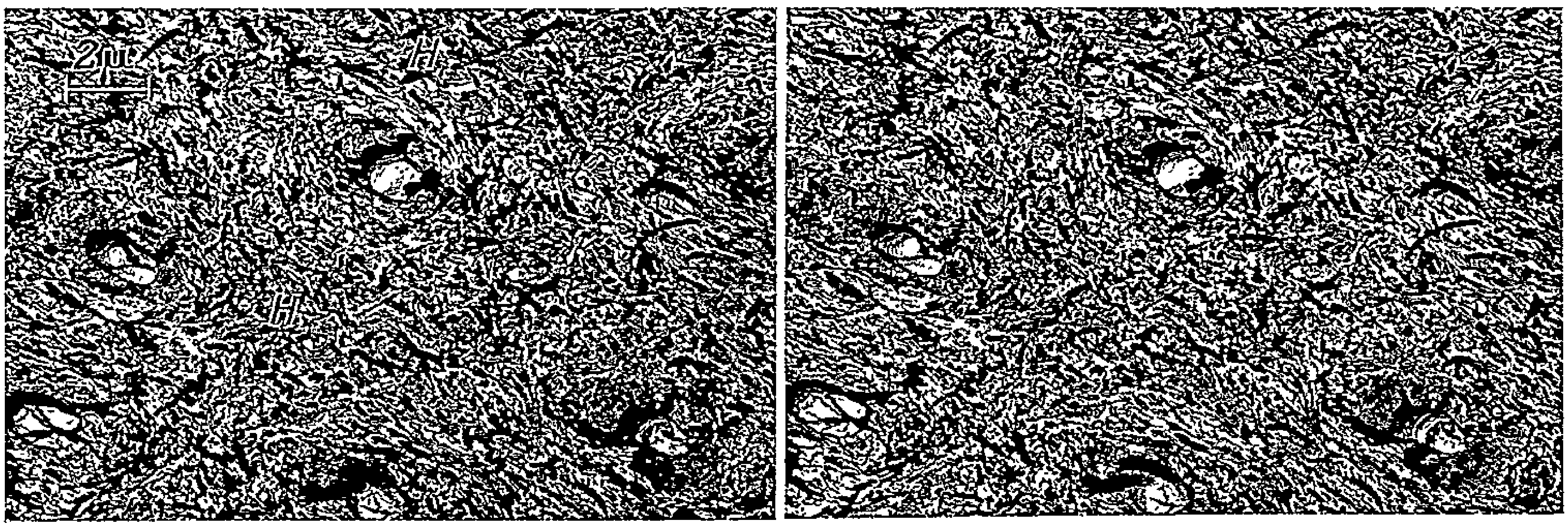

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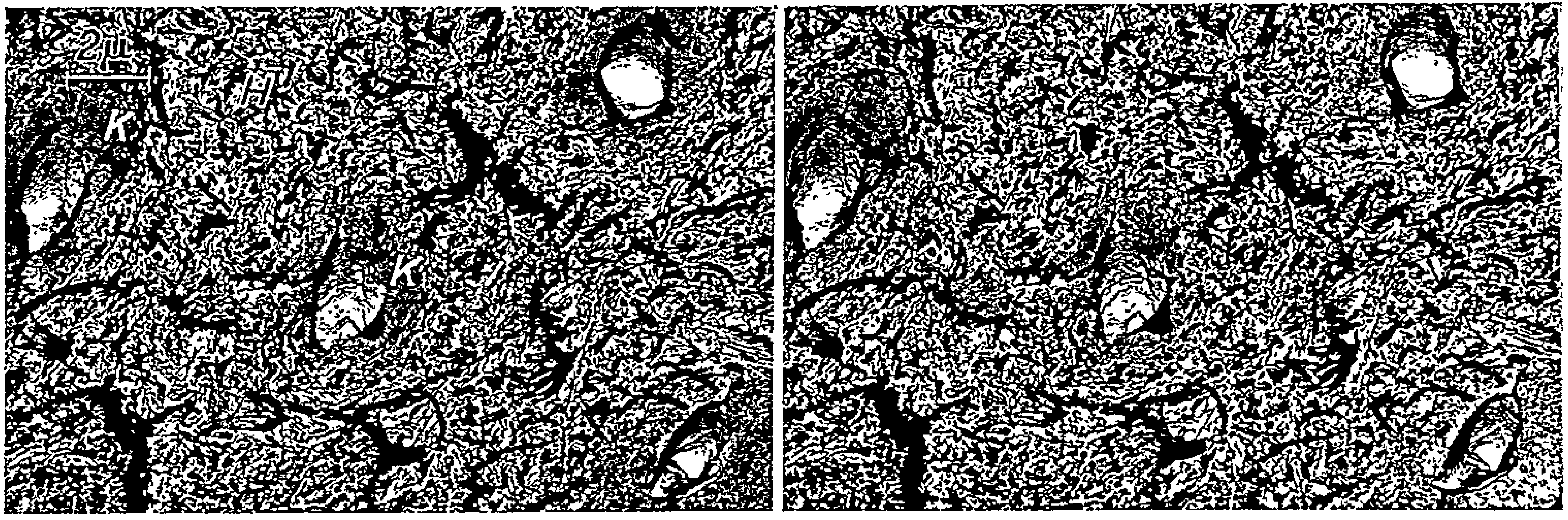

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Figs. 3—7 (for Legends see p. 127)

Dentinal Tubules. The tubules exposed at a fractured surface of any one of the dentines examined could be subjectively divided into tubules, large lateral branches and small lateral branches. There is a need however, for analyses of tubule diameters in order to determine whether there is a real basis for this commonly described subdivision, for it was apparent that not only did the average diameter vary from species to species but the degree of variation in diameter differed also. For example, the manatee dentine exhibited a marked variation in diameter of different, apparently parallel tubules over a limited area (Fig. 14) — as opposed to variation in diameter along the length of any one tubule — while the human and dolphin dentine showed a minimal variation in this respect.

Very occasionally, a lateral branch tubule could be seen to interconnect two major tubules (Figs. 3 and 4). The continuity of structure between two tubules illustrated in Fig. 5 appears to represent the beginning of an anastomosis between them. It is unlikely that the structure represents a single tubule as the curvature is too extreme to be a normal secondary curvature in the course of a single tubule.

Occurring very frequently in the calf material was a distinct, often very numerous type of small (ca. 0.12 μ in diameter) lateral branch (up to 60 per 100 μ length of whole dentinal tubule) which appeared to remain confined to the peritubular dentine tubule about the major (Figs. 9, 10 and 11). As a result of the assymetrical distribution of peritubular dentine, these 'peritubular branches' were typically localized to the side of a major tubule where the bulk of the peritubular dentine existed. Consequently, over a limited area of neighbouring major tubules, there was often an apparent polarization of these branches (Figs. 10 and 11). The peritubular branches themselves often branched along their very short course.

Dentinal Tubule Walls. The inner aspect of the walls of most tubules appeared as a very finely granular material (Figs. 10 and 13) which was very similar in appearance to the fractured surface of the peritubular dentine, although smoother and of an even finer texture. Where peritubular dentine occurred, the inner surface of the tubule wall appeared to represent its naturally occurring boundary.

Individual fibrils were often associated with the lining of the tubule wall and these could be broadly divided into two types. One exhibited clearly the striations typical of collagen and lay superimposed on the granular material of the wall (Fig. 18). These fibrils (ca. 600 Å in diameter) were, in the main, orientated essentially parallel to the longitudinal axis of the tubule. The other type of fibril lacked any obvious striation and was typically arranged transversely,

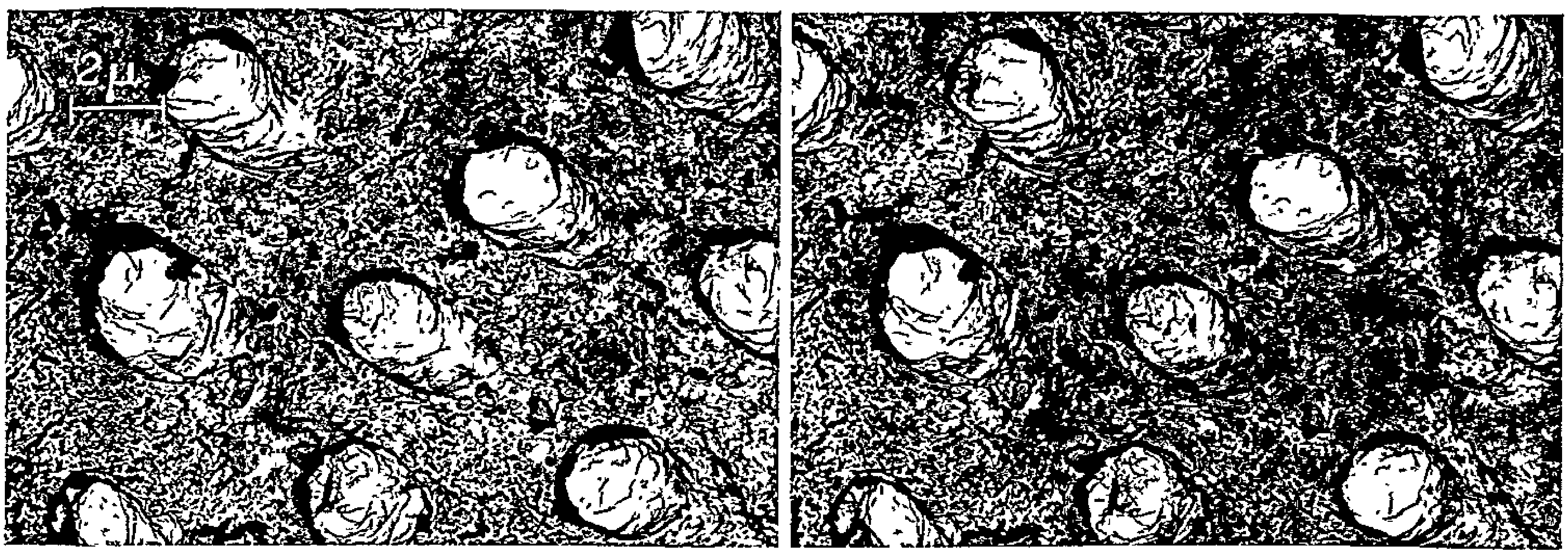
Figs. 3—20 (except Fig. 10). Stereo-pair electron micrographs of carbon replicas of fractured dentinal surfaces. Tilt angle = 8^0 40′. The two images are mounted 'orthoscopically' so that the appearance of the resulting fused image is as if looking into the tubules or viewing the inner surface of the tubule walls. Bar = 2 microns unless otherwise indicated

Figs. 3 and 4. Human permanent root dentine showing continuity between two tubules via a lateral connecting branch tubule

Fig. 5. Human permanent root dentine showing continuity between two tubules (at arrow)

Fig. 6. Monkey dentine showing an extremely fibrillar type of intertubular dentine

Fig. 7. Human permanent root dentine showing fibrillar intertubular dentine coexisting with well-developed peritubular dentine



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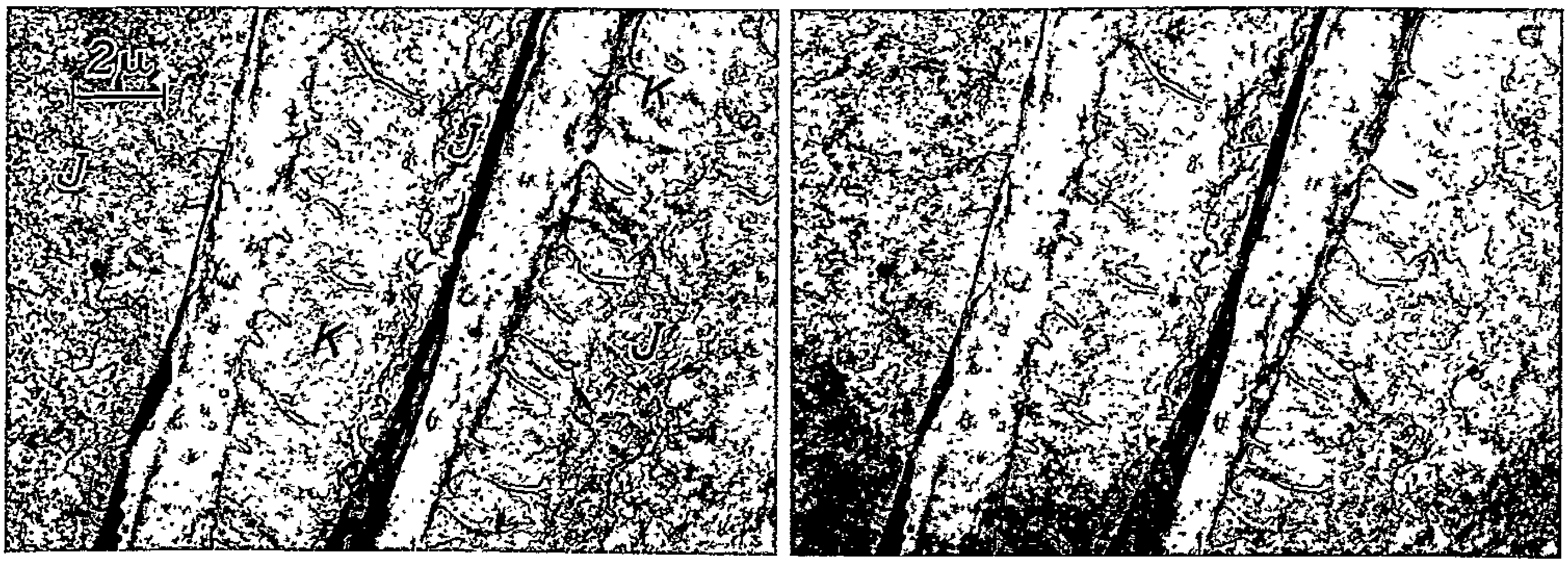

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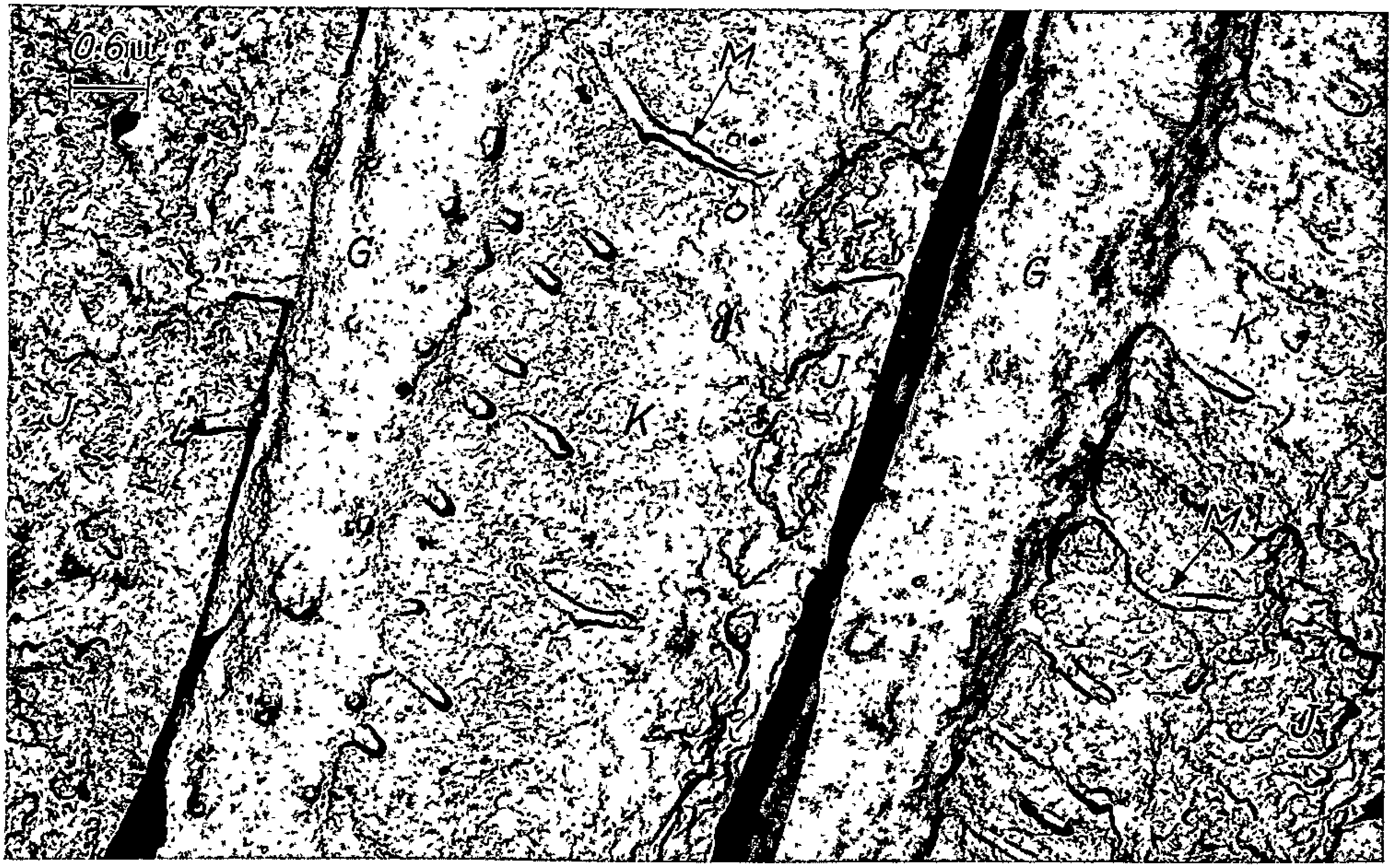

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Figs. 8—11 (for Legends see p. 129)

interwoven with its fellows (Fig. 19). These fibrils (ca. 0.1 μ in diameter) were continuous with the intertubular dentine and represented those fibrils of the intertubular dentine closest to the tubule. A more striking feature of some tubules however, was the presence of obviously striated collagen fibril "bundles" of very much larger diameter (up to 0.7 μ) (Fig. 20) and which lay inside the peritubular dentine where this was present. These fibrils formed part of the tubule wall but, at the point where the tubule was fractured, often left the wall to lie over the fractured dentinal surface (Fig. 15).

Very occasionally in what was otherwise 'typical' dentine, the usually smooth inner lining of tubule walls was replaced by a very roughened and irregular surface (Fig. 17).

Discussion

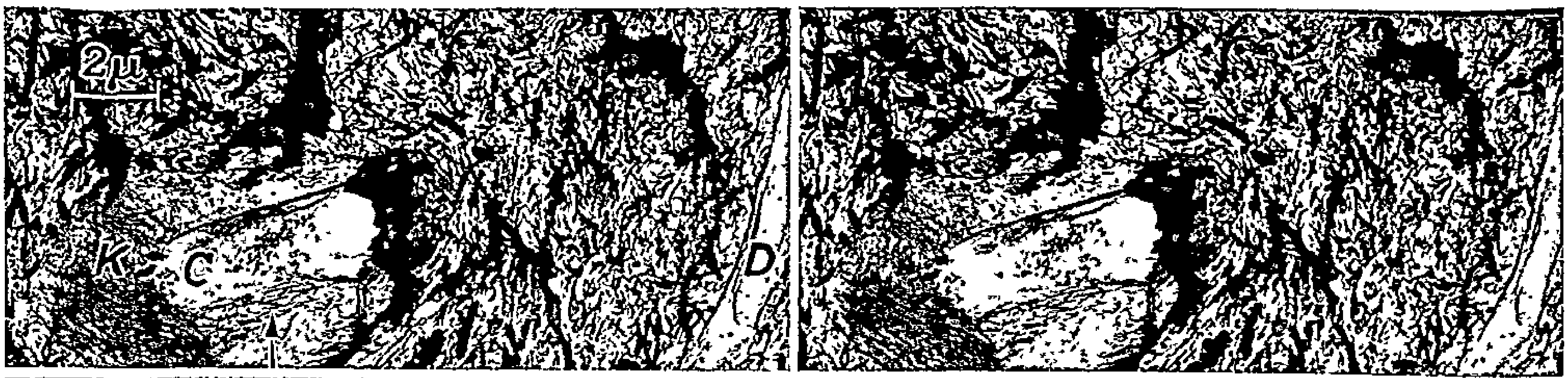
The well known and prolonged controversy as to the nature of the dentinal tubule wall, illustrated in the writings of, for example, HANAZAWA (1917), MEYER (1935) and LEHNER and PLENCK (1936), has not been fully resolved by the application of the electron microscope to the study of dentinal structure. In order to comment upon the structure of the tubule wall, it is first necessary, from the point of view of a replica study, to establish whether one is examining the inside of a tubule wall or the outside. Stereoscopic viewing of the photographic images will not, of itself, help to establish this fact for, by transposing the two photographic images, a previously concave surface (for example the inner wall of the tubule) will become a convex one (the outer wall of the tubule). Fortunately, the scanning electron microscope makes possible the direct observation of the original surface and it has been ascertained as a result that the preferred site of fracture exposes, almost without exception, the inner wall of tubules included in the line of fracture (Figs. 1 and 2). This point having been established, it is possible to make the following observations from the replicated material examined.

The inner aspect of a tubule most often presents as a smooth surface of very fine granular material which, in appearance, is almost identical with the peritubular dentine and, where the latter is present, blends imperceptibly with it. The basic lining may be added to by the appearance of fibrils in either one or both of two configurations.

Obviously striated individual collagen fibrils may lie on the inner aspect of the tubule wall (that is to say the side presented to the odontoblast process), in which case they lie essentially longitudinally with respect to the tubule although often appearing to spiral along it. These collagen fibrils could well correspond to those described as lying in the 'periodontoblastic space' by FRANK (1965) in his study of ultra-thin decalcified sections of dentine.

Fig. 8. Human permanent dentine showing a very slightly fibrillar type of intertubular dentine

Figs. 9—11. Deciduous calf dentine showing the 'peritubular branches' and the unequal distribution of peritubular dentine. Fig. 9 Tubules longitudinally fractured. Fig. 10. Enlargement of part of one member of the stereo-pair of Fig. 9. Bar = 0.6 microns. Fig. 11. Tubules transversely fractured



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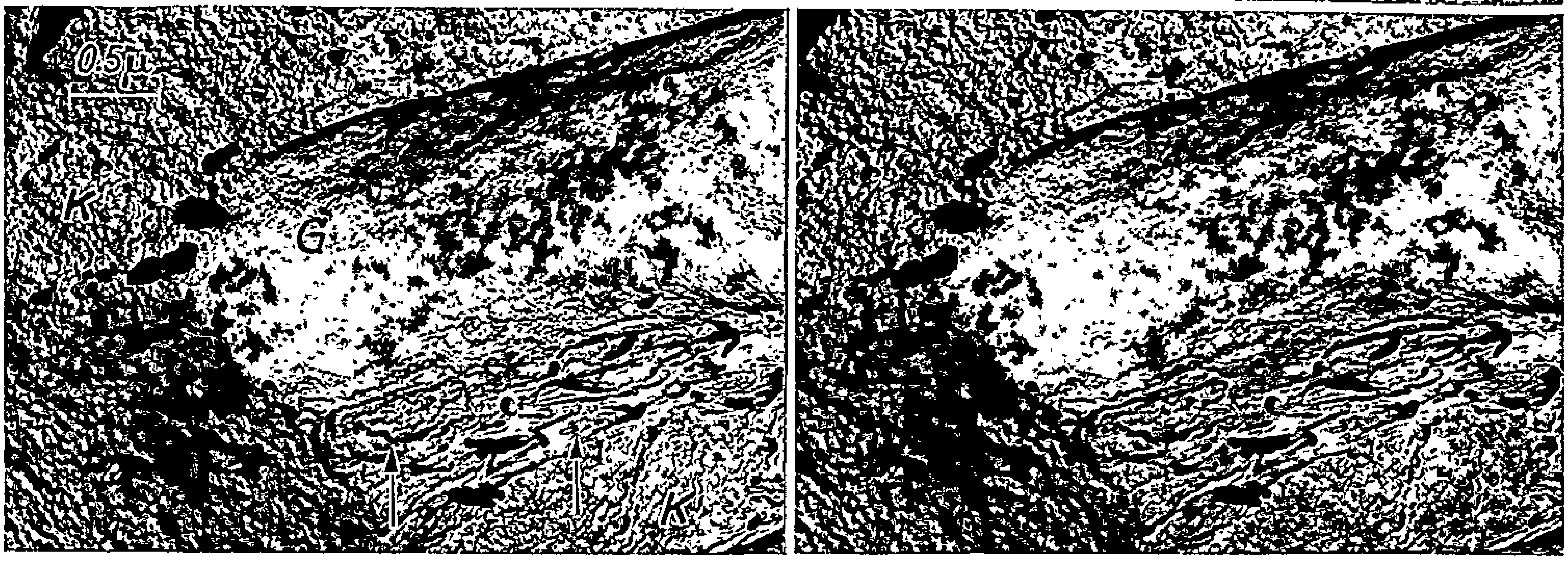

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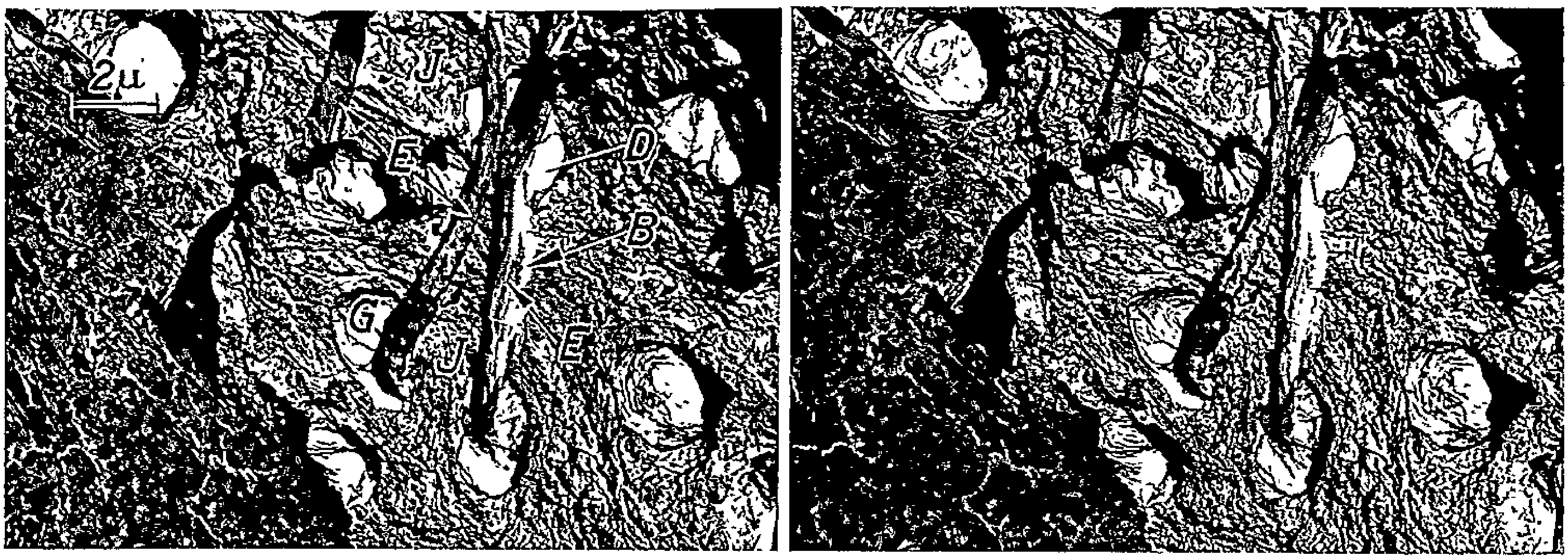

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Figs. 12—16 (for Legends see p. 131)

Fibrils in which the striation is rarely evident may lie on the outer aspect of the wall (external to the peritubular dentine where present) in a more transverse circumferential orientation to constitute a network about the tubule. It has been said (ROUILLER *et al.*, 1952 and KENNEDY *et al.*, 1953) that the clear visualization of these fibrils seems to depend in many instances on the partial removal of the finely granular material normally coating them and constituting the tubule wall. However, judging from the scanning electron micrographs (Figs. 1 and 2), it is possible that a complete lining of granular material is often absent in situations where no peritubular dentine exists. As noted by SCOTT (1955), these fibrils would correspond to those of the intertubular dentine reported by many workers as circumferentially orientated about dentinal tubules (for example KRAMER, 1951; FRANK, 1952; SCOTT, 1953; SHROFF *et al.*, 1956).

With regard to the much larger 'bundles' of collagen fibrils (Fig. 15), it is very difficult to establish to what degree this appearance is real or an artefact. It is certain that the fibrils are continuous with, and constitute part of the walls of the tubules with which they are associated and it is very probable that they are freed from the tubule wall as a result of the fracturing process and come to lie over the fractured dentinal surface. HELMCKE (1953) identified somewhat similar structures as odontoblast processes about each of which was bound a reticulum of collagen. However, in the present study of unfixed material, no regularly occurring structures were found which could be readily identified as odontoblastic processes.

The lateral branch system and the anastomosis of tubules have long been depicted by various means (for example, OWEN, 1840—1845; WALKHOFF, 1923; KAYE and HEROLD, 1966). There is however, some advantage in terms of clarity of detail in viewing these phenomena by the techniques employed in the present study. For example, the contour of lateral connections may be examined and the validity of their continuity ascertained (Figs. 3, 4 and 5). There are often tubules closer to connected tubules than these are to each other (Figs. 3 and 4). Tubules in the more immediate vicinity are apparently bypassed by the lateral connecting branch at some stage during its formation in favour of a more distant tubule.

Some consideration has been given elsewhere to the question of the mode of formation of the larger, more common type of lateral branch (LESTER and BOYDE, 1967). However, the small lateral branches so clearly demarcated in the peritubular dentine and difficult to trace beyond it raise a different problem. The fact that the formation of peritubular dentine has been shown to be a normal maturation process (BLAKE, 1958; SYMONS, 1961; DREYFUSS *et al.*, 1964) suggests that if these minute lateral branches are indeed limited to the peritubular dentine,

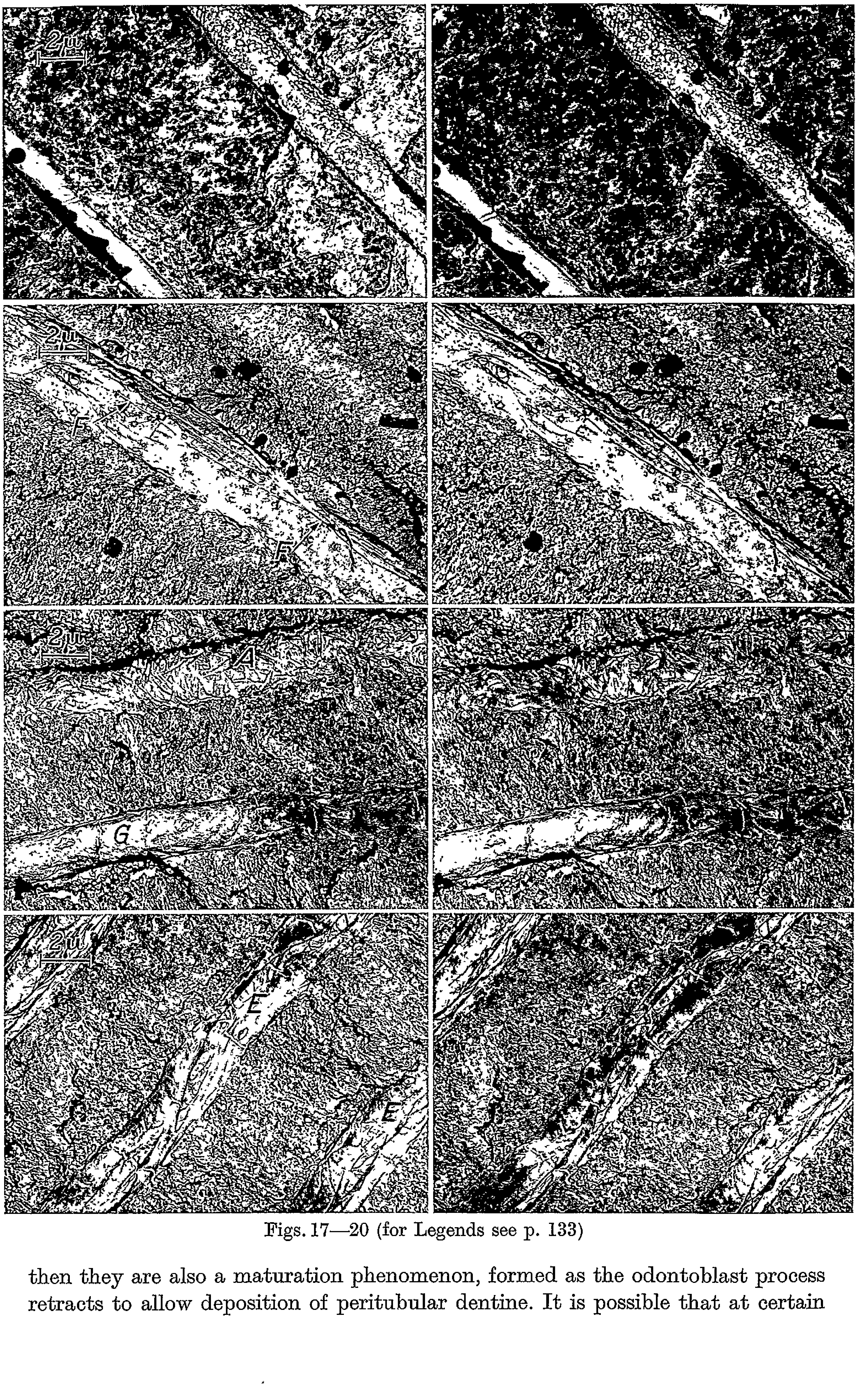
Fig. 12. Manatee dentine showing fibrils (at arrow) apparently plucked from the peritubular dentine

Fig. 13. Enlargement of part of Fig. 12. Bar = 0.5 microns

Fig. 14. Manatee dentine showing the characteristic difference in the size of apparently parallel tubules

Fig. 15. Human deciduous dentine. Note the collagen fibril 'bundles' continuous with the walls of fractured tubules

Fig. 16. Dolphin dentine showing tracts of large diameter fibrils (T) in the intertubular dentine



Figs. 17—20 (for Legends see p. 133)

then they are also a maturation phenomenon, formed as the odontoblast process retracts to allow deposition of peritubular dentine. It is possible that at certain localized points around the periphery of an odontoblast process the cytoplasm does not retract from the surrounding tubule walls with the major bulk of the odontoblast process but remains to result in the formation of these particular branches. It is more likely however, that the 'microvillous' or pseudopodial extensions of the odontoblast process (FRANK, 1965) existing before the formation of peritubular dentine account for these minute lateral branches.

The electron microscopic recognition of the existence of two apparently different types of dentinal substance separating neighbouring dentinal tubules was first achieved in replicas of polished and etched surfaces (SCOTT and WYCKOFF, 1947a, 1947b; MENKE, 1950; TSUCHIKURA and TAKUMA, 1951; ROUILLER *et al.*, 1952; SHROFF *et al.*, 1954 and others). Subsequently, the coarse, irregular nature of the intertubular dentine and the more homogenous, or very finely granular nature of what is now generally termed "peritubular dentine" (FEARNHEAD, 1957) were described in replicas of fractured surfaces (SYRRIST and GUSTAFSON, 1951; HELMCKE and JAHN, 1952; HELMCKE, 1953; SHROFF *et al.*, 1956).

Little is known of the matrix of the peritubular dentine although the existence of a fibrillar component has been reported by many workers (BRADFORD, 1950a, 1955; MATSUMIYA and TAKUMA, 1954; SHROFF *et al.*, 1956; TAKUMA *et al.*, 1956; FRANK, 1959, 1965; AWAZAWA, 1962; EDA and TAKUMA, 1965). Certainly the constant and characteristic evenness of the fractured peritubular dentinal surfaces suggests a homogeneity of structure which in turn would indicate a very fine fibrillar component — evidence of which was gained only in isolated instances in the present study (Figs. 12 and 13) — and an overall uniformity in the degree of mineralization.

Another common but unexplained characteristic of peritubular dentine is the unequal distribution of its bulk to either side of a fractured dentinal tubule (Figs. 10, 11 and 14). This has been well illustrated in recent times (for example by BRADFORD, 1950b, 1951, 1955, 1963; AWAZAWA, 1962; DREYFUSS *et al.*, 1964; EDA and TAKUMA, 1965) although only EDA and TAKUMA (1965) seem to have commented upon it. BRADFORD (1950a) related the abundance of peritubular dentine in the hypsodont teeth of the horse and the absence of it in the continually forming teeth of other species to its possible role in minimising the effect of heavy attrition to which the horse dentition is usually subject. The localization of peritubular dentine to the enamel side of the dentinal tubule in the horse (demonstrated by EDA and TAKUMA, 1965) and the proven defence role of the peritubular dentine in other situations (DREYFUSS *et al.*, 1964) tend to support this view. Little evidence is available in previous work, or could be gained from the present study, of the same marked degree of preferred localization of peritubular dentine, as found in the horse, in other species not usually subject to

Fig. 17. Human deciduous dentine. Note the different structure of the tubule walls

Fig. 18. Manatee dentine showing clearly striated collagen fibrils lying longitudinally along the tubule wall

Fig. 19. Human deciduous dentine. Note the different structure of the tubule walls

Fig. 20. Human deciduous dentine showing large bundles of collagen fibrils in association with the tubule walls

the same degree of attrition. Further study of a wider variety of species is necessary to assess this possible structural adaptation to function.

The fibrillar type of intertubular dentine (Figs. 5, 6 and 7) does not appear to have been described previously at a fractured surface, the intertubular dentine usually presenting little evidence of separate phases. However, fibrillar *bundles* in the intertubular dentine, arranged similarly to those found in the dolphin material (Fig. 16), have been previously identified as embedded Korff's fibres (Nylen and Scott, 1958), it being generally agreed that these are definitely associated with the formation of mantle dentine — although opinion differs as to their later role in the formation of circumpulpal dentine (cf. Orban, 1929; Bevelander, 1941; Symons, 1956; Nylen and Scott, 1960; Noble, Carmichael and Rankine, 1962; Zerosi, 1966). It is clear that carefully localized areas rather than pooled samples must be examined and information on the surface changes to collagen accompanying its mineralization must be acquired before the original site and true nature of these types of fibrillar intertubular dentine can be ascertained.

No replica technique can compete with the facility of direct observation of a surface, the great depth of focus and the ease of interpretation of the image provided by the scanning electron microscope. There seems little doubt, however, that with the improved resolution offered by the transmission electron microscope, the techniques employed in the present study are well suited to the visualization of dentinal structure. There is much scope for replica work and more extensive studies with the aid of etching and enzyme digestion are continuing, especially on a comparative basis, for it is obvious that, as previously shown by Bradford (1950a), a great deal of interesting species specificity exists among the mammalian ortho-dentines.

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Some Observations on the Enamel-Dentine Junction:

K.S. Lester and A. Boyde, The London Hospital
Medical College, London.

The region of the dentino-enamel junction was studied in fractured surfaces of the teeth of a number of mammalian species, by a single-stage carbon replica technique for transmission electron microscopy and also by direct scanning with an electron microscope. Where a wide peripheral layer of mantle or von Korff's dentin was found, as in the dolphin, it was characterised by the large size of the fibrils it contained and by their arrangement in bundles perpendicular to the plane of the junction. Within such a region, the major tubules did not reach the junction, although some small tubules approached it. Where the large fibril bundles penetrated into the dentin to some distance from the junction, the tubules ran parallel with them and, in the intertubular dentin, individual collagen fibrils were relieved from the fractured surface. Regions of intertubular dentine, in other than mantle dentine, most often presented a much more homogeneous appearance; however, the collagen fibrils remained masked within the fractured surface.

Where the mantle dentin was minimal in extent, as for example in the pig, large dentinal tubule branches crossed the dentino-enamel junction and penetrated several microns into the enamel, free from any extension of dentinal matrix. Nothing that could be interpreted as a membrane was seen at the dentino-enamel junction. The parallel crystallites that constitute the innermost layer of enamel appeared to fuse with the fibrillar matrix of the dentin.