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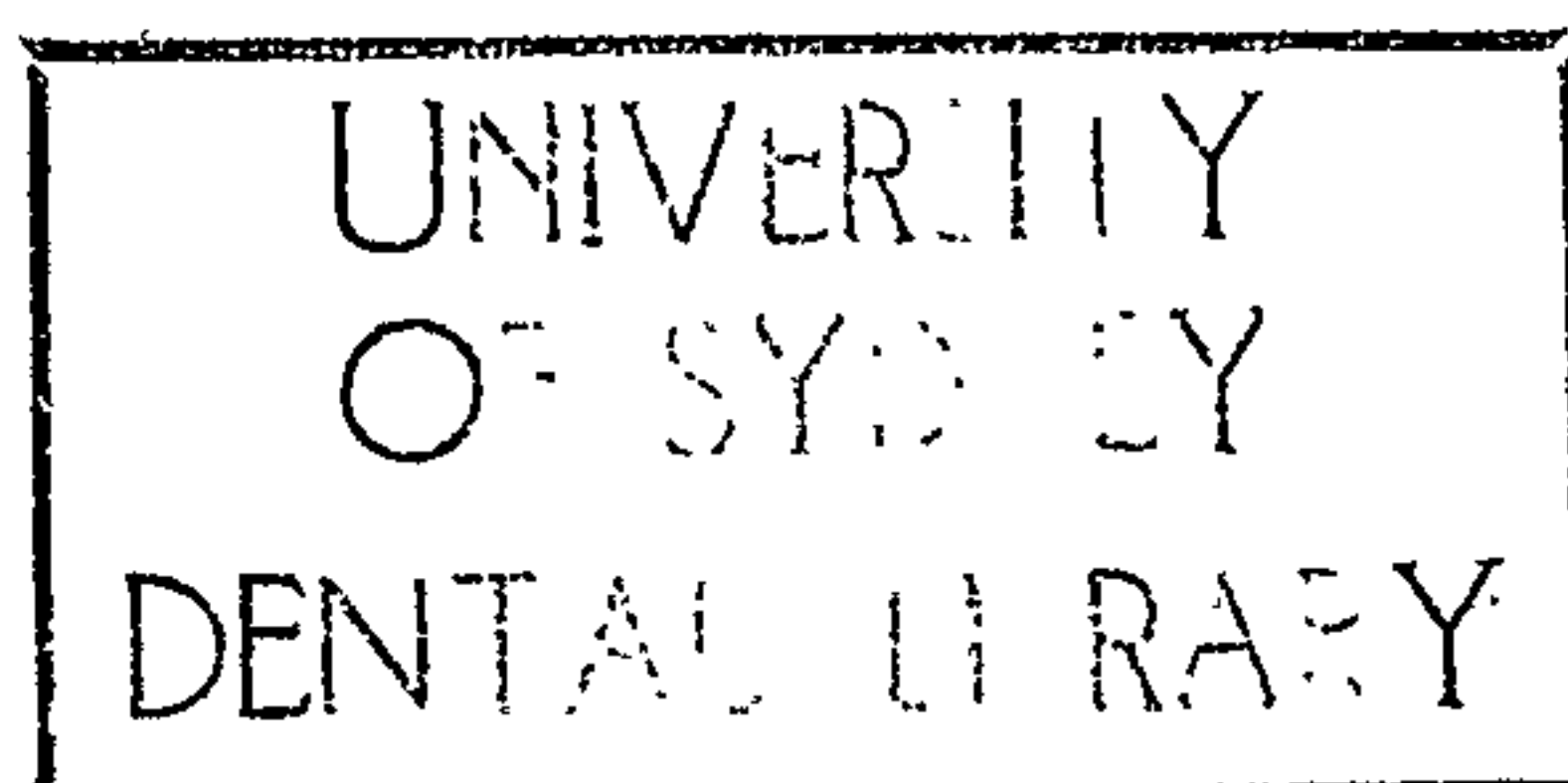
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A SCANNING ELECTRON MICROSCOPIC STUDY OF THE DISTRIBUTION
OF DENTINAL TUBULES IN HUMAN MOLAR TEETH.

A thesis submitted to the University of Sydney
as partial requirement for the degree of
Master of Dental Science (1985).



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The work submitted for examination in this thesis is the original work of the candidate alone. This investigation was conducted within the Department of Operative Dentistry, University of Sydney. No portion of this work has been submitted by the candidate to any other University in part or full for the award of any other degree.

A. O. Dourda.

This Thesis is dedicated
to my brother Abouzaid
for his encouragement and
support over the years.

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ABSTRACT

The dentinal tubule has been implicated in the transmission of stimuli from the external surface of the tooth to the pulp either via its contents or its morphology. The mechanism for the transmission of stimuli remains one of the major controversies in the dental literature. Direct stimulation of odontoblast processes and direct stimulation of nerves have been proposed. In addition a hydrodynamic mechanism for pain transmission in dentine is a widely accepted proposition. This theory implies that pain transmission is dependent upon movement of fluid in the dentinal tubules.

An accurate understanding of the morphology and size of dentinal tubules would seem important to any investigation of fluid movements through tubules. A review of the literature however revealed a considerable variation in the description of dentine and dentine tubules particularly with respect to the number and size of the dentinal tubules. It was considered that a further detailed study on the morphology of the dentinal tubule was warranted. Additionally, as it has been suggested that branching of the tubules in the region of the dentino-enamel junction may explain the relative hypersensitivity of the dentine at the dentino-enamel junction (Osborne and Ten Cate, 1976), it was thought that a comparative study of the means cross sectional area

of the dentine occupied by tubules at various levels in the tooth may be of value.

The advent of new computer assisted measuring methods has revolutionised many histometric measuring techniques. Comparative study between these new methods and the point count method of measurement could be found in the literature. It was considered that such a study would prove useful. The aim of this study was therefore:

- (a) To examine the surface morphology of fractured surfaces of dentine at various levels in the tooth by scanning electron microscopy.
- (b) To establish the number of dentinal tubules per square millimeter at different levels in the tooth.
- (c) To examine cross sections of dentinal tubules from different levels in the tooth to establish the percentage of dentine occupied by dentinal tubules at each level.
- (d) To establish whether there was any significant difference between the results obtained by Method A and by Method B.

Cross sections of dentine at different known levels in the tooth were prepared from intact human third molar teeth with completed root development, grouped according to level of section, and examined under the Scanning electron microscope. The surface morphology of the cross section was described, and the number of dentinal tubules and the percentage of area occupied by the dentinal tubules was measured for each level - using the point count method (Hilliard, 1961) (Method A) and a digitizing tablet and Bioquant II software (R & M Biometrics Co.) (Method B).

The morphology of the fractured dentinal surfaces was similar to that described in the literature. Structures resembling odontoblast processes were observed close to the dentino-enamel Junction. It was not possible to accurately identify these structures. It is considered that they may represent the "lamina limitans" of the dentinal tubule rather than the odontoblast process itself. Long style fibres were also seen extending from some dentinal tubules. These were thought to be collagenous.

Values for the number of dentinal tubules per square millimeter varied from 22,000 to 48,000 by Method A and from 25,000 to 50,000 in Method B. The number of dentinal tubules increased progressively from the dentino-enamel junction to the pulp. The size of the tubules also increased progressively from the dentino-enamel junction to the pulp as did the percentage of the dentinal surface

occupied by dentinal tubules. Area percentage figures varied from 3.63 at the dentino-enamel junction to 10.2 close to the pulp in Method A, and from 3.3 at the dentino-enamel junction to 7.8 percent closer to the pulp in Method B. Statistical analysis revealed no significant difference between results obtained from Method A and those obtained from Method B. However, it is recommended that Method A be used in future studies as it is quicker and easier than Method B although it has some disadvantages particularly in the time needed to calculate the statistics.

LITERATURE REVIEW

CHAPTER 1

CLASSIFICATION OF DENTINE

INTRODUCTION

Dentine is the hard tissue which forms the bulk of the tooth. It is composed of approximately 10% water, 20% organic material and 70% inorganic material by weight (22%, 33% and 45%, respectively by volume), the inorganic material consists mainly of hydroxyapatite, the organic phase is primarily collagen (Ten Cate, 1980). Dentine surrounds the coronal and radicular pulp and forms the walls of the pulp chamber and root. It constitutes the greatest part of the tooth. It is continuous and uninterrupted except for the foramina located at the apex of the root and is slightly harder than bone and softer than enamel. The difference in hardness can be readily distinguished in radiographs where the dentine appears more radiolucent than enamel and more radiopaque than alveolar bone. In its physical and chemical qualities the dentine closely resembles bone (Provenza, 1964; Avery, 1976).

Dentine is morphologically unique in that the odontoblasts do not become enclosed by their product, except for one long cell process which each cell leaves behind in the dentine. The cell bodies of the odontoblasts are located in the dental pulp at the inner surface of the dentine. Their processes are surrounded by dentine matrix (calcified in the mature dentine, uncalcified in the

immature predentine), so that each process lies in its individual dentinal tubule. All dentinal tubules run from the dentino-enamel junction to the dental pulp (Moss-Salentijn and Klyvert, 1980).

The first author to bring some order into early dentine taxonomy was Tomes (1876, 1879). The taxonomic situation has become confused by the introduction of many terms for one tissue for instance Mantle dentine (Pallial dentine, von Korff dentine) or Circumpulpal dentine (Circum-paollar dentine, von Ebner dentine) for the purpose of description the following terms will be used:

- 1.1 Primary dentine.
 - 1.1.1 Mantle dentine.
 - 1.1.2 Circumpulpal dentine.
 - 1.1.3 Interglobular dentine.
 - 1.1.4 Tomes' granular dentine.
 - 1.1.5 Incremental lines.
- 1.2 Secondary dentine.
 - 1.2.1 Regular secondary dentine.
 - 1.2.2 Irregular secondary dentine.
- 1.3 Predentine.
- 1.4 Transparent dentine.

1.1 Primary dentine.

The term primary dentine coined by Tomes (1848) was first defined by Bodecker (1879, 1894) as representing

regular orthodontin formation in contrast to irregular secondary dentine that was either adjoining primary dentine or was free within the pulp. The Federation Dentaire Internationale (Baume, 1980) has defined primary dentine as that portion of regular dentine (orthodontin) produced during the formation period up to near completion of the external shape of the tooth. Primary dentine forms most of the tooth and outlines the pulp chamber of the fully formed tooth. It can be divided into two layers; mantle dentine which forms its outer layer, and circumpulpal dentine. Kuttler (1959) reported that the primary dentine is formed by the thickening of the basic membrane between the internal enamel epithelium and the primary mesodermal pulp. First, the network of von Korff's fibres appear producing the first organic precollagenous (uncalcified) dentine that becomes the predentine. This is followed by the appearance of the odontoblasts, and by a process not yet determined, calcification of the dentine starts. The odontoblastic column begins to separate gradually and dentinogenesis advances from the incisal or occlusal portion toward the apex of the tooth forming the primary dentine which has also been referred to as primitive, initial, regular, normal, physiologic, etc.

1.1.1 Mantle dentine.

Historically the term "mantle dentine" was introduced by Weidenreich (1925). Osborn and Ten Cate (1976) refer to it as the layer of dentine immediately beneath the enamel in the crown and cementum in the root. It is the first formed dentine laid down by the newly differentiated odontoblast. Moss-Salentijn and Klyvert (1980) reported that, this layer is about 10 to 30 micrometres thick. It is different from the circumpulpal dentine because it contains, in addition to the normal collagenous fibres of the dentine, another group of collagen fibres which are considerably thicker. These thicker fibres have been called von Korff's fibres. The normal collagen fibres run parallel to the dentino-enamel junction. The von Korff's fibres run nearly perpendicular to the dentino-enamel junction. Moss-Salentijn and Klyvert have suggested that Mantle dentine is somewhat less calcific than the circumpulpal dentine because of the presence of the heavy von Korff's fibres. This lesser degree of calcification may explain the spread of caries along the dentinoenamel junction, once this junction is reached by the caries process.

Osborn and Ten Cate (1976) reported that there is a difference between coronal mantle dentine and root mantle dentine which has only been appreciated recently. The difference relates to the orientation of coarse collagen fibres. In root mantle dentine the fibres are aligned

parallel with, instead of perpendicular to, the basal lamina supporting the epithelium which in the root is Hertwig's root sheath.

Jones and Boyde (1984) described mantle dentine under two different categories; (1) coronal mantle dentine (2) radicular mantle dentine. The coronal mantle dentine is the first-formed, peripheral dentine which eventually lies adjacent to the enamel in the crown of a tooth. It is a layer of variable thickness, extending from virtually nothing to tens of micrometers. It is characterized by the presence of collagen fibres that are arrayed differently from those in the circumpulpal dentine that forms the bulk of the dentine. On the other hand, radicular mantle dentine is found peripheral to the granular layer of Tomes. It is said to be not as constant a layer as the coronal but, where present, contains similar, coarse collagen fibrils. Radicular mantle dentine has not attracted as much attention as its coronal counterpart and its position relative to other proper named layers is unclear. It has been reported to be absent below cementum in rat, rabbit, and guinea pig incisors, but present as a radio-opaque layer 8 to 15 micrometres thick below cementum in the dog, and to be 15 to 30 micrometers thick in primates (Jones and Boyde, 1984).

1.1.2 Circumpulpal dentine.

Circumpulpal dentine comprises the remainder of the primary dentine and has an organic matrix made up of much finer collagen fibrils arranged as a random network in a plane at right angles to the tubules. The Federation Dentaire Internationale adopted the following definition (1980): Circumpulpal dentine (von Ebner Dentine) is the main bulk of orthodentine characterized by fine fibres running approximately at right angles to the long axes of the tubules.

Weidenreich's (1925) original interpretation that these circumpulpal dentine types (amongst which he also included osteodentine) were phylogentic homologues of lamellar bone is no longer tenable. Provenza (1964) reported that the dentine intervening between the mantle dentine on the one side and the predentine on the other constitutes the circumpulpal dentine. The fibrous components mentioned above are of two varieties; the Korff and alpha fibre and the beta fibrils. The latter, which are the principal component of the circumpulpal dentine, are shorter and finer and possess characteristic banding. The orientation of the beta fibrillar components of the matrix in this area is generally agreed to be such that it is parallel with the dentine surface or the appositional lines of development. Expressed in terms of their relation to the dentinal tubules which are radially oriented the fibrils are

perpendicular or obliquely disposed. The path of these fibrils may be one in which they envelope individual tubules or one in which they meander through the matrix, avoiding the tubules. It has also been suggested that while the orientation of the fibrils of the circumpulpal dentine is such that they meet, encircle or pass the tubules perpendicularly or obliquely, the positional relation which the formed intercellular components of a given layer assume relative to the subsequently deposited layers is acutely angular. The variations in arrangement are believed to be a form of functional modification. Circumpulpal dentine forms the bulk of the calcified connective tissue in mamalian teeth and is laid down rhythmically as the odontoblasts move centripetally. It is penetrated throughout with a wealth of tubules housing the main processes of the odontoblasts and their side branches. It comprises two main regions, designated intertubular and peritubular dentine (Jones and Boyde, 1984).

1.1.3. Interglobular Dentine.

Interglobular Dentine is the term used to describe areas of uncalcified or hypocalcified dentine that persist within mature dentine (Ten Cate, 1980). Interglobular dentine represents areas where the globular areas of calcification (calcospherites) have failed to fuse into a homogeneous mass. It is seen most frequently in the

circumpulpal dentine just below the mantle dentine where the pattern of mineralisation is largely globular.

Ioskawa et al. (1963) have shown that interglobular spaces are absent or infrequent in dentine formed before birth. They also reported that Interglobular spaces were absent in most deciduous central and lateral incisor teeth, but occurred as a normal pattern with increasing frequency in canines, and in first and second molars. The interglobular areas were localized in patches, the arrangement of which below the central fissure and in the lateral bulges of the crown bore a closer correlation with coronal dentine thickness than with the incremental line pattern. The above authors also showed that the interglobular spaces limit the fusion of calcospherites but not the intensity of their hypercalcification (sclerosis).

In dry ground sections the interglobular dentine is sometimes lost and replaced by air. In cross-section these interglobular "spaces" appear black. Osborn and Ten Cate (1976) and Avery (1976) shown that although dentinal tubules run interruptedly through these areas, peritubular dentine is absent. This suggests some correlation between peritubular dentine formation and the presense of mineralized dentine but this point has not been studied.

Brand and Isselhard (1982) reported that interglobular dentine is found next to the dentino-enamel junction in the

crown and the dentinocemental junction in the root. The root interglobular dentine is generally called the granular layer of Tomes.

Interglobular spaces of unmineralized dentine are especially prevalent in human teeth where there has been a deficiency in vitamin D during dentine formation, or conditions such as vitamin D resistant Rickets or severe fluorosis. Because of the limitation of their growth, calcospherites tend to remain spherical in these cases. Interglobular spaces may be found in most human teeth, and are usually found in poorly mineralized teeth of, for example, some odontocetes (Jones and Boyde, 1984).

1.1.4 Tomes granular dentine

This layer was named after Tomes in 1838. Seltzer and Bender (1975) described Tomes' Granular layer as a thin layer of dentine adjacent to the cementum which appears granular in ground sections of the teeth.

Microradiographic, polarized light, phase-contrast and scanning electron microscope studies of this layer have been made by Shackelford (1971). He found that it consisted of a narrow zone of hypomineralized, hyperorganic dentinal matrix. One component of Tomes' layer appeared to be fibrillar in nature. The dentinal tubules pass through Tomes' layer and terminate in the cementum.

The exact function of Tomes' granular layer has not been elucidated. Shackleford believed that, in dogs, it protected the tooth from sudden insults or large occlusal forces transferred to the dentine via the periodontal ligament.

Ten Cate (1980) reported that although the significance of this layer has not been established, it has been suggested that it plays a functional role in "cementing" cementum to dentine, and that it may be product of root sheath cells.

Avery (1976) reported that, the configuration is found only in the tooth, and it does not follow the incremental pattern. It is believed to represent an interference with mineralization of the entire surface layer of the root dentine prior to the beginning of cementum formation. Recent evidence indicates that the granular layer of dentine underlying the cementum may result from the looping of the terminal portions of the dentinal tubules. This would be the result of a slightly different orientation of the odontoblast process in the initial stages of root dentine formation as compared to that adjacent to the enamel (Avery, 1976).

Moss-Salentijn and Klyvert (1980) have shown that the non-calcified dentine islands in root dentine have the same shapes as the interglobular dentine areas in the crown, but they are considerably small.

1.1.5 Incremental lines.

The term "incremental lines" was first coined by von Ebner (1891). Throughout life, dentine formation proceeds rhythmically with alternating phases of activity and quiescence. These phases are represented in formed dentine as incremental lines which, although often not clearly detectable, can best be seen in longitudinal ground sections of teeth. They run perpendicular on the dentinal tubules and generally mark the normal rhythmic linear pattern of dentine deposition in an inward and rootward direction (Ten Cate, 1980).

Avery (1976) reported that the distance between the lines corresponds to the daily rate of apposition. In the crown it varied from 4 to 8 micrometres and became decreasingly less in the root. In addition he claimed that in the deciduous teeth and in the first permanent molars, where the dentine is formed partly before and partly after birth, the prenatal and postnatal dentine are separated by an accentuated contour line, the so-called neonatal line. This line is the result of incomplete calcification and represents the abrupt changes in environment and nutrition that occur at birth.

A. Incremental lines of von Ebner (Imbrication lines).

Moss-Salentijn and Klyvert (1980) reported that the dentine deposited at night differs from that deposited

during the daytime in amount, nature of the matrix and degree of calcification. Thus, if the daily deposition rate of dentine is about 4 micrometres, each 4 micrometres of dentine in a ground section is composed of a somewhat lighter (more calcified) and somewhat darker (less calcified) band. In decalcified and stained histologic sections such bands, or lines, are visible as well. These lines, representing alternate bands of dentine deposited during daytime and during nighttime, are called imbrication lines (von Ebner).

B. Contour lines of Owen.

In case of major changes in the body metabolism such as in periods of illness, the dentine deposition may be somewhat affected as well. Provenza (1964) shown that, the contour lines of Owen are related to the lines of Retzius in enamel in both number and density. The relation of these two lines is believed to be developmentally comparable and reflects the same differences in mineralization initiated by disturbed calcium metabolism. The width of the lines is of course correlated with the duration of the disturbance.

C. Man-made contour lines.

Certain antibiotics, such as tetracyclines, are chemically bound to matrix components and even more to hydroxyapatite at the growing surfaces of bone, cementum and

dentine. Bone and cementum are well embedded in soft tissues, so that any discoloration of these two tissues, as the result of the binding of tetracycline, remains undetected. Furthermore, bone is constantly remodelled and so, in time, rids itself of the tetracycline. But dentine is permanently marked because the tetracycline remains fixed in this nonremodelling tissue for the life of the tooth. Tetracycline has a deep golden colour and because of the transparent and thin of the enamel tissue, a dark band may be visible through the enamel. As a result, the tooth may become aesthetically unattractive (Moss-Salentijn and Klyvert, 1980).

1.2 The secondary dentine.

The changes that occur in the structure and in the distribution of dentine is a problem of interest not only in the study of histology, but to clinical dentistry as well. This is particularly true in regard to the variety of this tissue usually referred to as secondary dentine (Bevelander and Benzer, 1943).

The Federation Dentaire Internationale (Baume, 1980) had defined the secondary dentine as following: "Secondary dentine" comprises that circumpulpal portion of regular dentine (Orthodentine) in continuity with the primary one produced circumpulpally throughout the later periods of the vital tooth. Philippas and Applebaum (1966) reported that,

after completion of the primary dentine and the "closure" of the apex, two types of secondary dentine were formed in increasing amount with increasing age. The regular secondary dentine was slowly formed on the walls of the pulp chamber of crown and root. The irregular secondary dentine was first formed more on the lingual than on labial and incisal walls of the pulp chamber of the crown of the upper central incisor.

Morphologically, secondary dentine may be recognized by an abrupt change in the course of the dentinal tubules at the transition between primary and secondary dentine. Also there is a slight reduction in the number of the dentinal tubules that can be explained only by the cell death of some of the odontoblasts at the completion of the primary dentine production. Above all, the secondary dentine matrix is somewhat more calcified than the primary dentine matrix (Moss-Salentijn and Klyvert, 1980).

Provenza (1964) reported that not all of the secondary dentine produced is of the same structure and quality, and suggested that two types of secondary dentine could be recognized: regular secondary dentine and irregular secondary dentine.

1.2.1 Regular secondary dentine.

When the dentine has increased in quantity to the point that encroachment upon the pulp cavity is more

appreciable, the odontoblasts of the area respond by a slight modification in orientation. The newly formed dentine is referred to as regular secondary dentine or functional dentine. The term "functional dentine" is used because it is that dentine deposited as a result of more severe functional stimulation (Provenza, 1964). Bevelander and Benzer (1943) have reported that their observations in regard to the zone of circumpulpal dentine incline them to favour the term physiologic secondary dentine as proposed by Hopewell-Smith (1918) for the following reasons: It was present in all adult teeth and was not the result of any external force or environmental influence.

Rushton (1944) believed that the changes in dentine in the absence of attrition may be "a progressive degeneration of the fibrils and occlusion of the tubules from without inwards". The change in the structure from primary to secondary dentine may be caused by the progressive crowding of the odontoblasts, which finally leads to the elimination of some and to the rearrangement of the remaining odontoblasts (Avery, 1976). As secondary dentine formation proceeds, a decrease in the size of the pulp canals, chambers, and pulp horns occurs (Brand and Isselhard, 1982).

Osborn and Ten Cate (1976) reported that regular secondary dentine is that dentine laid down on the pulpal walls at very much slower rates after the normal anatomy of

the tooth has been established. Its structure is essentially the same as the primary dentine from which it is demarcated by a resting line. The dentinal tubules bend more or less sharply at this line (Avery, 1976).

Kuttler (1959) has shown that the regular secondary dentine is less permeable and also has less dentinal tubules due to the reduction in the number of odontoblasts per square millimeter and consequently in the number of Tomes' fibres. It is somewhat darker in colour than the primary dentine, and its tubules are more curved, sometimes angulated, less regular and smaller in diameter.

1.2.2 Irregular secondary dentine.

Irregular secondary dentine or (tertiary dentine) is produced in reaction to noxious stimuli such as caries or restorative dental procedures. Unlike primary or secondary dentine, which is formed along the entire pulp-dentine border, irregular secondary dentine is produced only by the odontoblasts directly affected by the stimulus. The quality or architecture and the quantity or degree of the irregular dentine produced is related to the intensity and duration of the stimulus. For example, if the stimulus of an active carious lesion causes extensive destruction of dentine and considerable pulp damage, tertiary dentine is deposited rapidly, up to 3.5 micrometres per day, and displays a sparse, irregular tubular pattern with frequent cellular

inclusions. Tertiary dentine with such cellular inclusions is sometimes called osteodentine. On the other hand, if the lesions is less active, tertiary dentine is deposited less rapidly, its tubular pattern is more regular, and there are fewer, if any cellular inclusions (Ten Cate, 1980).

Avery (1976) confirmed that, some areas of reparative dentine contain few or no tubules. Dentin-forming cells were included in the rapidly produced intercellular substances, such cells degenerated and vacated the spaces that they formerly occupied. Frequently reparative dentine was separated from primary or secondary dentine by a deeply staining line.

Philippas and Applebaum (1966) examined 273 permanent upper central incisors taken from different age groups and they showed that there was no secondary dentine in the age group from 6 to 10 years. In the second age group, 11 to 15 years both regular and irregular secondary dentine were described. In regular secondary dentine the course of the tubules was continuous with that of the primary dentine. In irregular secondary dentine, the course of the tubules was different from that of the primary dentine; moreover, irregular secondary dentine had fewer tubules. This earliest stage of irregular secondary dentine formation occurred more on the lingual wall of the pulp chamber and less on the labial wall. An upward curve of the tubules in this irregular secondary dentine differed from the curve of

the tubules in the primary dentine. In the third age group 16 to 20 years, there was a small amount of irregular secondary dentine formed at the incisal tip of the pulp chamber. There were also deposits on the lingual and labial walls but a greater deposit on the lingual than on the labial wall. A pronounced increase in the amount of irregular secondary dentine in the pulp chamber of the crown was observed in the age group 47 to 50 years, the increase was greatest on the lingual of the pulp chamber, extending below the cervical line and continuing with the regular secondary dentine of the root canal there. In the age group 61 to 65 years, the irregular secondary dentine filled the entire pulp chamber and one fourth of the root canal. Philippas and Applebaum concluded that there was a general trend toward increase of irregular secondary dentine formation with age.

Brand and Isselhard (1982) reported that, reparative (irregular) dentine is formed in response to local trauma. They stated that this trauma was generally of two varieties occlusal trauma and chemical trauma such as caries. Where present, reparative dentine was located immediately beneath the area of trauma.

The atubular "irregular" dentine is that dentine which has been formed in those sites where odontoblasts have not survived. The matrix in such cases has been formed by

the dying odontoblasts or by their replacements (Provenza, 1964). Moss-Salentijn and Klyvert (1980) reported that, if odontoblasts perish, neighboring undifferentiated mesenchymal cells move to the affected dentine and become odontoblasts these cells produce a small, localized patch of reactive (irregular) dentine. The new tissue has irregular dentinal tubules and occasionally no tubules at all, sometimes odontoblasts become trapped in this dentine.

The dentinal tubules, which run between the dentinoenamel junction and this new patch of reactive dentine, usually no longer contain any odontoblastic processes. These processes are lost when the odontoblasts themselves perish. During the preparation of a ground section, such empty tubules may fill up with air. Because of the presence of air in a band of dentinal tubules, such a band appears dark in ground sections and is called a dead tract.

The interface between dentine and irregular secondary dentine may vary markedly, and it cannot be directly related to the type of destructive process or experimental procedure. Undoubtedly this type of increased dentine formation is important biologically. However, it should be kept in mind that irregular secondary dentine may contain more organic material and be less mineralized than primary dentine. The more organic material the dentine comprises the more permeable it will be. It is possible, therefore,

that from a clinical point of view, irregular secondary dentine represents an inferior type of dentine as compared to primary dentine (Mjor 1972).

Kuttler (1959) listed the difference between the irregular secondary dentine and the other types of dentine as follows:

1. It is localized exclusively in front of the irritated zone.
2. The dentinal tubules are greatly irregular, even tortuous.
3. The tubules are reduced in number or are absent.
4. The calcification is deficient.
5. There are cellular inclusions which convert into spaces.
6. There is a tonality difference.

1.3 The preentine.

The preentine is a layer of unmineralized matrix 10 - 20 micrometres wide which is found between the layer of odontoblasts and the mineralized dentine. It is present during dentinogenesis and remains throughout the life of the tooth, because there is a continuous, slow deposition of dentine during life (Mjor and Pindborg, 1973). Provenza (1964) described the preentine as a narrow band of dentinal matrix observed immediately external to the layer of

odontoblasts. This dentine also referred to as dentinoid and is analogous in position and staining reaction to osteoid in developing bone. The presence of preentine is a constant feature as long as the dentinogenic cells are present.

The first sign of preentine development is the appearance of bundles of fibrils between the differentiating odontoblasts. von Korff (1905, 1907) suggested that these fibres contributed to the organic matrix of the mantle dentine. Later studies have shown that "von Korff fibres" were present during mantle dentine formation, but that there was a reduction in their size and number during the formation of circumpulpal dentine (Nylen and Scott, 1960; Noble et al., 1962; Ten Cate, 1968).

Lester and Boyde (1968) reviewed the controversy regarding the presence of 'von Korff fibres' could be visualized in prepared surfaces of dentine matrix, and their specific location and conformation in the tissue could be related to the developing surface.

However, Ten Cate et al. (1970) and Ten Cate (1978), in an electron microscopic study, were unable to demonstrate collagen fibrils in the region and resolved that the fibres of von Korff did not represent collagen fibrils, but rather silver impregnation of extracellular material. Costacurta et al. (1974) also failed to detect any fibrils or collagenous fibres between developing odontoblasts, and

emphasized that the intercellular spaces amongst the odontoblasts were too small to allow sufficient passage of collagen to be deposited in the mantle dentine layer. In contrast, Whittaker and Adams (1972) and Adams and Whittaker (1972) were able to demonstrate collagen fibres both at the light and electron microscopic levels. These authors also demonstrated that silver impregnation revealed silver deposition associated mainly with collagen fibrils. the identity and function of von Korff fibres is controversial.

1.4 Transparent Dentine (Sclerotic Dentine).

A condition in which the dentinal tubules are filled with a dentine like material is called sclerotic dentine. This type of dentine takes place especially in the peripheral dentine and root dentine. The cause of this is related to occlusal trauma or decay. However, when the contents of the tubules are calcified, the light rays pass relatively straight through the section, without deflection, making it a transparent tissue.

Ten Cate (1980) reported that sclerosis of dentine is brought on by age. It is frequently seen in the apical third of the root and in the crown midway between dentinoenamel junction and the surface of the pulp. Sclerosis reduces the permeability of the dentine and may help to prolong pulp vitality.

Provenza (1964) demonstrated that sclerotic dentine is found most frequently at those sites where enamel thickness is reduced (subjacent to fissures and pits). It has been shown that those areas in which the primary dentine has been converted to sclerotic dentine are more resistant to caries. Avery (1976) reported that Roentgen ray absorption tests and permeability studies have shown that such areas are denser, and hardness tests have demonstrated that they are harder than normal dentine.

Weber (1974) surveyed a large sample of human teeth by microradiography attempting to discern differences between what he termed physiologic and pathologic dentinal sclerosis. He reported that perhaps the most striking difference between physiological and pathological sclerosis was in the percentage of tubules which were obliterated. Tubular obliteration in physiological sclerosis was most extensive in the region mid-way between the dentinoenamel junction and the surface of the pulp. Even at this level however, less than 50% of the tubules were obliterated. Near the dentino-enamel junction and the surface of the pulp, the number was usually less than 20%. The alterations seen in latent pathological sclerosis were more diverse. Usually, more than 80% of the tubules were obliterated. In addition no evidence of intraluminal mineralization was seen in physiological sclerosis. The absence of intraluminal

mineralization suggests that the tubules were obliterated by an active centripetal appositional process.

Moss-Salentijn and Klyvert (1980) reported that, with age, the contents of the dentinal tubules, excluding the odontoblasts process, may slowly undergo calcification. This takes place especially in the peripheral dentine and root dentine. Transparent or sclerotic dentine is also formed adjacent to carious dentine during the caries attack.

The development of sclerotic dentine is clearly related to age but surprisingly the process is much more rapid in the dentine of the apex, which develops last, than elsewhere. Indeed the closure of the tubules spreads coronally from the apex as well as from the cementum-dentine junction toward the pulp. In addition, even at the apex, the process begins in the mesial and distal dentine and only spreads to the lingual and buccal dentine much later (Vasiladis et al. 1983).

The absolute increase in the amount of mineral in the zones of sclerotic dentine indicated that the occluding mineral is not derived from the adjacent intertubular dentine but originates from another source. This other source can only be the pulp because, if it arose from an external source and passed through the cementum the process of sclerosis would be self-limiting. Furthermore, the observation of Bang and Ramm (1970) that the percentage of translucent dentine in non-vital teeth is well below that of

vital teeth of corresponding ages supports this conclusion and suggests that the maintenance of a blood supply to the pulp is necessary for the appearance of sclerosis in the root dentine (Vasiliadis et al 1983).

CHAPTER 2

THE STRUCTURE OF DENTINE

2.1 Odontoblast Cell.

The cell associated with the formation and maintenance of dentine are the odontoblasts which form a single layer lining the periphery of the pulp and have processes extending into the dentine. Avery (1967) has shown that the odontoblasts are approximately 5 to 7 micrometres in diameter and 25 to 40 micrometres in length. The cell bodies of the odontoblasts are columnar in appearance with large oval nuclei. In comparison, Osborn and Ten Cate (1976) have been reported that in the mature tooth the morphology of the odontoblasts may vary considerably, ranging from columnar to almost squamous in shape. The odontoblasts in the crown are larger than the odontoblasts present in the root. In the crown the cell bodies of the odontoblasts are columnar in shape and measure approximately 35 micrometres in length, whereas in the midportion of the pulp the cells are more cuboid and in the apical part they are flattened in appearance. Similarly Avery et al. (1984) has shown that the odontoblasts maintain a somewhat different relationship to each other throughout the various regions of the root pulp, such as the bifurcation zone, the central pulp, and the coronal pulp. Along the root pulp-dentine interface, the odontoblasts are seen as cuboidal-to low columnar-

appearing cells with some extracellular spaces between them, in the central columnar appearance. In the pulp horns, the odontoblasts are densely packed together with many junctional complexes evident between the cells.

Osborne and Ten Cate (1976) have stated that it is unwise to give too precise a description of the odontoblasts as their morphology can vary depending upon the functional activity of the cell. Odontoblasts may be in either an active synthetic state or a resting state. The resting cell is flattened, with relatively little cytoplasm while the active odontoblast is a big, plump cell with much intensely basophilic cytoplasm. In its fine structure, the organelles of an active odontoblast are prominent, consisting of a large nucleus located in the basal portion of the cell body (adjacent to the pulp), numerous vesicles, much rough endoplasmic reticulum, a well-developed Golgi complex located on the dentinal side of the nucleus, and numerous mitochondria scattered throughout the cell body (Ten Cate 1980).

The fully differentiated odontoblast cell is columnar and in close contact with its neighbours and the advancing front of dentine matrix formation (Noble et al., 1962). The morphology of the odontoblasts also changes during differentiation. Differentiation requires quantitative as well as qualitative changes. The quantitative changes involve cell elongation with no appreciable change

in the cell width. The qualitative changes in the odontoblasts are manifested by the complex organizational structure of the intracellular constituents (Provenza, 1964). The newly differentiated odontoblasts is characterized histologically by its location and its size, histochemically by its high RNA content and marked oxidative and hydrolytic enzyme activity, and ultrastructurally by a well-developed Golgi complex, numerous mitochondria, many vesicular structures, and a well developed microtubular system (Osborn and Ten Cate 1976).

2.2 The odontoblastic process (Tomes' fibres).

The cytoplasmic extension of the odontoblasts, which are membranous tubes filled with cytoplasm and housed by the dentinal tubules, are variously known as odontoblastic processes, Tomes' processes, dentinal fibres or Tomes' fibres. The latter two terms should be avoided because of their tendency to be confused with the fibrous components of the matrix (Provenza, 1964).

Avery (1976) reported that the cytoplasmic contents of the process are sparse, and that the predominant structures include microtubules of 200 to 250 A° diameter and filaments of 50 to 75 A° diameter. Other contents include an occasional mitochondrion, some dense bodies resembling lysosomes, coated vesicles that may open to the extracellular space, and microvesicles. There is a conspicuous absence of ribosomes and endoplasmic reticulum. Frank (1968) has

reported that the odontoblasts process is filled mainly with small filaments, some microtubules, large vacuoles and dense droplets. The two latter structure seem to take part in the elaboration of collagen fibrils and ground substance in extracellular location.

The odontolastic processes divide near their ends near the dentino-enamel junction into several terminal branches. Along their course from the pulp to the periphery they send out thin secondary processes enclosed in fine tubules, which seem to unite with similar lateral extensions from neighboring odontoblastic processes. Some terminal branches of the odontoblastic processes extend a short distance into the enamel (Avery, 1976). The latter statement is controversial.

Some studies (Meyer, 1932, 1951; Bradford, 1967; Martens, 1968; Holz and Baume, 1973; Langeland, 1976) have shown that the processes extend right out to the dentine-enamel or dentine-cementum junction. Langeland (1976) claimed that the absence of the process was probably an artifact caused by laboratory processing. Kaye and Herold (1966) revealed with vital staining reaction an extremely fine branching system of the odontoblastic processes just below the dentino-enamel junction, whose refractive index corresponded to values generally given for cytoplasm. Boyde, (1980) and Kelly et al. (1981) have also found

evidence of odontoblast processes in the peripheral parts of dentinal tubules in the rhesus monkey.

On the other hand other studies in rats (Garant, 1972), in cats (Holland, 1975, 1976), and in humans (Thomas 1979; Tsatsas and Frank, 1972; Brannstrom and Garberoglio, 1972) have concluded that the process does not extend into the dentinal tubules more than 0.7 millimeter from the pulp.

The concept that an odontoblast process is present only in the inner part of the dentine has become generally accepted and is included in most current texts on dental histology (Mjor, 1979; Avery, 1980; Torneck, 1980). But recent studies by Maniatopoulos and Smith (1983) and Grossman and Austin (1983) reported that the odontoblast processes traverses the full length of dentinal tubules. Szabo et al. (1984) observed a few odontoblast processes near to the dentine-enamel junction and they were divided into longitudinal branches with lateral subdivision entering or leaving the tubules which were 0.1-0.8 micrometres in diameter. The most peripheral odontoblast processes were about 60 micrometres from the dentine-enamel junction.

Szabo et al. (1984) reported that odontoblast processes were observed in the inner-third, middle-third, and peripheral dentine. The processes in the inner-third dentine closely approximated the walls of tubules and could be found in most of tubules. Some tubules contained a thin fibre besides the odontoblast processes. In the middle

third of dentine, the processes became thinner. Their appearance was cable-like with lateral branches and a wide periodontoblast space could be seen. Processes were seen only in a few tubules of outer-third dentine in both young and aged subjects. They divided into longitudinal and lateral branches and seemed to be connected with one another.

Tube-like processes were observed by Grossman and Austin (1983) protruding from the opening of transversely and obliquely fractured dentinal tubules adjacent to the enamel-dentine junction. These structures appeared to be tethered in the tubules and were assumed to have been withdrawn from the portion of the dentinal tubule in the opposing fragment. The tubular structures emerged from the openings of the dentinal tubules which were fractured within 20-30 micrometres of the dentino-enamel junction. Many of the processes which emerged from the exposed dentinal tubules were reflected against the surface of the fractured dentine. This resulted in a flattening of the process as it traversed the margin of the tubule opening. Occasionally, fine side-branches were observed protruding from the processes .

2.3 The sheath of Neumann.

Neumann (1863) suggested that the pipe-stem appearance of ground transverse sections of dentine was due to the presence of a sheath around the dentinal process of the odontoblast cell and that this was the structure which could be isolated from the dentine by hydrolysis. The hydrolysis of dentine provides a good starting point for the investigation into the identity of Tomes' fibre. Romer (1899) Hanazawa (1917) and Churcill (1935) maintained that the structure isolated from dentine by hydrolysis of the matrix was identical with Tomes' fibre (the odontoblast process), whereas Walkhoff (1915), Fleishchmann (1905), and Kantorowicz (1911) considered that the structure isolated by hydrolysis and Tomes' fibre were two different structures.

Hoppe (1862) and others have shown that structures similar to Tomes' fibre could be isolated from the teeth of prehistoric man, teeth which have been subject to putrefaction and fossilification for thousands of years. Romer (1899), had explained this phenomenon that Tomes' fibre consisted of two parts;

1. An inner portion of a soft protoplasmic nature.
2. An outer elastic membrane which was continuous with the cell wall of the odontoblast (Bradford, 1950b).

Wislocki et al. (1948) considered that the basophilic character of the sheath of Neumann, combined with

metachromatic staining with toluidine blue, after fixation with basic lead acetate, indicated that it consist essentially of acid mucopolysaccharides. The pulpodentinal membrane gives very similar staining reactions which would indicate that it is probably of similar chemical constitution, and also that the two structures are likely to be one continuous membrane separating the cells of the pulp from the dentine (Bradford, 1950a).

Yasuzumi and Obata (1955) have shown that when the surfaces of slabs of dentine are etched with 1.4N HCL for 2 minutes, the sheath of Neumann, which is enclosed with collagenous fibres, becomes clearly visible.

Bradford in his studies (1950b, 1951, 1955) has termed this structure the line of junction, and he said the familiar terms such as the "sheath of Neumann" been used as they are not sufficiently precise and have been used by different workers to describe different structures. For example (Provenza, 1964) reported that the interface between the peritubular and the intertubular dentine has been referred to as the sheath of Neumann.

In (1955) Bradford reported that, if the "sheath of Neumann" is to be retained, its use should be confined to denoting the original structure described by Neumann. Bradford stated that, in view of the historical confusion, it would be better to use some other terms to denote this structure. His term "translucent area" which was introduced

purely for the purpose of describing the appearance of ground sections of dentine, is unsatisfactory from many points of views, but could be used until some term denoting the structure or function of the area is introduced.

The confusion regarding the naming of this structure is readily apparent. The following terms have been used in the literature : Neuman's sheath (Wislocki et al. 1948), Peritubular matrix (Takuma and Tsuchikura, 1950), Translucent area (Bradford, 1950b), Hypercalcified ground substance (Rouiller et al., 1952), Calcified sheath (Shroff et al. 1954), Tubulo-fibrillar complex (Frank, 1956), Outer organic sheath (Shroff et al., 1956), Peritubular dentine (Fearnhead, 1957), Peritubular translucent zone (Black, 1958), Limiting membrane (Thomas, 1979), Lamina limitans (Thomas and Carella, 1983) and membrane-like structure (Thomas and Carella, 1983).

Shroff et al. (1956) suggested that the term Neumann's sheath is best forgotten. None of the structures now known to exist fits Neumann's original description and, therefore, it is not known to what he was referring. The organic fibrillar sheath which surrounds the outer surface of the calcified sheath could be known simply as the "outer organic sheath" to differentiate it from the similar "inner organic sheath" which lines the lumen of the canal and forms the outer surface layer of the odontoblast process. In some studies the later structure "inner organic sheath" has been described as Neumann's sheath. Shroff et al. (1956)

concluded that what previously were considered to be the dentinal tubule and the odontoblast process consist of the following structures, all of which are intimately connected with each other:

There is (Fig. 1) an outer sheath (A) consisting of collagenous fibrils arranged principally in a helical manner. This organic sheath separates the intertubular matrix from the calcified sheath (B) but is intimately connected, through a series of interlacing fibrils, with both of these structures and with the outer layer of the odontoblast process (C). The calcified sheath encloses on its inner aspect what appears to be a similar organic sheath (C). This organic sheath appears to be the outer layer of the odontoblast process. Within this is a further thicker sheath (D) of material which has some of the properties of myelin and which is resistant to the action of acids and alkalies and yet is soluble in hot alcohol. It also takes specific stains for myelin. There is a further organic sheath (E) which surrounds a central core (F). This core is susceptible to acids and alkalies but is not soluble in fat solvents.

Bradford (1967) reported that in sections in which the tubules were cut transversely, however, each tubule appeared to be surrounded by a frequently eccentric cuff or halo. Fleischman (1905) published the results of a series

of observations whereby he showed conclusively that in demineralized sections a odontoblast process 1-2 micrometers in diameter was contained within a dentinal tubule 5 micrometers in diameter. He found no evidence of any tissue between the fibre and the dentine matrix. Therefore he concluded that the sheaths of Neumann seen in ground sections were an artifact probably produced optically due to the observation of the opaque tubule in a thick section (50-100 micrometer) of a translucent material. He suggested that "in vivo" the fibres of Tomes (the odontoblast process) are approximately 5 micrometers in diameter and completely fill the tubule and that demineralization and embedding caused the fibre to shrink to 1-2 micrometers. He found that the tubule wall in demineralized sections stained more heavily than the rest of the dentine and regarded this stained layer as the sheath of Neumann.

Churchill (1932) tried to find a more satisfactory explanation for the appearance of the sheath of Neumann, but without success, and came to the conclusion that even the heavily stained tubule boundary seen by Fleischman (1905) and in demineralized section was an artifact. The basic reason for the controversy is therefore seen to be that the two methods of observing the structure of the dentine, derived from ground sections on the one hand and decalcified sections on the other, are irreconcilable (Bradford 1967).

Jones and Boyde (1984) have reported that the dissenting report published by Plackova et al. (1959), in which replicas of etched dentine showed some patterning in the peritubular regions that they believed to be unmasked collagen, is probably unreliable because etching procedures can dissolve peritubular dentine and probably allow translocation of material. The peritubular matrix may be well preserved with some fixation and decalcification procedures and be seen as "Neumann's sheath" in fortuitous SEM preparation.

Kennedy et al. (1953) have however shown that a definite thin membrane lines the tubule. Demineralization of the teeth resulted in the loss of peritubular dentine and the appearance within each tubule of a sheet-like structure which extended from the predentine-dentine junction to the dentine enamel junction. It has been suggested that this sheet-like structure, here termed the lamina limitans, may play a role in the control of peritubular dentine formation (Thomas and Carella, 1983).

2.4 Periodontoblastic space.

The potential space between the wall of the dentinal tubule and the odontoblast process is called the periodontoblastic space. It contains tissue fluid and a few collagen fibres. This interface is important for secondary intratubular changes, e.g., growth of the peritubular

dentine. The transition between the hypomineralized structure lining the tubules and the periodontoblastic space has not been clarified (Mjor 1984). Thomas (1979) reported that a short distance into the dentine a constriction of the odontoblast process was noted. This constriction was usually found on one side and resulted in a space between the tubule wall and the odontoblast process. Collagen fibrils and a fine granular material were present in this space. However Arwill and Bloom (1955) reported that a surrounding empty space cannot be seen.

2.5 Dentineal tubules.

Dentineal tubules are small, canal-like spaces within the dentine filled with tissue fluid and occupied for part or all of their length by odontoblast processes. They extend through the entire thickness of the dentine from the dentino-enamel junction to the pulp and their configuration indicate the course taken by the odontoblast during dentinogenesis (Ten Cate, 1980). The dentineal tubules are probably the most striking feature of dentine (Mjor, 1984). They lodge the odontoblast processes. The size of their lumen is dependent on their location within the dentine and the age of the tooth. The dentineal tubules are formed as the result of deposition of dentine around the odontoblastic cell processes which, during dentine formation are left behind by the odontoblasts. Since the odontoblasts are moving

moving backward, the shape of the odontoblastic processes and of the dentinal tubules in which they are located provide us with an accurate record of the path taken by the odontoblasts. The dentinal tubules are not straight, but rather gently curved in an S-shape. The S-shape curvature of the dentinal tubules in the crown and cervical region of the tooth has clinical significance. Caries, or any other external insult that affects the peripheral dentine may have a local effect in the dental pulp at a more apical level than the level at which the external insult occurred (Moss-Salentijn and Klyvert, 1980).

The dentinal tubules are tapered in outline, measuring approximately 2.5 micrometres in diameter near the pulp, 1.2 micrometres in the midportion of the dentine, and 0.9 micrometres near the dentino-enamel junction. In coronal dentine there are approximately 20,000 tubules per square millimeter near the enamel and 45,000 per square millimeter near the pulp (Ten Cate, 1980). A number of studies has examined the number of dentinal tubules per square millimeter of dentine. These are summarised in Table 1. All studies have shown more tubules and larger tubules towards the pulp, with progressively smaller and fewer tubules being found towards the dentino-enamel junction.

Studies by Forssell-Ahlberg et al. (1975) on rat, cat, dog, and monkey have shown that the tubules were found to be widest at the pulpal wall, where the mean diameter

ranged from 1.7 to 1.8 micrometres, the highest value being found in monkey and the lowest in the incisors of rat. In the middle part of the dentine the mean diameter varied from 1.0 to 1.3 micrometres and at the periphery it was 0.6 to 0.9 micrometre. The largest number of tubules per unit area was found immediately incisal to the pulp horn and the smallest number cervically, near the enamel. At the pulpal wall the mean number of the tubules ranged from 50,000 to 90,000 per square millimetre, in the middle of the dentine it was between 37,000 and 50,000 per square millimetre and at the periphery it was between 10,000 and 25,000 per square millimetre. In all species examined the dentinal tubules showed a straight course, except in incisors of rat, where local irregularities were seen.

Avery (1976) reported that there are more tubules per unit area in the crown than in the root. The dentinal tubules have lateral branches throughout dentine, which are termed canaliculi. These canaliculi are 1 micron or less in diameter and originate more or less at right angles to the main tubule.

At the dentino-enamel junction, however, the tubule density becomes suddenly higher than in the rest of the peripheral dentine. This is because the terminal parts of the dentinal tubules, near the dentino-enamel junction, are branched into 2 or 3 short branches. This higher tubular

density makes this part of the dentine sensitive on exposure, almost as sensitive as the dentine near the pulp.

The terminal branching of the dentinal tubules is produced during the earliest stages of dentine formation. At that time the odontoblasts have several cell processes, some of which contact the processes of the pre-ameloblasts across the broken-down basal lamina. As the odontoblasts recede, the original processes come together into one single odontoblastic process. The dentine is first deposited around the large number of smaller processes and later around the single process, producing the terminally branched dentinal tubule (Moss-Salentijn and Klynert, 1980).

There are only a few studies which deal with the area of the dentinal tubules. One of these studies (Garberoglio and Brannstrom, 1976) reported that the total tubule area was 10 percent of the total coronal area of dentine being 28 percent near the pulp and 4 percent near the dentino-enamel junction. Another, (Hoppe and Stuben, 1965) estimated that the total tubule volume was 21 percent of the total coronal volume of dentine being 27.7% near the pulp and 19.1% near the dentine enamel junction. The reason for the difference between the two values may be that Hoppe and Stuben used decalcified sections. The value found by Hoppe and Stuben for the value near the pulp (where peritubular dentine is absent) was 27 percent which compares favourably with that of Garberoglio and Brannstrom (1976).

If 25% of the volume of dentine is occupied by tissue fluid as suggested by Kreudenstein and Stuben (1956), and if incornal dentine only about one fifth of a tubule contains an odontoblast process, Brannstrom and Garberoglio (1972) calculated that only about 32% of the dentinal tissue fluid is present in dentinal tubules and the remaining 68% is in peritubular and intertubular dentine. The main free dentinal fluid may thus exist in the intertubular dentine (Garberoglio and Brannstrom, 1976).

2.6 Peritubular dentine.

The term peritubular dentine was coined by Fearnhead in 1957 (Baume, 1980), and has been defined by Baume as being that component of orthodentine which constitutes the highly mineralized matrix found within the tubules adjacent to the odontoblastic process or its remnants. It can be observed when undemineralized ground sections are viewed by transmitted light. A ring-shaped transparent zone of peritubular dentine can be seen surrounding the odontoblastic process as can be differentiated from the remaining darker matrix, the intertubular dentine zone. Studies with soft roentgen rays and with the electron microscope have shown convincingly that the peritubular dentine is more highly mineralized than the intertubular dentine (Avery, 1976).

Takuma (1960) has shown that the peritubular matrix can be visualised more clearly following acid treatment which

results in the intertubular matrix being left at a slightly higher level than the intertubular matrix. It can also be easily distinguished from the intertubular matrix by its characteristic granular appearance.

Osborn and Ten Cate (1976) established that peritubular dentine first develops in the fully mineralized dentine matrix near the predentine-dentine border. Its development coincides with the narrowing in width of the space formerly occupied by the odontoblast processes. From this point it lines the canal and surrounds the odontoblastic process throughout its length terminating at the dentino-enamel junction and separating the process from the calcified matrix. Where a tubule passes through an interglobular space the peritubular zone is not found but the tubule enlarges within the space to meet the collagen matrix boundary. The peritubular zone is widest nearest the dentino-enamel junction (0.75 micrometers) where the odontoblastic process is narrowest, and conversely is narrowest (0.4 micrometer) close to the pulp where the process is widest (Miller, 1954; Atkinson and Court, 1961; Ten Cate, 1980).

In an electron-microscopic and microradiographic study (Takuma and Eda, 1966) confirmed that peritubular dentine was present in both developing and mature teeth and that there was no difference in the structure of the peritubular matrix in the crown and root dentine. In the root dentine,

however, the peritubular matrix was generally small and even seemed to be missing in some teeth.

Ten Cate (1980) reported that the formation of peritubular dentine is a continuing process, which can be accelerated by environmental stimuli causing a progressive reduction in the size of the tubule lumen and, on occasions, eventually obliterating the tubule space. When this occurs in several tubules in a small area, the dentine assumed a glassy appearance and seems brighter than the surrounding dentine when calcified sections are viewed under transmitted light. The term used to describe the continued deposition of peritubular dentine and the obliteration of the tubule is sclerosis, and dentine so affected is called physiological sclerotic dentine. Sclerosis of dentine is a feature of ageing. It is frequently seen in the apical third of the root and in the crown midway between the dentino-enamel junction and the surface of the pulp. Sclerosis reduces the permeability of the dentine and may help to prolong pulp vitality.

Jones and Boyde (1984) reported that the extra density of peritubular dentine is its most remarkable feature, and that it is 40% more highly mineralized. Ten Cate (1980) reported that it was 9% more mineralized.

2.7 Intertubular dentine.

Baume (1980) defined the intertubular dentine as that component of orthodentine which constitutes the mineralized matrix between tubules or peritubular dentine. The intertubular dentine is evenly mineralized except in the zone with low mineral content close to the pulp, where it is less mineralized (Mjor and Pindborg, 1973).

Avery (1976) has shown that the main body of the dentine is composed of intertubular dentine. Although it is highly mineralized, over one half of its volume is taken up by the organic matrix. This matrix consists of large numbers of collagen fibrils enveloped in an amorphous ground substance.

The intertubular dentine represents the prime secretory product of the odontoblasts and consists principally of a tightly interwoven network of collagen fibrils measuring between 0.05 micrometres and 0.2 micrometres in diameter in which apatite crystallites are deposited. The crystals average 0.1 micrometres in length and are generally oriented with their long axes parallel to the collagen fibril. The collagen fibrils are aligned roughly at right angles to the dentinal tubules and are formed parallel to the developing dentinal front (Ten Cate, 1980).

Posner and Tannerbaum (1984) reported that the crystals which form the mineral phase of intertubular dentine are though to be a calcium hydroxyapatite and are usually described as plates or needles.

CHAPTER 3

SENSITIVITY OF DENTINE

Introduction.

Anderson (1975) reported that dental enamel is completely insensitive, and when mechanical, thermal or electrical stimuli applied to the tooth surface cause pain, they do so by simple stimulus spread to adjacent innervated tissues - either dentine and pulp, or periodontal disease, enamel wear or dental operative procedures. Dentine on the other hand is generally assumed to be a very sensitive tissue from which only a sensation of pain can be evoked. There are, however, two important questions: Firstly, it is really dentine which is sensitive? Secondly, is it really very sensitive?

Dentine contains many thousands of tubules which can provide access from an exposed surface of the tissue to the tooth pulp. It is therefore possible that its sensitivity depends on stimuli reaching pulpal receptors via the tubules, without the intervention of any biological mechanisms in the dentine. So when we talk of sensitivity of dentine we may mean sensitivity of dental pulp (Brannstrom, 1979).

From clinical experience it is well known that unprotected dentine, exposed by the effect of acid,

grinding, abrasion, and bacterial plaque, can be hypersensitive. Dentine hypersensitivity would appear to be a ubiquitous problem, the diagnosis of such often being consigned in the absence of any clear cut alternative pathology. The pain arising from exposed dentine, typically in response to chemical, thermal, tactile osmotic stimuli is varied in both frequency and severity. Thus it may be rapid in onset, sharp in character and of short duration or in some cases the pain may persist as a dull or vague sensation in the affected tooth. When such painful symptoms arise, the term dentine hypersensitivity is used to describe the condition. In fact for most patients pain from exposed dentine only occurs on the application of a stimulus and the term dentine sensitivity would appear more appropriate (Dowell et al., 1985).

From experimental studies it has been established factors contributing to the development of hypersensitive dentine are (1) a lowered pain threshold due to local inflammation in the corresponding pulp and (2) facilitated transmission of pain-producing stimuli, particularly dehydrating and mechanical stimuli, because of opened and widened apertures of dentinal tubules. Brannstrom et al. (1979) found that a fractured or acid-treated surface with wide open tubules was more sensitive than a ground dentine surface.

A number of questions have been posed (with respect to dentine sensitivity) by Brannstrom (1966).

- How can the neck of a tooth sometimes be so sensitive to the slightest touch?
- Why may just an air blast cause so much pain while careful grinding on the same surface under a stream of water may occasionally elicit only the slightest reaction?
- How can we explain the sensitivity to temperature?
- How is it that sugar can cause pain, but not Perhydrol and many other chemical agents which are known to elicit pain where there are pain fibres.
- Why do we obtain no anaesthetizing effect at all when an anaesthetic is applied to the dentinal surface?

Anderson et al. (1958) reported that many workers who examined this problem from a histologic standpoint were not in agreement in their conclusions and that it has been impossible as yet to provide a satisfactory histologic basis for the sensitivity of dentine, especially in the amelo-dentinal region which is said to be a very sensitive part of the tooth.

Osborn and Ten Cate (1976) asked that if dentine were not tubular would it be sensitive and suggest that it should be worth while to examine the sensitivity of other dentines which are non-tubular, such as vaso-dentine, which represents a complex folding of the pulp dentine interface.

These forms of dentine and, of course, tubular dentine represent anatomical configurations to provide proper nutrition of the dentine matrix, and it is just possible that tubular dentine may only coincidentally provide the needed anatomical basis for dentine sensitivity.

Anderson et al. (1967) investigated the mechanism of sensation in human teeth by a method in which various solutions are applied to dentine with the object of evoking a sensation of pain or modifying that sensation. These authors have established that there appears to be a relationship between the osmotic pressure of solution and its ability to cause pain. This relationship was established first with solutions of calcium chloride and later with other solutes. Anderson et al. reported that a saturated solution of CaCl_2 at 37°C causes fluid movement through dentine when applied to a small occlusal cavity. Measurement of the rate of flow was made photographically, and the maximum value obtained in a group of eight teeth was 4.8 ml/sec. During a sequence of eight applications, the rate of flow declined. Anderson et al. suggested that with osmotic stimulation;

- (1) flow through dentine and pain production are related events.
- (2) The decline in the number of reports of pain during repeated stimulation is due to some change in the dentine, rather than in the pulp.

Anderson and Matthews (1967) have shown that solutions with high osmotic pressures are more likely to cause pain than solutions with low osmotic pressures.

Brannstrom (1966) has found that fifty-four applications of dry paper elicited pain with a mean duration of 25 seconds, but the paper which moistened with potassium chloride solution did not produce pain and he reported that pain elicited by the application of sugar and salt to the dentine might be due to dehydration where, as in case of an air blast, the liquid is removed from the apertures of the tubules. Brannstrom (1963) has also shown that the aspiration of odontoblasts into the dentinal tubules as an immediate effect of physical stimuli applied to exposed dentine seems to result from loss of substance at the distal apertures of the tubules and subsequent outward flow of the tubular contents through capillary action. On the other hand, Kramer (1955) concluded that the aspiration must have been accompanied by violent disturbances in the content of the dentinal tubules, and stated that the lack of correlation between the incidence of this disturbance and pain experience would seem to provide evidence that dentine sensitivity cannot be explained in terms of movement of tubule contents.

3.1 Innervation of the dentine.

The innervation of the dentine has been the source of much controversy. The main reason for the controversy has probably been related to difficulty in the demonstration of nerves in histologic sections of teeth.

Silver impregnation techniques employed are difficult and not specific. Electron microscopic studies have now shown that fine nerves are found in the periodontoblastic space in the predentine and in the most pulpal part of the mineralized dentine. However, no technique has demonstrated nerve fibres in the main and peripheral part of the dentine although the dentine in this location is particularly sensitive (Mjor and Pindborg, 1973). Arwill (1963) reported that in (1866) Cutler suggested that nerve fibrils accompany the odontoblastic processes through the dentinal tubules. Ten Cate (1980) has suggested that most of nerve bundles terminate in the subodontoblastic plexus as either small (2000 to 1600 nanometres) fibres or a larger (2 to 5 micrometres) unmyelinated fibres, but that not all the nerve axons terminate in the subodontoblastic region. Some apparently lose their Schwann cell coating, pass between the odontoblast cell bodies and enter the dentinal tubules running very close (20 micrometres) to the odontoblast process occupying that tubule. Such intertubular nerves are found only in about one of ten tubules or less in human coronal dentine and are fewer in number in root dentine.

A diagrammatic representation of the various theories of dentine innervation is illustrated in Figure. 2 (Arwill, 1963).

Lilja (1979) reported that the innervation in the coronal dentine was more compact than in the root dentine. Within the coronal dentine itself, the most densely innervated area was the dentine covering the pulp horns. No nerves were identified more than 100 micrometres from the pulp-dentinal border. No nervous structures were found in the mineralized dentine of the root. Nervous structures which seemed to have no connection with the odontoblastic processes were frequently observed in the predentine of the root. Signs of degeneration of the nervous structures of the predentine of the root was also a common finding.

The observation that the intradentinal nerves were confined to the predentine and the most pulpal part of the dentine supports the theory that surface stimulation on dentine has a indirect effect on the nerves activated by movements in the liquid of the dentinal-tubules. On the other hand, some authors claim to have in a few morphological studies evidence of nerve fibres in the pulpal part of the dentine has been reported (Fearnhead, 1957; Stella and Fuentes, 1963; Frank, 1966), but in the majority of investigations the results of the search have been negative (Arwill, 1958).

Byers and Kish (1976) used radioautography to study the distribution of somatic nerve endings in rat teeth by direct injection of the right trigeminal ganglion. These authors claim to have established that there is extensive innervation of cusp pulp and dentine in rat molars. The incisors appeared completely different from the molars, perhaps because they are continuously erupting teeth. Lilja and Fagerber-Mohlin (1984) have shown that the nervous structure were found in the predentine and the dentine of the impacted third molars in different parts of the crown and also in the predentine of the root.

Despite the above uncertainty still exists with respect to how far peripherally the nerves extend (Linde, 1984). Nerve fibres seem to be present in the predentine and possibly also in the adjacent dentine, but it is generally agreed that they do not extend beyond the pulpal one third of the dentine. Their frequency is also low and they can only be demonstrated in one out of several hundred dentinal tubules.

Many stimuli evoke a painful response when applied to dentine, including some related to clinical dental practice such as thermal stimuli from air water sprays, mechanical stimuli caused by touching exposed dentine with a probe or by cutting with a bur, and the dehydration of dentine with cotton wool or a stream of air.

Avery et al. (1984) have summarised the three basic theories of pain conduction through dentine (Fig. 3). The first one is that of direct neural stimulation. This theory presumes the presence of nerves and endings in the predentine and dentinal tubules. The nociceptive stimuli are picked up at or below the dentino-enamel junction and carried to the pulp via nerve ending activation and then directly without synapse to the descending nucleus of the trigeminal system. The second is the fluid or hydrodynamic theory. Various stimuli such as heat, cold, air blast, dessication, mechanical pressure, and other factors affect the fluid movement within the dentinal tubule. It is this fluid flow, inward or outward, which stimulates the nociceptive mechanism within the dentinal tubule, predentine, and/or odontogenic zone, ultimately resulting in a painful response at higher centres. The third transduction theory presumes that the odontoblastic process which is located at, or near to the dentino-enamel junction, is the primary structure excited by a stimulus, causing a mechanoreceptor-like effect at the nerve endings located in the tubules and odontogenic zone. Each of these theories of pain transmission has supportive factors as well as information that is nonsupportive in regard to that mechanism of conduction through dentine.

No single proposed mechanism fully explains all the facts related to dentine sensitivity. It may well be that more than one mechanism operates at any one time (Ten Cate, 1980).

3.2 Transducer theory.

The odontoblast and its processes have been perceived as a transducer mechanism. The concept of impulses being propagated along a stimulated nerve by a chemical agent was first suggested by Elliott in 1904 (Rapp et al. 1968). Loewi (1921) demonstrated that perfusion fluids obtained from hearts under the influence of vagal stimulation produced an inhibitory effect when returned to the same heart. He concluded that a chemical substance was present which could mimic the action of vagus stimulation, and termed this material "vagusstoff". Loewi and Navratil (1926) later compared the similarities of vagusstoff and acetylcholine, but the acceptance of these two substances being the same was not forth-coming, as acetylcholine had not yet been shown to be a constituent of the body. Dale and Dudley (1929) isolated and characterized acetylcholine, and it was at this point that the two substances were realized to be identical. From this time, the concept of neuro-humoral transmission of nerve impulses became more firmly established.

Acetylcholine and the enzyme acetylcholinesterase play an essential role in the transmission of neural impulses. Both of these substances are present in all cholinergic neurons. Acetylcholinesterase is responsible for the inactivation of acetylcholine within a nerve fibre. The rapidity of action of acetylcholinesterase ensures a rapid cessation of the passage of nerve impulses.

In support of neurohumoral transmission it was at one time reported that acetylcholinesterase was present adjacent to the bodies and processes of the odontoblasts. This enzyme is typically found in association with nerves and its presence in dentine suggested an affinity between nerves and odontoblasts. Secondly, because odontoblasts are probably of neural crest origin it is not unreasonable to suggest that they may retain the ability of many neural crest cells (i.e. peripheral sensory nerves and postganglionic sympathetic nerves) to propagate an impulse. Thirdly, electron microscopical studies have demonstrated a seemingly close functional relationship between nerve endings and the processes of odontoblasts and odontoblast cell bodies. Finally, the fact that odontoblast processes branch profusely in the region of the dentino-enamel junction could explain the reported hyper-sensitivity of the dentinoenamel junction on the bases of a summation phenomenon (Osborn and Ten Cate, 1976).

Rapp et al. (1968) reported that the presence of acetylcholinesterase in the pulp and dentine, extending from the area of darker staining at the dentino-enamel junction, along the Tomes' fibres and secondary processes to the area of the peripheral pulp, over the odontoblast cell bodies and parietal nerve plexus (where its presence is greatly intensified), along the smaller nerve fibres to the larger neural trunks to the apical foramen suggests a pathway of impulse transmission in teeth. Stimuli received at the dentino-enamel junction could be passed along the odontoblast process to the odontoblast cell body, where a synapse might occur with the pulpal nerves so that the impulse is able to continue through the pulp chamber to the root to the supplying neural trunk (Fig. 4).

The membrane potentials of odontoblasts have been measured by Kroeger et al. (1961) and Winter et al. (1963) (Narhi and Antila, 1973). Winter et al. (1963) obtained a mean value of 30mV. They also applied electrical stimulation at the amelodentinal junction and observed no response in the membrane potential of the odontoblasts although this might have been expected, if these cells are receptors. Kroeger et al. (1961) obtained potential values ranging from 25 to 45mV, but it was not certain that the cells than they measured were odontoblasts. Berman (1985) reported that there is no direct evidence for the presence

of acetylcholine activity in the neural transmission in the pulp.

Whenever accepted theories do not adequately explain both clinical observations and laboratory findings, it is time to reexamine these theories. In light of the present evidence, there may be a need to modify some of the prevailing theories of dentinal physiology, morphology, and sensitivity.

3.3 Hydrodynamic theory.

Perhaps the most widely accepted theory (Dowell and Addy, 1983) for the transmission of stimuli to the pulp is the hydrodynamic theory (Brannstrom 1962, 1966). The concept of fluid in dentinal tubules was first presented by (Gysi, 1900) who attempted to explain dentinal pain on the basis of fluid movement in the dentinal tubules (Avery et al., 1984).

Analysis of human dentinal fluid suggested that it was extra-cellular (Coffey et al., 1970). The wall of the dentinal tubule was found to be considerably more mineralised than the rest of the dentine (Johansen and Parks, 1962). Brannstrom (1966) theorised that the contained fluid would obey the same physical laws as liquids in glass capillaries. The contents of the tubules have been described as transparent fluid, devoid of cells, with a lower protein and

sugar content than plasma and a composition similar to that synovial or cerebrospinal fluid. The rate of flow in a dentinal tubule due to capillary attraction was calculated to be about 4 millimetres/sec at a distance of 2 millimetres from the pulp (Berggren and Brannstrom, 1965).

Berman (1985) reported that there is no direct support for any type of specialized terminal nerve receptors for hot, cold, electrical, osmotic, dehydration or chemical stimuli in dentine. The interpretation of most of these stimuli can be explained by the hydrodynamic theory (Figure 5) will help to visualize this mode, as explained below. The thermal coefficient of expansion of the substance composing the tubule wall seems to be only 5% or so of that of the tubule contents (Brannstrom and Astrom, 1972).

The dehydration of dentine (i.e., by air-blasts) is probably the clearest example for understanding dentine sensation. When Brannstrom applied absorbent paper to exposed dentine it caused pain; but no pain was elicited using wet paper. The outward movement of the dentinal fluid into the dehydrating source (i.e., air-blasts or absorbent paper) is thought to stimulate the "mechano-receptor" of the odontoblast, causing pain.

The perception of acute thermal stimulation can also be explained by the hydrodynamic theory. The coefficient of thermal expansion of the dentinal tubule fluid is about ten

times that of the tubule wall. Therefore, heat applied to dentine will result in a contraction of the fluid, with both creating an excitation of the "mechano-receptor". This fluid-flow model is further re-enforced by the finding that pain is felt before temperature changes can be measured on the pulpal side of the dentine.

It is also a common clinical finding that pain is produced when sugar or salted solutions are placed in contact with exposed dentine. When the irritant is rinsed or brushed away, the discomfort subsides. This again can be explained by dentine tubule fluid movements. Fluids of a relatively low osmolarity (i.e. dentinal tubule fluid) will have a tendency to flow towards solutions of higher osmolarity (i.e. salt or sugar solutions). When iso-osmotic solutions are applied, no stimulus is felt.

Brannstrom and Astrom (1972) reported that the pain elicited from the dentine may be of two types. The main might be that a larger volume of the dentine must be heated before a sufficient pronounced dislocation.

This basic hydrodynamic considerations suggest that, if fluid is removed from the outer surface of the dentine, a rapid flow in the tubules might constitute a mechanical force which could deform nerve structures in the pulp or at the pulpal end of the tubules.

Brannstrom et al. (1981) reported that experimental findings and theoretical calculations have shown that the

magnitude of capillary attraction force is sufficient to elevate a column of water at 7 meters in a tube like the dentinal tubule. Furthermore, the rate of flow due to capillary attraction in the tubule near the pulp would be around 2mm/sec. This means that if a tubule could be emptied it would be refilled again within one second. One type of response is an immediate sharp pain which can be elicited by most stimuli such as drilling, probing, an air blast and cold. The necessary stimulus for this response would seem to be a rapid outward movement of the contents of the tubules at the pulpal end with resulting deformation of nerve fibres. The second type of pain sensation is that which may be elicited by heat. The required stimulus for this response is evidently a more extensive inward movement of the tubule contents. This conclusion is supported by the observation that pain due to heat takes longer to develop than pain due to cold. The explanation of this difference the other hand Brannstrom (1968) reported that the application of isotonic potassium chloride solutions to exposed dentine has failed to produce pain (Anderson, 1958; Brannstrom, 1962; Anderson and Naylor, 1962; Brannstrom and Astrom, 1964). This seems to rule out the possibility that any nerves in the dentine are alone responsible for its sensitivity. Nor is there any evidence that the odontoblasts play an important role in the transmission of pain as it has been shown that the dentine surface may be sensitive

despite loss of all the odontoblasts in the corresponding area of the pulp (Brannstrom and Astrom, 1964). Evidence of a hydrodynamic transmission mechanism has been provided by many experiments in which it was found that the removal of small parts of the contents of tubules at the outer apertures may produce pain through the mobilisation of capillary forces and a rapid outward flow in the dentinal tubules (Brannstrom, 1966). It would seem that an outward flow of only a few microns in the tubules by applying dry absorbent paper to the dentine is sufficient to produce pain (Brannstrom and Astrom, 1964).

SCOPE OF THE INVESTIGATION

CHAPTER 4

Scope of the investigation

It would appear from the review that the dentinal tubule is implicated in the transmission of stimuli from the external surface of the tooth to the pulp. It is also apparent that there is considerable variation in the description of dentine and dentinal tubules in the literature. It was thought that these variations warranted further investigation.

Additionally as it has been suggested that branching of the tubules in the region of the dentino-enamel junction may explain the relative hypersensitivity of the dentine at the dentino-enamel junction (Osborne and Ten Cate, 1976), it was thought that a comparative study of the mean cross sectional area of the dentine occupied by tubules at various levels in the tooth may shed some light on reasons for this reported sensitivity.

The advent of new computer assisted measuring methods has revolutionized many histometric measuring techniques. To date no comparative study between these newer methods and the point count method could be found in the literature. It was considered that such a study would prove useful.

The aim of the study was therefore:

- (a) To examine the surface morphology of fractured surfaces of dentine at various levels in the teeth by Scanning Electron Microscopy.
- (b) To establish the number of dentinal tubules per square millimetre at different levels in the tooth.
- (c) To examine cross sections of dentinal tubules from different levels in the tooth by Scanning Electron Microscopy, to establish the percentage of dentine occupied by dentinal tubules at each level.
- (d) To establish whether any significant difference existed between results obtained from the point counting method and the digitizing method of measurements.

MATERIALS AND METHODS.

CHAPTER 5

MATERIALS AND METHODS.

Thirteen intact human permanent third molar teeth with completed root development taken from patients aged between 18 and 28 years were used in this study. Immediately after extraction the teeth were placed into normal isotonic saline in which a small amount of disinfectant was placed to inhibit bacterial growth*. Initially a histological pilot study involving several teeth was undertaken to establish the direction of dentinal tubules at various areas in the tooth.

Subsequently, the teeth were split in a mesio-distal direction using a tube cutter (Fig. 6A, 6B). Each half of the tooth was then reduced to a thickness of approximately 2

* Hibitane, ICI Australian Operations Pty. Ltd.,
Pharmaceutical Division, Christina Road,
Villawood 2168. N.S.W.

F.H.P. No. TC10. 1/8 to 1-1/8 capacity.

millimetres by cutting with a 0.5 millimetre diamond disc from the buccal or lingual (outer) surface of the tooth (Fig. 6C).

The 2 millimetre mesio-distal sections of the tooth were then further divided occluso-pulpally in a bucco-lingual direction into two smaller blocks of tooth structure using a 0.3 millimetre diamond disc⁺⁺ Fig. 6D) to produce separated cross sections of the crown of the tooth from the pulp to the outer surface.

A line (MN) (Fig. 7) was then drawn along the dentino-enamel junction. A second line (XY) was drawn at right angles to this passing from the pulp to meet the first line at the point "A" on the dentino-enamel junction. The enamel was then fractured from the tooth block using wire cutters (Fig. 6E, 6F).

The distance YA was measured with a Combimike* digit outside micrometer (Fig. 6G). The average length of this distance was 3.4mm. The dentine block was then further sectioned transversly close to the dentino-enamel junction

+ Horico No. 547190) Hopf, Ringleb and Co., GMBH & CJE
) Gardeschietzeneg 82 0-1000 Berlin

++ Horico No. 557220) 45 West Germany.

* Combimike Micrometer, Mitutoyo MFG. Co., Ltd. Japan.

at a new level B to provide a cross-sectional specimen of dentine for examination. The distance YB was measured the distance YA - YB was measure and recorded. This distance represented the distance the plane of section was from the dentino-enamel junction. The specimens were then numbered and placed in individual bottles of 50% alcohol for processing after the exposed surface was marked with a pencil to allow correct evaluation in the microscope.

Subsequently, the original block was resectioned and remeasured to give separate, additional specimens at different levels. Three to four specimens were obtained from each separate block. Up to ten specimens were obtained from each tooth.

In all 160 specimens were obtained. These were arranged in order according to the distance from the dentino-enamel junction and then divided into six groups:

Group 1. Less than 0.5mm

Group 2. From 0.5mm to less than 1.00mm

Group 3. From 1.00mm to less than 1.5mm

Group 4. From 1.5mm to less than 2.00mm

Group 5. From 2.00mm to less thant 2.5mm

Group 6. From 2.5mm to 3.00mm

Ten specimens were randomly selected for each group for use to the study. Each specimen was routinely dehydrated through graded alcohols (50%, 70%, and 100%) for three days

in each. They were then immersed in small amount of absolute alcohol in a petrie dish and placed in a silica gel for another three days until all the alcohol had evaporated from the specimen.

In addition to the above, several teeth were sectioned in a longitudinal direction so that a comparison could be made between the morphology of the dentinal tubules at the dentino-enamel junction compared with that at the pulp.

Specimens were then mounted on a 7mm aluminium stub with the fractured surface as close to parallel as possible to the surface of the stub in order to maintain perpendicular deflection of the secondary electrons. Specimens were luted in position by use of adhesive tape*, and Dag 154+ was applied to their periphery in order to increase conductance. Each specimen was then covered in vacuo with a film of gold about 20 nanometres in thickness. The specimens were examined in Jeol 35C++ Scanning Electron Microscope (Fig. 8) operated at an accelerating voltage of 10Kv.

* Scotch Pressure Sensitive Tape, 3M Australia Pty. Ltd.
Sydney, Australia.

+ Acheson A.N.Z. Pty. Ltd. 63 Dawes Road, Padstow, Sydney,
Australia.

++ JSM 35 Scanning Microscope, Jeol Ltd. Tokyo, Japan,
Serial No. EP 711009-53

Before each specimen was examined the magnification of the microscope was checked by using standard size plastic (0.48 micrometres) spheres to calculate the magnification of the photographic print (Fig 9).

Five separate photographs were taken at different areas within each specimen making a total of 300 photographs (6 groups x 10 specimens x 5 fields). The photographs were magnified exactly four times to facilitate the area measurement. Photographic prints were made with black borders to ensure that the whole negative was printed. Two separate methods were used to measure the cross sectional area of the dentinal tubule in each photograph. The first method (A) was used on the photographs from all the six groups. The second method (B) was used on Groups 2, 4 and 6.

5.1 Method A. Point counting. (Hilliard, 1961)

A regular point grid was constructed to estimate the area fraction (Saunders and Kahles, 1942) of the photograph occupied by dentinal tubules. A grid was constructed such that there were 100 regularly spaced points on a 13.5 cm x 25 cm rectangular area on a sheet of clear acetate film, each point being arranged in rows 1.5 cm apart. Along each row the points were 2.5 cm apart. Each second row was staggered 1.25 cm to the left of the first row. The grid was enclosed in a solid border (16.8 cm x 26.4 cm) which

corresponded to size of the photographs being examined (Fig. 10).

The clear acetate strip was overlayed onto each enlarged photograph so that the bottom solid border and the right hand solid border corresponded to the edges of the enlarged photograph. This ensured that all points were located on some part of the photograph.

An examination of the relationship between the points and the dentinal tubules was made, and the number of points which intersected major dentinal tubules were counted. Each photograph was measured twice and the mean of the two readings recorded.

The per cent of the area of the dentinal tubules was calculated by the following formula:

$$\begin{array}{lcl} \text{Percent} & \text{Number of Points of} & \\ \text{of the} & = \frac{\text{intersecting dentinal tubules}}{\text{Total No. of points}} & \times 100 \\ \text{area} & & \end{array}$$

Where a point crossed the edge of a tubule it was recorded as being within the tubule.

To calculate the number of dentinal tubules per square milli- meter all the tubules contained in one photograph were counted. Where tubules intersected the edges of the photograph, only those which intersected the top and right hand margins were included in the total.

The number of dentinal tubules per square millimetre was then calculated by the following formula:

$$\text{Number of tubules/mm}^2 = \frac{\text{Number of tubules per photograph} \times (\text{Magnification})^2}{\text{Area of photograph in square mm}}$$

To determine the final magnification of the photographic prints. The diameter of the standard sized plastic spheres were measured on the final photographic prints with a travelling microscope and these diameters were compared with the original diameter of the plastic spheres before magnification (0.48 micrometres).

The magnification was calculated by the following formula:

$$\text{Magnification} = \frac{\text{Diameter of enlarged spheres in mm} \times 1000}{\text{Diameter of original sphere in micrometers}}$$

i.e. Diameter of enlarged spheres in mm x 1000=Magnification

.00048

5.2 Method (B): Determination of area of tubules using a digitizer tablet.

An Apple IIe* computer and Hipad Digitizing table with magnifying Cursor (Fig. 11) was used to determine the cross sectional area of dentinal tubules using Bioquant II software programmes .

The total area of each of the 50 photographs in each group was first digitized by point mode and counted via the computer to square micros. This area was designated A0.

The sum of the areas of all the 50 photographs in each group was thus obtained.

The total area occupied by dentinal tubules in each photograph was measured and the sum of the areas for each group was obtained via the computer software. This area for each group was designated A1.

The number of dentinal tubules (i.e. the number of individual measurements) was recorded by the computer and the sum of the number of tubules contained in each group was obtained. This was designated N1.

* Apple Computer 20525 Mariani Avenue Cupertino,
California 95014

† Bioquant II software, R & M Biometrics Corporation
6301 Robertson Avenue Nashville. TN 37209.

The number of dentinal tubules per square millimeter for each was calculated by the following formula:

$$\text{Number of tubules per square millimeter} = \frac{N1 \times 106}{A0}$$

The actual percentage of area occupied by dentinal tubules in each group was calculated using the Bioquant statistical package and results tabulated.

Only photographs for group 2, 4 and 6 were measured by digitization.

RESULTS

CHAPTER 6

RESULTS

6.1 Magnification of prints.

Measurement of the diameter of plastic spheres revealed that there was very little difference between the magnification of prints at one session compared with another session. Mean magnified diameters of the spheres ranged from 3.18 to 3.39 millimetres the average being 3.25 millimetre. The original spheres had a diameter of 0.48 micrometer and therefore the final magnification of the enlarged prints was calculated at X6771.

The magnifications shown in captions to figures indicate the actual magnification of the photographic print.

6.2 Morphology of Dentine.

Typically, sections showed dentinal tubules, large amounts of intertubular dentine and variable amounts of peritubular dentine.

Both the diameter and the number of the dentinal tubules increased in size from the dentino-enamel junction to the pulp (Fig. 12-14) this appearance was reflected in longitudinal sections of the dentine (Fig. 15, 16).

While the diameter of individual tubules was similar within the one cross section, at times considerable variation was observed.

Cross sections of lateral branches were often encountered. At times it was difficult to distinguish between these lateral branches and small tubules (Fig. 17). Longitudinal sections of lateral branches (Fig. 18) were also seen. Interconnecting branches between one tubule and another were observed.

Odontoblast processes were observed in all sections of dentine from the pulp to the dentino-enamel junction. Structures which were interpreted as odontoblast processes were seen as close as 0.2 millimeter from the dentino-enamel junction (Fig. 19) and 0.7 millimeter from the dentino-enamel junction (Fig. 20).

The processes did not fill the whole lumen of the dentinal tubules. lateral branches of the processes were also observed, either as true branches (Fig. 21) or as projections on the surface of the process (Fig. 22). Cable like fibres (Fig. 23) and twisted membranous extensions from the dentinal tubules were often observed (Fig. 24). These processes were elongated and had a smaller diameter than the odontoblast processes. At times very long fibres (40 microns) could be seen passing from the dentinal tubule (Fig. 25, 26). These fibres were smooth and most appeared to lack the characteristic cross banding of collagen

although suggestions of cross banding were seen in some fibres. Occasionally a twisted cable like or fibrous structure could be detected (Fig. 27, 28).

6.3 Number of Dentinal tubules per square millimeter.

In general the number of dentinal tubules increased from the dentino-enamel junction towards the pulp, being more numerous towards the pulp. Mean number of tubules per square millimeter varied from 22,000 to 48,000 by Method A and 25,000 to 50,000 by Method B.

6.3.1 Estimation of number of tubules per square millimeter. Using Method A.

The number of tubules per enlarged 50 photographs of each group is illustrated on Table 2. These results converted to number of tubule per square millimeter of dentine are illustrated in Table 3. When statistical comparisons were made between the results from one group and another the exact figures for number of tubules per photograph were used rather than the number per square millimeter.

The number of tubules per square millimeter varied from 22,000 at the dentino-enamel junction to 49,000 2.0-2.4 millimeter from the pulp. Mid-way between the pulp wall and

dentino-enamel junction the mean number of dentinal tubules varied from 34,000 to 37,000 per square millimeter.

A comparison of the number of dentinal tubules per square millimeter for the six groups was made using the student's t-test. Values were calculated for $n_1 + n_2 - 2$ degrees of freedom (Table 4). No significant difference was established between groups 1 and 2 ($P 0.05$), groups 3 and 4 ($P 0.01$) and groups 5 and 6 ($P 0.05$). There was a statistical difference between all other pairs of groups.

6.3.2 Estimation of number of tubules per square millimeter using Method B.

The number of tubules per square millimeter obtained by Method B are illustrated in Table 5 together with the raw data used to obtain the results.

The number of tubules per square millimeter varied from 25,000 near the dentino-enamel junction to 50,000 near to the pulp.

6.3.3 Comparison of the estimation of the number of tubules using Method A and Method B.

A graphic illustration of the difference in number of tubules per 1,000 square millimeter is illustrated in Figure 30. The actual number of dentinal tubules per square

millimeter obtained for each of the group 2, 4 and 6 by Method A and Method B is illustrated in Table 6. In general the numbers obtained by Method B were slightly higher than those obtained in Method A. It was not possible to do a statistical analysis because it was not possible to get the mean of the number of dentinal tubules using the computer softwear available. However, it can be seen that there is very little difference between the two numbers.

6.4 Percentage cross sectional area of dentine occupied by dentinal tubules.

Both methods of investigation established that the percentage of the cross sections of dentine occupied by the dentinal tubules increased significantly from the dentino-enamel junction to the pulp.

6.4.1 Estimation of the cross sectional area of dentine occupied by dentinal tubules using Method A.

Values for the percentage area of dentine occupied by dentinal tubules increased progressively from the dentino-enamel junction to the pulp. Values ranged from 3.63 percent near the dentino-enamel junction to 10.2 percent near the pulp. the mean values obtained from Group 1-6

together with the standard deviation and range of values obtained are illustrated in Table 7.

A graphic representation of the percentage of area occupied by dentinal tubules in the different groups using Method A is illustrated in Figure 31. A comparison of the areas occupied by dentinal tubules for the six groups was made using the student's t-test. t value were calculated for $n_1 + n_2 - 2$ degrees of freedom. As can be seen in Table 4 there was no significant difference between Groups 1 and 2 ($P < 0.05$), Groups 3 and 4 ($P < 0.05$) or Groups 5 and 6 ($P < 0.01$). There were significant differences between all other pairs of groups.

6.4.2 Estimation of the percentage cross sectional area of dentine occupied by dentinal tubules using Method B.

The results obtained for Method B for Groups 2, 4 and 6 are illustrated in Table 8. Values ranged from 3.3 percent in Group 2 (0.5-0.9 millimeter from the dentino-enamel junction) to 7.8 percent close to the pulp. Corresponding values for Method B are illustrated for comparison.

6.4.3 Comparison of the results obtained by Method A and Method B for percentage of cross section area of dentine occupied by dentinal tubules.

The difference in values obtained for percentage cross sectional area occupied by dentinal tubules can be seen in Table 8 and in Figure 32.

In general figures obtained by Method B were lower than those obtained by Method A varying from 3.8 percent to 10.2 percent in Method A and 3.3 percent to 7.8 percent in Method B. However statistical analysis of the difference between the two methods of measurement showed the difference to be not significant ($P < 0.05$) (Table 9).

DISCUSSION

CHAPTER 7

DISCUSSION

7.1 Morphology of Dentine

The scanning electron microscopic appearance of fractured surface of dentine has been studied by a number of authors (Yasuzumi and Obata, 1955; Kettler, 1961; Johansen and Parks, 1962; Noble et al., 1962; Kaye and Herold, 1966; Frank, 1968; Lester and Boyde, 1968; Kubota et al., 1969; Isokawa et al., 1970a; Isokawa et al., 1970b; Kuwajima et al., 1970; Tronstad and Langeland, 1971a; Tronstad and Langeland, 1971b; Kuvam, 1972; Brannstrom and Garberglio, 1972; Isokawa et al., 1972; Tronstad, 1973a; Tronstad, 1973b; Weber, 1974; Garberglio and Brannstrom, 1976; Kubota et al., 1978; Mendis and Darling, 1979; Thomas, 1979; Tidmarsh, 1981; Thomas and Carella, 1983; Vasiliadis et al., 1983; Grossman and Austin, 1983; Maniatopoulos and Smith, 1983; Weber, 1983; Carrigan et al., 1984; Fox et al., 1984; Szabo et al., 1984). Also Forssell-Alberg et al., 1975 have studied the dentine in rat, cat, dog and monkey. Kelly et al. (1981) studied the dentinal tubules in monkeys.

The morphological feature of the cross sections of dentine were very similar to those observed in the literature.

The observation of odontoblast processes with the dentinal tubules near the dentino-enamel junctions confirmed the previous finding of Langeland (1976), Boyde (1980), Tidmarsh (1981), Hawkinson and Eisenmann (1983), Maniatopoulos and Smith (1983). Grossman and Austin (1983) and Szabo et al. (1984), Fox et al. (1984) who reported that odontoblast processes extended to the dentino-cemental junction at all levels of the developing roots. Also, Kelly et al. (1981) identified odontoblast processes near the dentino-enamel junction of demineralized macaque monkey dentine.

On the other hand Brannstrom and Garberoglio (1972), Frank (1972), Garant (1972), Tronstad (1973a), Holland (1975, 1976), Thomas (1979), and Thomas and Carella (1983) all claimed that the odontoblast processes are limited to the inner pulpal third of dentine. In addition to the above, Tsatsas and Frank (1972) in a transmission electron microscopic study found no structure which resembled odontoblast processes within dentinal tubules near the dentino-enamel junction.

Maniatopoulos and Smith (1983) raised the question as to whether the structures identified here as odontoblast processes are true processes or not. They also stated that this could be resolved by examination of these structures with transmission electron microscopy but there were difficulties to be overcome and additional work was

required. Maniatopoulos and Smith stated there is good reason for believing that the structures are odontoblast processes by virtue of their observed characteristics in the scanning electron microscope. They extend the whole length of the dentinal tubules: Although they present variations in diameter due to shrinkage, many fill most of the lumen of the tubules: They have considerable tensile strength - in some cases being twisted around their long axis without fracturing: They have exactly the same appearance as the processes in the predentine and inner portion of dentine and were observed at various distances from the pulp in the outer, middle and inner dentine as well as in the predentine, where they appeared as a clear extension of the odontoblast cell body. The processes gave rise to lateral branches in the outer portion of dentine either as true branches or as projections on the surface of the processes in close relation with holes on the surface of the dentinal tubules (openings of lateral canals): In the outer dentine, the processes have a tubular appearance similar to that found in the inner portion of dentine by Garberoglio and Brannstrom (1972).

Maniatopoulos and Smith suggested that the failure of previous investigators to observe the process at distances greater than one- third of the dentine thickness may have been due to inadequacies in the fixation or preparation techniques. They reported that in their study variations in

preparation techniques varied the length of the extension of the odontoblast process in the tubule.

Interestingly, Kelly et al. (1981) identified odontoblast processes in the inner and outer third of dentine but not in the middle third as the structure found near the dentino-enamel junction resembled odontoblast processes closer to the pulp in every manner except for their size, as they followed the branching course of the dentinal tubule, and as their smooth surface and tubular nature were identical to that of the odontoblast processes seen in dentine close to the pulp, Kelly et al. concluded that the structures observed were extensions of the odontoblast process. Kelly et al. also revealed that not every tubule beyond the inner pulpal third of dentine contained an odontoblast process. They speculated that this lack of processes in the middle third could be artifact of mechanical preparation. They state that it is not unreasonable to assume that the process is anchored at both ends and that shrinkage during processing, and the process of sectioning itself may result in loss of the middle section. They drew the readers attention to the fact that 26 percent linear shrinkage occurs using the critical point drying method (Boyd, 1978) of specimen preparation. Further substantiation of the feasibility of a mechanical artifact can be found in the fact that they did not find any odontoblast processes in sample prepared by freeze-drying.

A process in which a large amount of soft tissue shrinkage can occur.

Not all the extensions from dentinal tubules can be regarded as odontoblast processes. Many investigators have described a hypomineralized structure lining the peritubular dentine (Isokawa et al., 1972; Johansen and Parks, 1962; Takuma, 1967). Thomas and Carella (1983) described the appearance of the lining which they termed the "lamina limitans" in decalcified and undecalcified sections of dentine. Initial decalcification selectively removed the peritubular dentine and revealed a sheet like structure which was separated from the walls of the tubules by a space corresponding to the peritubular matrix. As the lining material resisted demineralization it was presumed to be organic in nature. Thomas and Carella (1983) have suggested that the "odontoblast processes" described by Kelly et al. (1981) in outer dentine could have been the portions of the lamina limitans. Part of the controversy surrounding the presence or absence of odontoblast processes may be related to confusion surrounding the identity of the hypomerized layer surrounding the process. The membranes structures seen in Figures 23 and 24 could represent the "lining membrane" of the dentinal tubule.

Fox et al. (1984) in an interesting study demineralized fractured sections of dentine and then digested them in collagenase to remove the collagen. In developing teeth,

collagenase to remove the collagen. In developing teeth, odontoblast processes were observed passing all the way from the pulp to the dentino-enamel junction. while Fox et al. suggested that the processes were at times coated with a layer which could be the lamina limitans. In many places these smooth-walled tubular structures were observed travelling all the way to the dentino-cemental junction. They interpreted these structures as odontoblast processes. Fox et al. examined developing roots when the thickness of dentine is narrow and dentinogenesis still active. A further study using their techniques on mature coronal dentine is indicated.

Long single fibres extending from or contained within the dentinal tubules similar to those illustrated in Figure 25 and 26 have been reported by Tronstad (1973a) and Tidmarsh (1983) reported that the great majority of these fibres observed in his study had a smooth patternless surface although a periodic cross banding could occasionally be seen.

Lateral branches similar to those seen in this study (Fig. 18) have been reported by many authors. Kaye and Herold (1966) have demonstrated the presence and extent of these branches by phase contrast microscopy. Weber (1983) described the morphology of the labial branches using resin casts of dentinal tubules. They were also well illustrated

by Fox et al. (1984) in their studies of demineralized, collagenase treated dentine.

Tronstad stated that the occasional observation of periodic cross banding at the surface of the fibres as well as the demonstration of the presence of fibres with the same characteristics and dimensions intra tubularly in root dentine of teeth in some of his unpublished work imply that these long fibres are not odontoblast processes but rather collagenous in nature. Collagen fibres have been demonstrated by transmission electron microscopy within dentinal tubules by Frank (1966, 1968), Johansen (1967), Symon (1970) and Tronstad and Langeland (1971b). Tronstad (1973a) suggests that the smooth appearance of the fibres in the Scanning electron microscope may be attributed to masking of the surface structural details by ground substance (Nyten and Scott, 1958; Frank and Nalbandian, 1963). Tidmarsh (1983) also suggests that the fibres are collagenous although he states that this point still requires clarification as the evidence afforded by transmission electron microscopy has not been clearly correlated with the appearance and distribution of intra tubular fibres seen in the scanning electron microscope. Grossman and Austin (1983) illustrated fibres similar to these long fibres but identified them as odontoblast process.

Szabo et al. (1984) describe the presence of a thin fibre beside the odontoblast process and suggest that it is

possibly a nerve fibre or that it was a longitudinal branch of the same tubule nearer the pulp or alternatively a branch that had entered from a neighbouring process.

7.2 A number of dentinal tubules per square millimetre.

A progressive increase in the number of dentinal tubules from dentino-enamel junction towards the pulp has been reported previously by Kuttler (1959), Ketterl (1961), Tronstad (1973a) and Garberglio and Brannstrom (1976) of the above only those studies by Ketterl (1961) and Garberglio and Brannstrom (1976) have examined the subject in any detail.

Ketterl (1961) studied histological sections of dentine using a Leitz measuring eyepiece which was calibrated so that the area under investigation was known. Garberglio and Brannstrom used the Scanning electron micrograph in a similar manner used in this study.

Ketterl studied the number of tubules per square millimeters in three age groups (15 to 30 years, 30 to 50 years and 50 to 75 years) at three different levels in the tooth. He established that there was an average of 16,000 tubules per square millimeter close to the dentino-enamel junction, 36,000 per square millimeter in intermediate dentine and 64,000 close to the pulp. As would be expected there was very little difference in the numbers of tubules

found for each age group although the numbers for the 15-30 year age groups (which corresponded in age to the teeth used in this study) were slightly lower than for the other two groups.

Ketterl's numbers his intermediate group were very similar to those found in the intermediate groups in this study. However, he found fewer tubules near the dentino-enamel junction and more tubules closer to the pulp than were found in the current study.

The results obtained by Garberglio and Brannstrom (1976) were very similar although slightly lower than those found in this study. Many reflect slight differences in individual teeth, (Garberglio and Brannstrom used 24 premolar, 5 molars and 1 incisor in their study while only third molar teeth were used in this study), to different creases within the tooth being studied (Garberglio and Brannstrom measured diameters under the cusp whereas in this study measurements were made accross the occlusal surface of the tooth) or to differences in processing techniques.

Also a direct comparison of the results is not really possible as Garberglio and Brannstrom made measurements from the pulp outwards whereas in this study measurements were made from the dentinoenamel junction downwards. It could be argued that the measurements used in this study were more accurate as measurements from the pulp may be inaccurate due to the complexity of the pulpal anatomy particularly in the

pulp horn region which would often result in measurements being taken from a sloping or undermined surface.

In a recent study Carrigan et al. (1984) reported a decrease in the number of tubules with increasing age. However their specimen preoperative is suspect and the results poorly displayed. No attempt was made to check the magnification of the microscope or to use duplicate areas in the same tooth for examination. Slightly higher numbers found by computer estimation (Method B) compared with individual counting (Method A) was expected as the computer software could not differentiate between whole tubules and tubules intersecting the four edges of the photograph. In the direct counting method only those tubules intersecting the top and right hand borders of the photograph were counted to avoid double counting. As there was a progressive change in both numbers of dentinal tubules and their cross sectional area from the dentino-enamel junction it is not surprising that there was no significant difference statistically between one group and its immediately adjacent group. It was of interest however that there was a significant difference between Group 2 and Group 3, and between Group 4 and Group 5. It is possible that at these particular points in the formation of the dentine a more rapid change occurred in the tubular structure of the dentine.

7.3 Percentage cross sectional area occupied by dentinal tubules.

Three studies have attempted to measure the percentage cross sectional area occupied by dentinal tubules. Ketterl (1961) estimated that dentinal tubules accounted for 4 percent of the dentine at the dentino-enamel junction for all age groups studied (15 to 30, 30 to 50, 50 to 75), but that towards the pulp (Table 1) values ranged from 79 percent in the younger age groups to 42 percent in the older groups. Hoppe and Stuben (1965) reported much higher values close to the dentino- enamel junction 19.1 percent. However they used decalcified material. As decalcification removes peritubular dentine, it would be expected that their values would be high. Garberoglio and Brannstrom reported the percentage at the dentino-enamel junction was 4 percent. Similarly this study showed that the percentage at the dentino-enamel junction was 3.63 percent.

The result 10.2 percent close to the pulp found in this study is lower than 27.7 percent reported by Hoppe and Studam (1965) and 28 percent by Garberoglio and Brannstrom (1976).

Garberoglio and Brannstrom (1976) reported a slight increase in the number of dentinal tubules very close to the dentino-enamel junction and suggested that this may be due to branching of processes in the region. It had been

expected that an increase in the cross sectional area of tubules would be expected at the dentino-enamel junction . In this study, however this was not apparent in the sections examined. While the differences were not statistically significant it is difficult to explain why the results obtained by use of Method B were numerically lower than those obtained by Method A. Possible paralax errors in the use of the cursor on the digitizing table may explain some of the differences.

7.4 Diameter of dentinal tubules.

A number of studies have measured the diameter of dentinal tubules at different levels in the tooth. Ketterl (1961) reported the diameter close to the periphery as being 1.73 micrometres in the peripheral dentine. Similar results were found by Fromme and Riedel (1970) and Maniatopoulos and Smith (1983) for peripheral dentine. However Tronstad (1973) and Garberglio and Brannstrom (1976) report much lower values for peripheral dentine 0.5 and 0.8 micrometres respectively.

All studies have shown that the diameter of the tubules are greater towards the pulp. Ketterl (1961) reported that the diameter ranged from 3.98 micrometres in lower age group to 2.91 micrometres in the older age groups. Other research work show lower values (Table 1). While the diameter of the

dentinal tubules were not measured directly in this study some comparisons can be made.

Assuming the tubule is circular in cross ssection, then the following calculations can be made:

- 1) Area occupied by dentinal tubules in one square millimeter of dentine = 1 X percentage of area occupied by dentinal tubules.
- 2) Area of one dentinal tubule in square millimeters = $\frac{\text{Area occupied by dentinal tubules in one square mm.}}{\text{Number of Dentinal tubules per square millimetres}}$
- 3) Diameter of the dentine tubule in square micrometers = $10^6 \times \sqrt{\frac{\text{Area of one Dentinal Tubule in square millimeters}}{2}}$

If the above calculations are used to estimate diameter of dentinal tubule in this study the values obtained range from 1.4 micrometers to 1.6 micrometers (Table 10) whcih are of the same order as most of the published studies (Table 1) although lower than those studies which used decalcified material (Kettrel, 1961).

If the above calculations are applied to the work of Garberglio and Brannstrom (1976) then some discrepancies appear. The above authors reported that the percentage at the pulpal wall is 28 percent which is somewhat higher than their calculated 22.1 percent.

They report a value of 4 percent at a level of 3.1 to 3.5 millimeter from the pulp. Recalculation shows that, for a diameter of 0.8 micrometers and a tubule concentration of 19,000 tubules per square millimeters, then value is four times the theoretical value of 0.95 percent. It is possible that Garberglio and Brannstrom's values for diameters may be incorrect. Their method of calculation of tubule diameter is not clear. It would appear that diameter measurement may have been recorded directly from longitudinal sections in the Scanning electron microscope.

The value of percentage area established in the present study for level 2.5-3.0 millimeter from the dentino-eneamel junction (10.2%) are almost equivalent to the recalculated percentage from the work of Garberglio and Brannstrom (1976) (Table 11). 2.5-3.0 millimeter from the dentino-eneamel junction in the present study is roughly equivalent to about 0.5 millimeter from the pulp (average width of dentine in this study from dentino-eneamel junction to pulp 3.4 millimeter) i.e. an average between Group 2 and Group 3 (9.9%) in Garberglio and Brannstrom's study.

7.5 Comparison of Method A and Method B.

The results of this study did not show any significant differences between the two methods of measurement although the values for Method B gave slightly higher values for number of dentinal tubules (which could be explained by the incorporation of all half tubules in the total number) and slightly lower values for percentage area (which could possibly be explained by parallax error during measurement). From these results it can be recommended that Method A for any future investigation be used because it is easier and faster than Method B. However there are two disadvantages of Method A:

- (1) It is difficult to get the total area if you wanted only the percentages available.
- (2) More time is needed to calculate the statistics.

7.6 Range of Results.

Attention should be drawn to the wide range of results obtained for both the number of tubules per square millimeter and for the cross sectional area. A similar range of results were observed by Garberglio and Brannstrom (1976). Some of the variation in figures obtained in the literature could be related to the small sample sizes used by some authors. In studies describing the morphological character-

istics of dentine should include examination of large numbers of specimens and a large number of fields within each specimen.

7.7 Implications for the hydrodynamic theory of pain transmission.

It would appear from the results of this study, and from the theoretical recalculation of the results of Garberglio and Brannstrom (1976) that dentinal tubules are larger in diameter at the dentino-enamel junction than claimed by the above authors. Berggren and Brannstrom (1965) requires calculated theoretical values for the speed of movement of fluid through dentinal tubules based on his smaller diameters. If the tubules are in fact larger at the dentino-enamel junction these theoretical calculations need to be revised. In his theoretical calculations Brannstrom did not take into consideration the large number of lateral branches between tubules or the progressive change in the diameter of the tubules from the dentino-enamel junction to the pulp.

7.8 Summary of Results and Discussion.

- 1) The morphological appearance of the fractured surface of dentine were similar to those described in the literature.
- 2) Studies resembling odontoblast processes were observed close to the dentino-enamel junction. Identification of these processes was difficult. It is possible that these "processes" may represent the "lamina limitans" of the dentinal tubule.
- 3) Long single fibres were observed extending from some dentinal tubules. It is possible that these may be collagenous. Further studies are indicated to establish the identity and function of these fibres.
- 4) Values for the number of dentinal tubules per square millimeter varied from 22,000 near to the dentino-enamel junction to 48,000 near to the pulp by Method A and from 25,000 near to the dentino-enamel junction to 50,000 near to the pulp by Method B. These figures were similar to those established by Garberglio and Brannstrom (1976). The number of dentinal tubules increased progressively from the dentino-enamel junction to the pulp. The higher values in Method B could be related to the incorporation of all half tubules into the total number.

- 5) The size of the dentinal tubule increased progressively from the dentino-enamel junction to the pulp.
- 6) The percentage cross sectional area of dentine occupied by the dentinal tubules increased progressively from dentino-enamel junction to the pulp. The values obtained varied from 3.63 percent to the dentino-enamel junction to 10.2 percent near to the pulp in Method A, and from 3.3 percent near to the dentino- enamel junction to 7.8 percent near to the pulp in Method B. The differences were not statis- tically significant, however values for Method B could be related to paralax errors during measuring.
- 7) Theoretical calculation of the diameter of dentinal tubules show the diameter of tubules to range from 1.4 micrometers to 1.6 micrometers which is similiar to most published work. Recalculation of the results of Garberglio and Brannstrom revealed anomolies with respect to tubule size and percentage area occupied by dentine.
- 8) It is recommended that in future calculations Method A be used as it is quicker and easier than Method B although it has some disadvantages.
- 9) Further work is indicated to establish the identity of the so called odontoblast processes at the dentino-enamel junction, to establish the identity of the long single fibres extending from tubules near the dentino-

enamel junction. To investigate the extent of the odontoblast process into dentine, to investigate the distribution of lateral branches at various levels of the tooth.

Table 1. Literature review of the number of dentinal tubules per square millimeter, percentage of area occupied by dentinal tubules, and the diameter of dentinal tubule at various distances from the dentino-enamel junction in human teeth.

Author's name	Year	Age of Sub-ject	Number of Dentinal tubules per square millimeter			Diameter of dentinal tubules in microns			Percentage of dentine occupied by tubules	
			Periphery	Middle	Pulpal	Periphery	Middle	Pulpal	Near DEJ	Near Pulpal wall
Kuttler	1959	-	15000	-	70000	-	-	-		
Ketterl	1961	16-30	13000	34000	61000	1.73um	3.09	3.98	4%	79%
"	1961	30-50	16000	40000	68000	1.71	2.56	3.12	4%	49%
"	1961	50-75	18000	35000	64000	1.75	2.36	2.91	4%	42%
Hoppe and Studam	1965	Young	-	-	-	-	-	-	19.1%	27.7%
Fromme & Riedel	1970	Young old	-	-	-	1.77 1.21		1.8 1.54	-	
Brannstrom	1972		-	-	-	1	1.8-2.0	2.7		
Tronstad	1973		7000	-	60000	0.5	-	2-3		
Garbeoglio & Brannstrom	1976		20000	29000	45000	0.8	1.2	2.5	4%	28%
Maniatopoulos & Smith	1983		-	-	-	1	1.2-1.5	2.6-4.0		

Table 2. Mean number of dentinal tubules occupied by tubules per photograph, obtained for each of the six groups using Method A.

Group	Distance from the D.E.J. towards the pulp in mm.	Number of Dentinal Tubules per 50 photographs		
		Means	S-D	Range
1	0.2 -- 0.4	21.94	8.1	10-39
2	0.5 -- 0.9	23.42	10.7	9-49
3	1.0 -- 1.4	33.08	8.1	21.50
4	1.5 -- 1.9	36.74	8.8	23.59
5	2.0 -- 2.4	47.66	11.1	29.69
6.	2.5 -- 3.0	47.00	9.6	28.68

Table 3. Mean number of dentinal tubules per square millimeter occupied by tubules standard deviation and range for various distances from the dentino-enamel junction.

Group	Distance from the D.E.J. in mm.	Number of Dentinal Tubules 1000 per square millimeters		
		Means	S-D	Range
1	0.2 -- 0.4	22	8.2	10.2 - 39.7
2	0.5 -- 0.9	24	10.9	9.2 - 49.9
3	1.0 -- 1.4	34	8.2	21.4 - 50.9
4	1.5 -- 1.9	37	9.0	23.4 - 60.1
5	2.0 -- 2.4	49	11.3	29.5 - 70.2
6.	2.5 -- 3.0	48	9.8	28.5 - 69.2

Table 4 Incidence of significant differences ($P < 0.05$) and ($P < 0.01$) between various distances from dentino-enamel junction.

GROUPS	Student t-test value	
	Number of Dentinal tubules	Area %
1 and 2	0.8 N.S.	0.25 N.S.
1 and 3	6.8	5.00
1 and 4	8.7	4.96
1 and 5	13.1	8.17
1 and 6	14.0	9.59
2 and 3	5.1	4.22
2 and 4	6.7	4.29
2 and 5	11.0	7.43
2 and 6	11.5	8.98
3 and 4	2.1 N.S.	0.8 N.S.
3 and 5	7.4	4.31
3 and 6	7.8	6.4
4 and 5	5.4	3.12
4 and 6	5.5	5.26
5 and 6	0.3 N.S.	2.49 N.S.

N.S. No Significant difference between groups.

Table 5. Number of tubules 1000 per square millimeter together with raw data used to obtain the results for Group 2, 4 and 6 using Method B.

Group No.	Total No. of tubules in 50 fields	Total area of 50 fields in square micrometers	Number of tubules 1000 per square millimeters
2	1255	49358	25
4	1924	49300	39
6	2483	49211	50

Table 6. Estimation of the number of dentinal tubules 1000 per square millimeter obtained by using Method A and Method B.

GROUP NUMBER	Number of dentinal tubules 1000 per square millimeter	
	METHOD (A)	METHOD (B)
2	24	25
4	37	39
6	48	50

Table 7. Percentage cross sectional area of dentine occupied by dentinal tubules obtained for each group using Method A.

Group	Distance from the D.E.J. towards the pulp in mm.	Percentages of area occupied by Dentinal Tubules		
		Means	S-D	Range
1	0.2 -- 0.4	3.63	2.08	0-10.5
2	0.5 -- 0.9	3.75	2.57	0-10
3	1.0 -- 1.4	5.78	2.17	1.5-10.5
4	1.5 -- 1.9	6.21	3.08	1-14
5	2.0 -- 2.4	8.25	3.37	2-16.5
6.	2.5 -- 3.0	10.2	4.32	3-26.5

Table 8. Percentage cross sectional area of dentine occupied by dentinal tubules obtained for Groups 2, 4 and 6 using Method A and Method B.

GROUP	Distance from DEJ towards the pulp in millimeter	Area occupied by Dentinal Tubules	
		METHOD A	METHOD B
2	0.5 -- 0.9	3.8%	3.3%
4	1.5 -- 1.9	6.2%	5.2%
6	2.4 -- 3.0	10.2%	7.8%

Table 9. Incidence of significant differences ($P < 0.05$) between Method A and Method B for measurement the percentage area occupied by dentinal tubules.

GROUPS	Student's t-test value
2 and 2	0.42
4 and 4	0.32
6 and 6	0.55

Table 10. Calculation of diameters of dentinal tubules according to the number of tubules per square millimeter and percentage area occupied by dentinal tubules.

	Distance from DEJ	Number/mm ²	Percentage of area occupied by dentinal tubules	Diameter in microns
1	0.2 - 0.4	22000	3.6%	1.4
2	0.5 - 0.9	24000	3.8%	1.4
3	1.0 - 1.4	34000	5.8%	1.5
4	1.5 - 1.9	37000	6.2%	1.5
5	2.0 - 2.4	49000	8.3%	1.5
6	2.5 - 3.0	48000	10.2%	1.6

Table 11. Recalculation of the percentage area occupied by dentinal tubules according to the diameter and number of dentinal tubules/mm² of Garberoglio and Brannstrom (1976).

Groups	Distance from the Pulp	Number/mm ²	Diameter in um	Percentage Reported	Recalculated Percentage
1	Pulpal Wall (0)	45000	2.5	28%	22.1%
2	0.1 - 0.5	43000	1.9	-	12.2%
3	0.6 - 1.0	38000	1.6	-	7.6%
4	1.1 - 1.5	35000	1.2	-	4%
5	1.6 - 2.0	30000	1.1	-	2.9%
6	2.1 - 2.5	23000	0.9	-	1.5%
7	2.6 - 3.0	20000	0.8	-	1%
8	3.1 - 3.5	19000	0.8	4%	0.95%

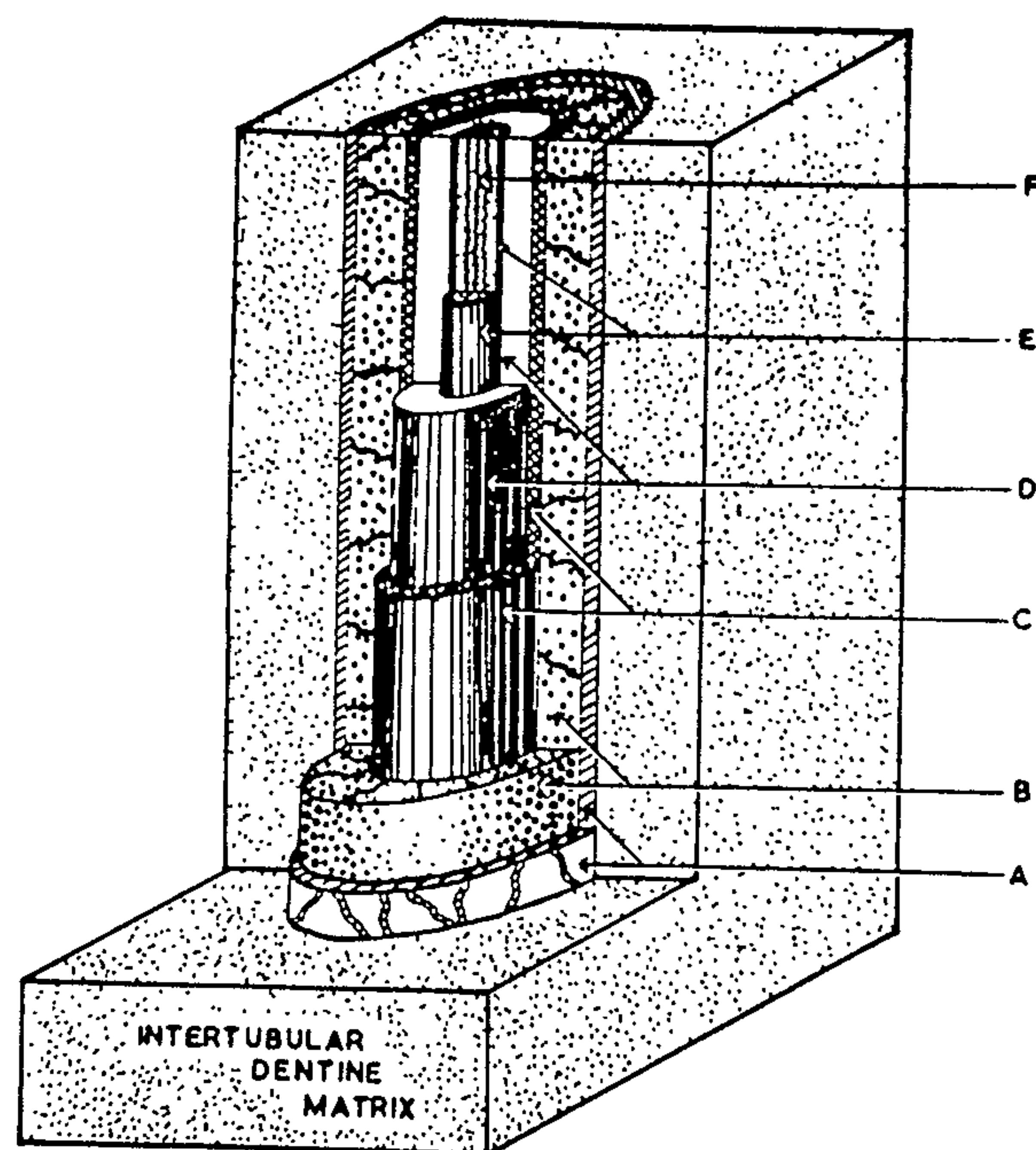


Figure 1. Diagrammatic representation of the structure of the dentinal tubule and odontoblast process. (From Shroff et al. 1959).

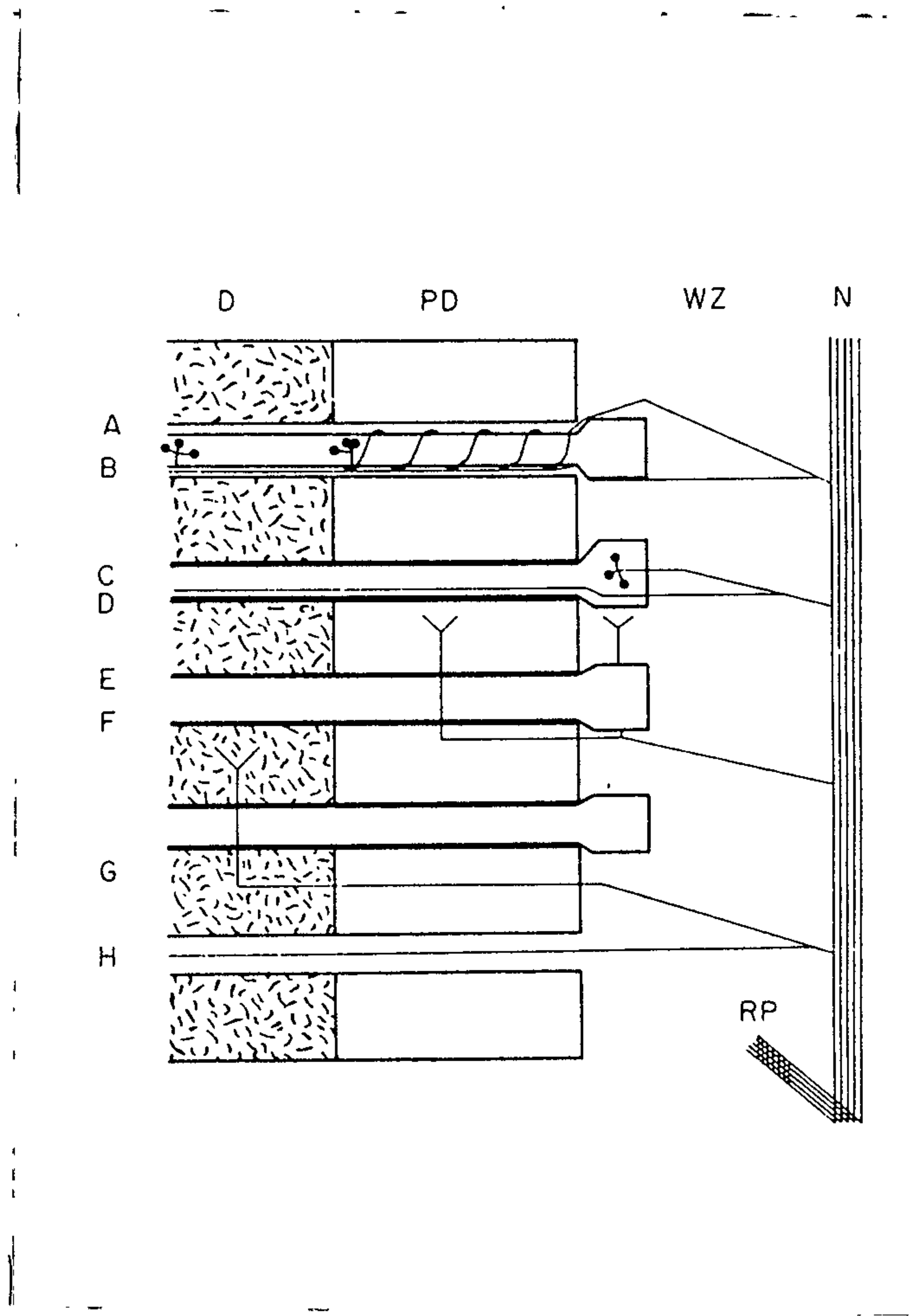


Figure 2. Schematic drawing of the different views on the innervation of the teeth.

- A. The nerve fibrils follow the odontoblastic process in a spiral course with nerve endings on the surface of the process.
- B. The nerve fibrils follow the odontoblastic process in a straight course in the space between the process and the tubular wall with nerve endings on the surface of the process.
- C. The nerve fibrils terminate with nerve endings on the surface or within the body of the odontoblast.
- D. The nerve fibrils run within the odontoblastic process.
- E. The nerve fibrils end in the odontoblastic zone with free nerve endings not in direct contact with the odontoblasts.
- F. The nerve fibrils run in the predentine ground substance crossing the tubules.
- G. The nerve fibrils run in the ground substance of the mineralized dentine crossing the tubules.
- H. The nerve fibrils run in separate canals.

D = dentine; PD = predentine; WZ = Weil's zone; N = nerve trunk; RP = Raschkow's plexus. From Arwill, 1963.

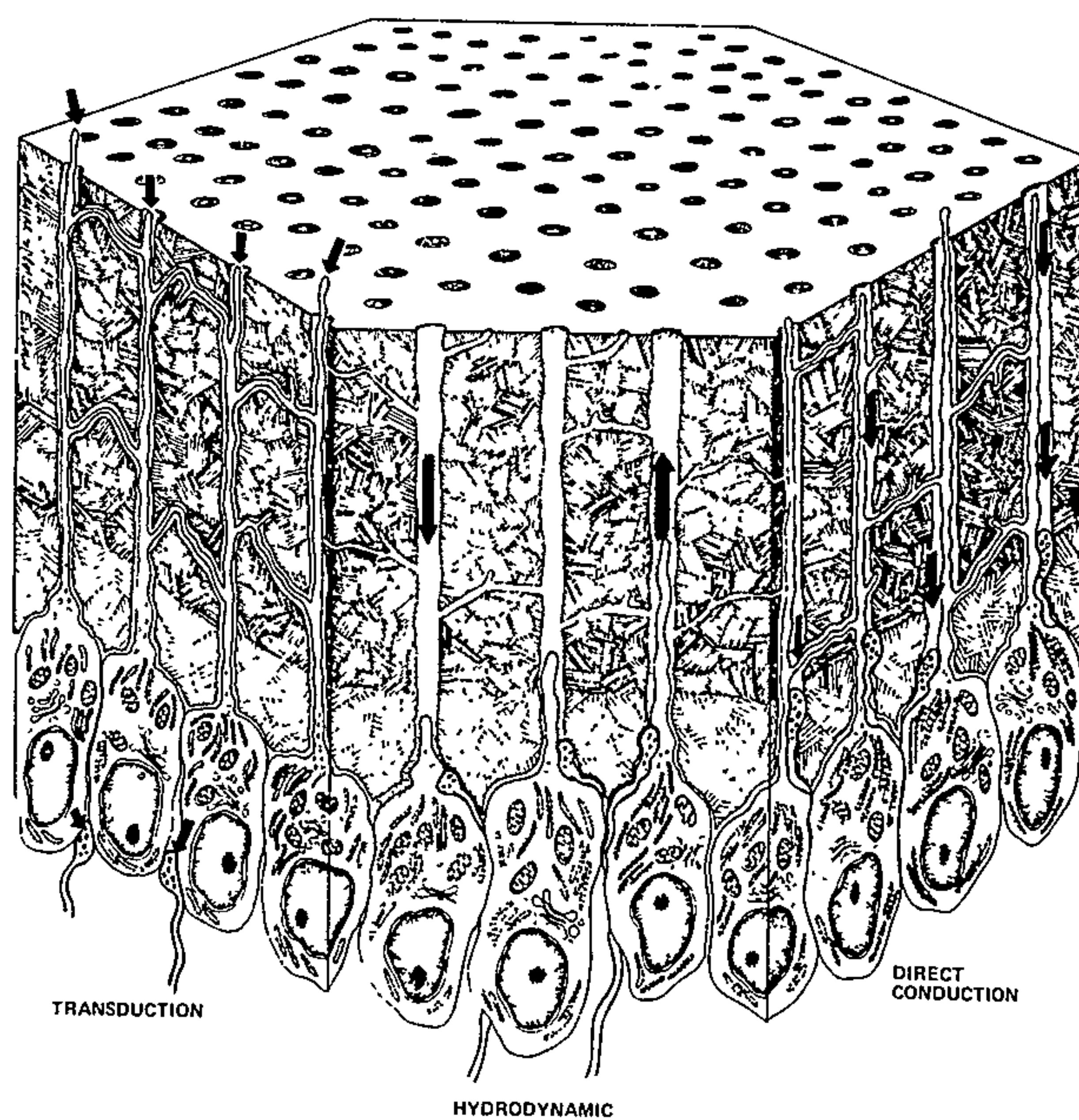


Figure 3. Diagram of the three main explanations of pain perception through dentine. (From Linde, 1984).

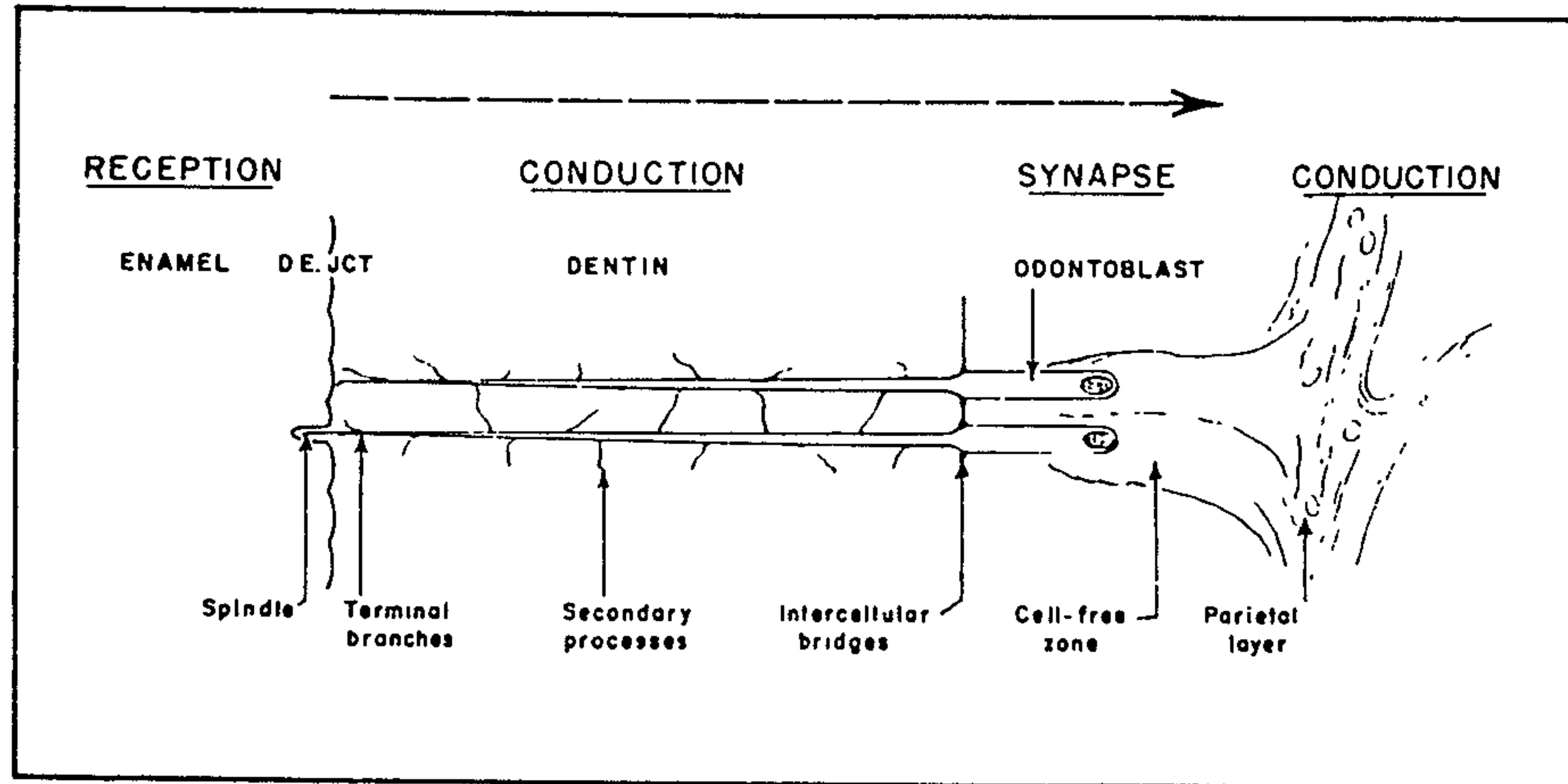


Figure 4. Diagram representing possible pathway of, and mechanism of, pain transmission from the dentino-enamel junction by means of the odontoblast process and a synapse between this cell and adjacent neural fibres in the pulp. (From Avery and Rapp, 1959).

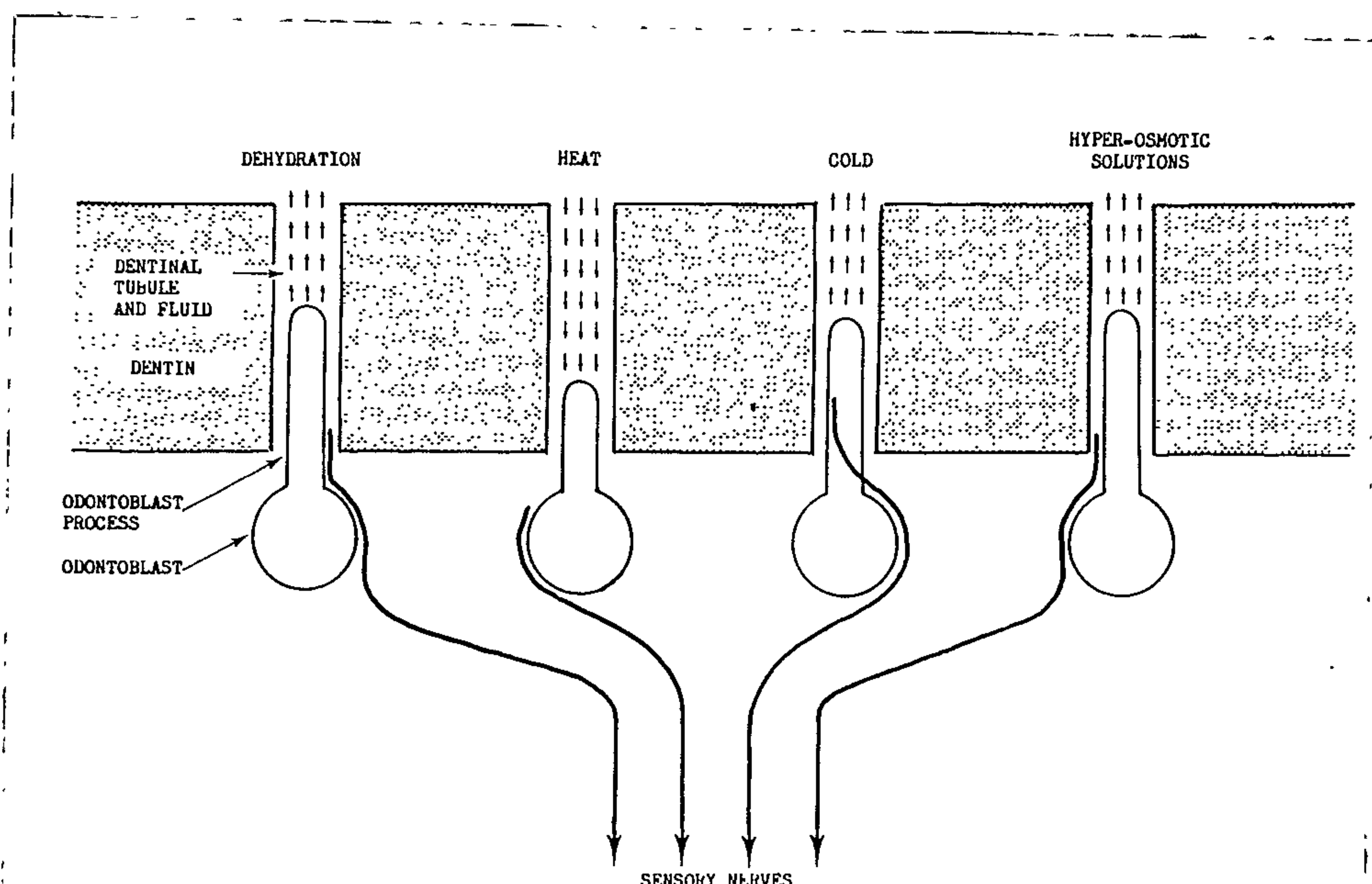


Figure 5. Diagrammatic representation of dentinal components. (From Berman, 1984).

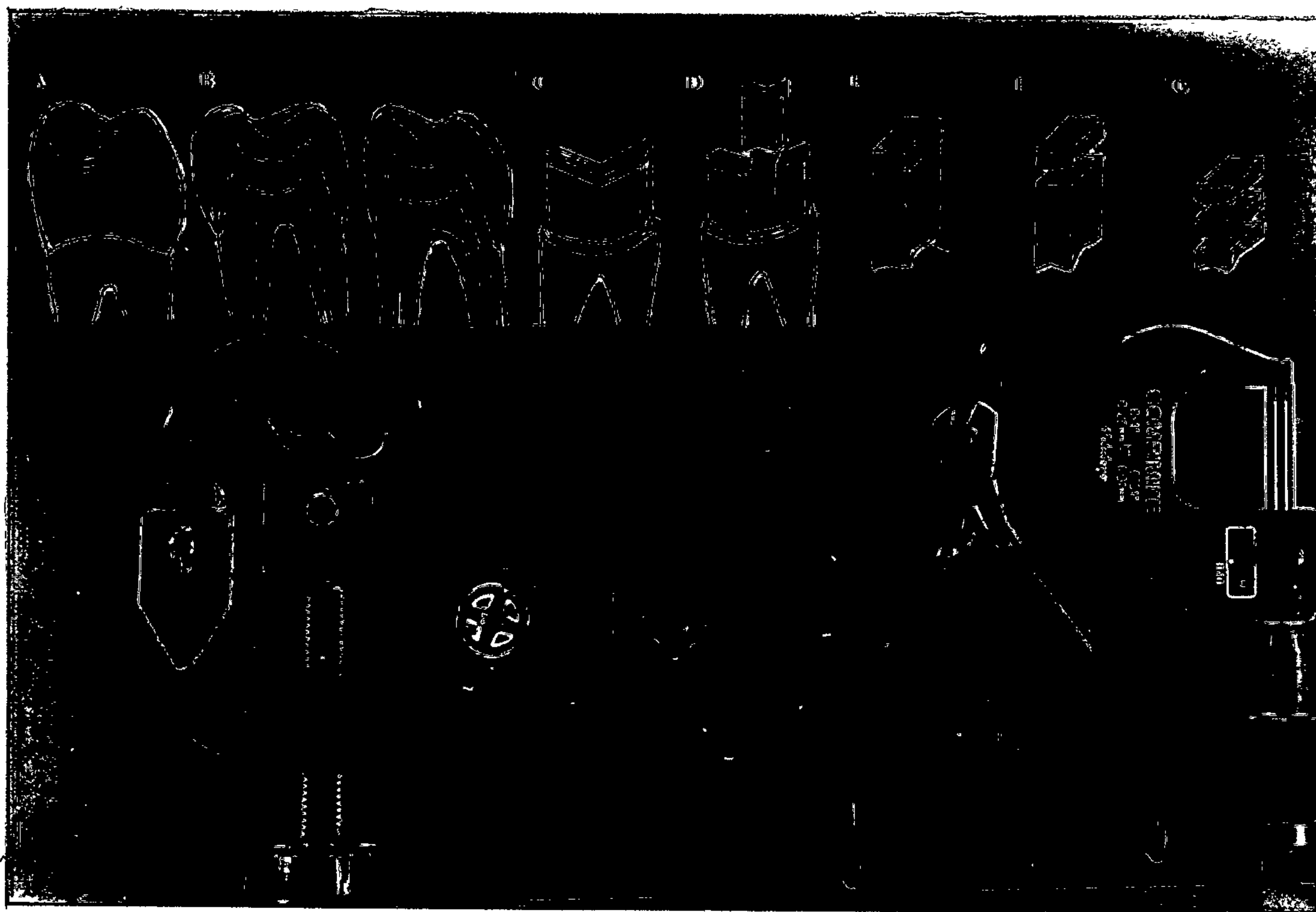


Figure 6. Diagrammatic representation of the method of obtaining sections of the tooth, together with photographs of equipment used to obtain the sections.

- A. Intact tooth.
- B. Tooth split into two halves occluso-cervically in a mesiodistal direction using a tube cutter.
- C. Reduction of the tooth half to 2mm using a 0.5mm diamond disc cutting from the buccal or lingual (outer) enamel surface.
- D. Tooth cross section sectioned bucco-lingually in an occluso-pulpal direction using a 0.3mm diamond disc.
- E. Tooth section with enamel intact.
- F. Tooth section with enamel removed at dentino-enamel junction using wire cutters.
- G. Specimens for examination and combike micrometer used for measurements.

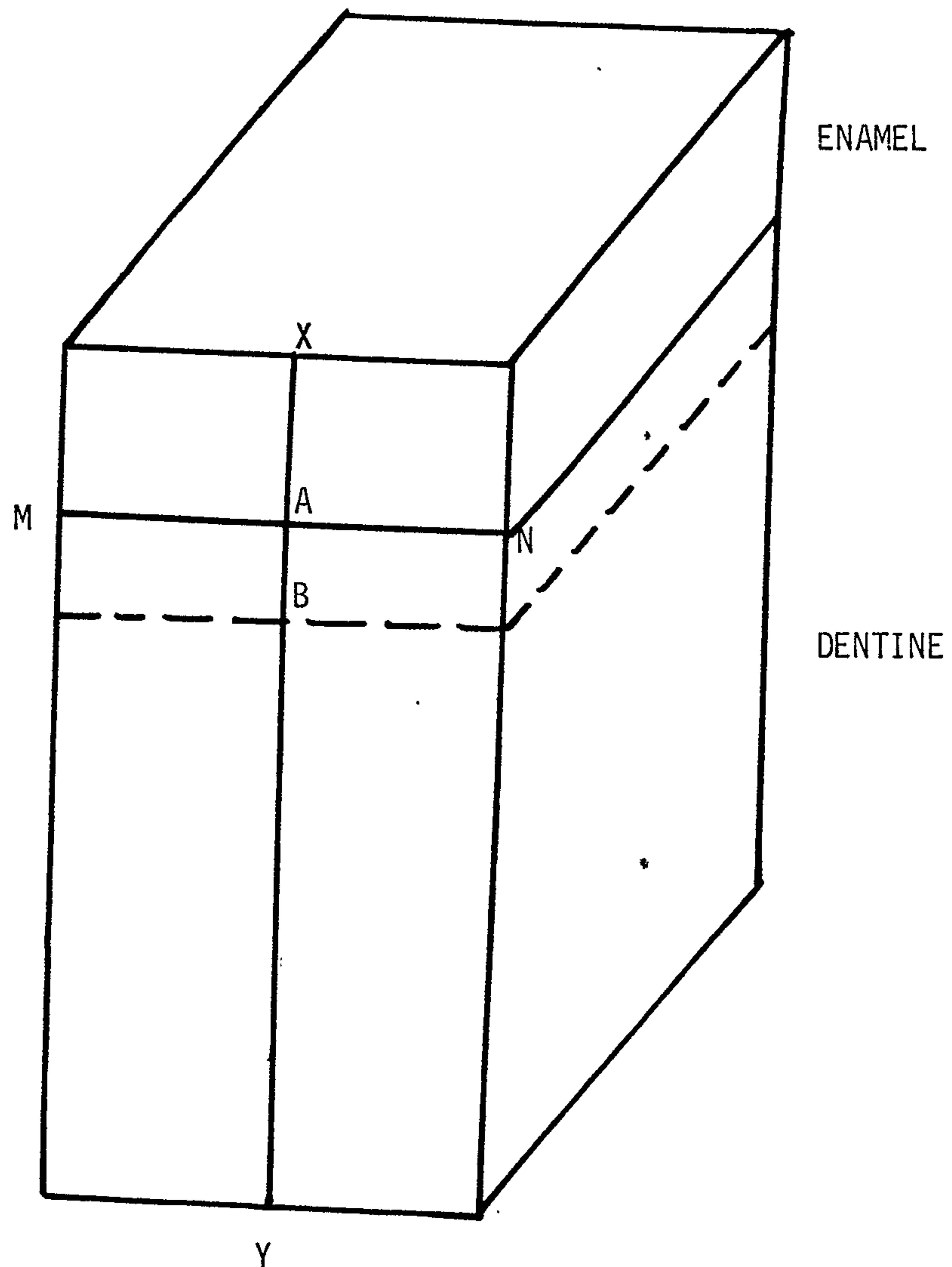


Figure 7. Diagrammatic representation of the sectioning and measuring technique to obtain the sections at various levels in the tooth.

- MN - Line drawn along dentino-enamel junction.
- XY - Line drawn from the pulp at right angles to MN intersecting it at point A on the DEJ.
- A - Point of intersection of two lines at DEJ.
- B - Plane of section.
- AB - Distance from dentino-enamel junction.

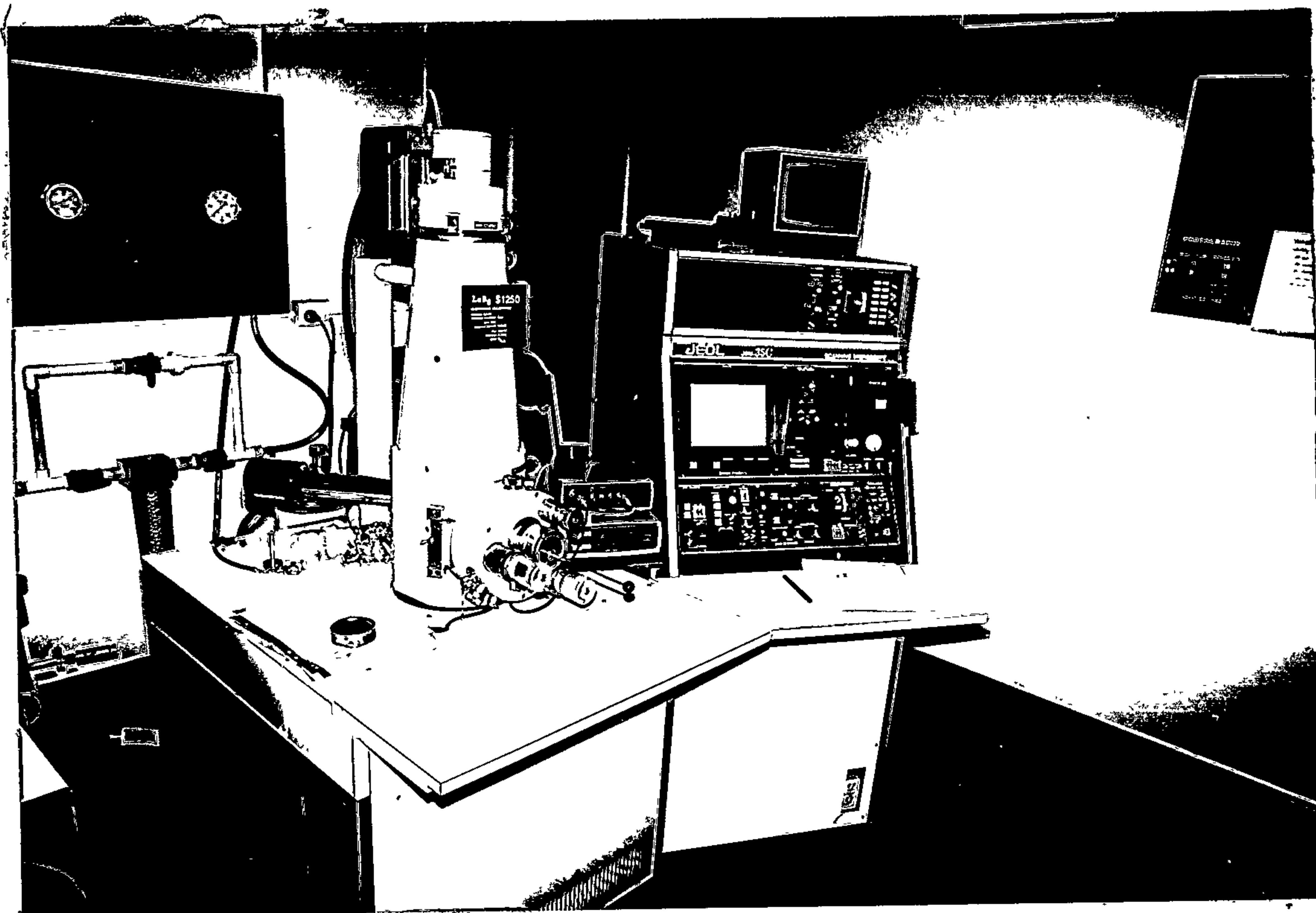


Figure 8. Jeol 35C Scanning Electron Microscope used in this study.

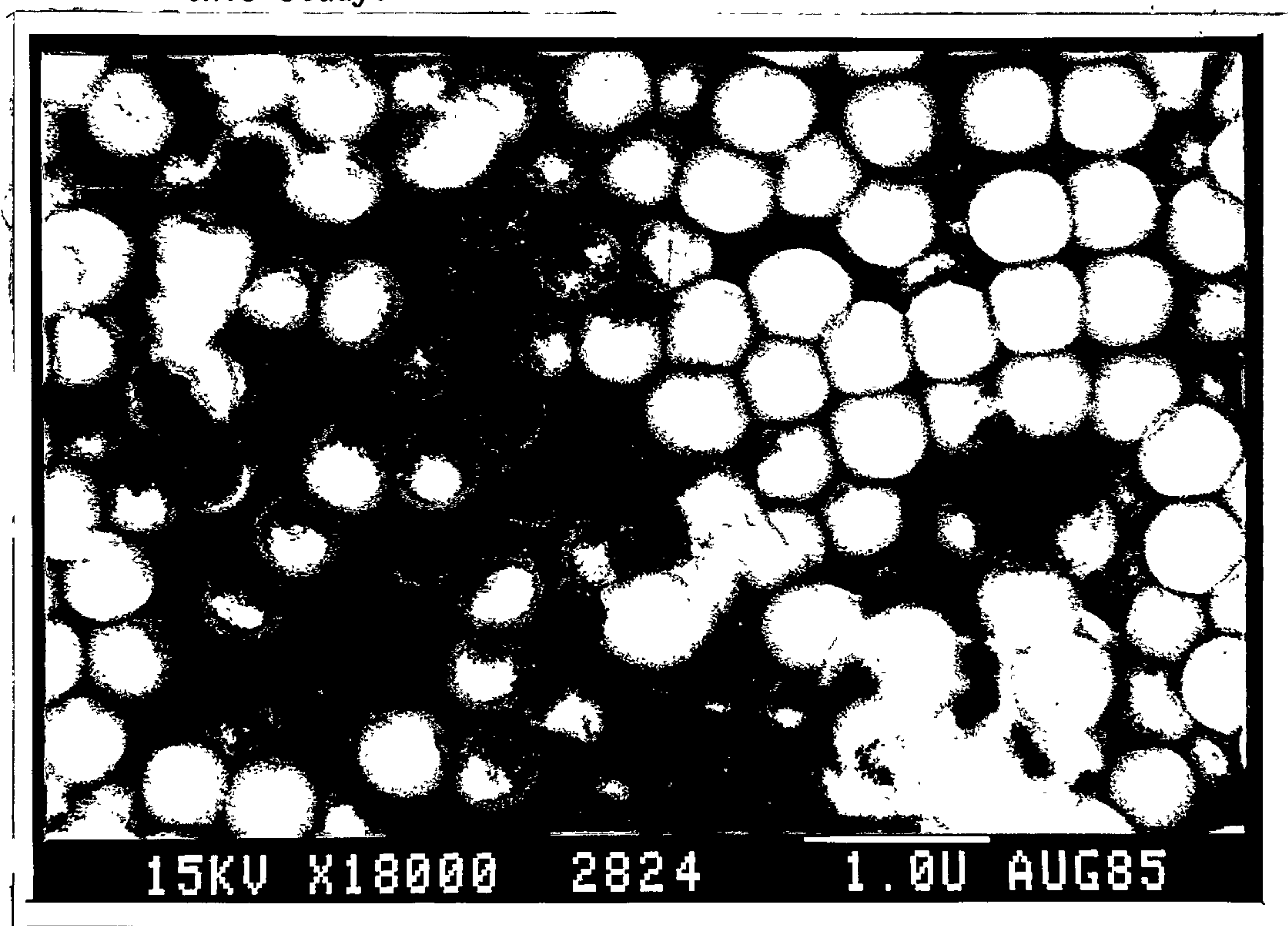
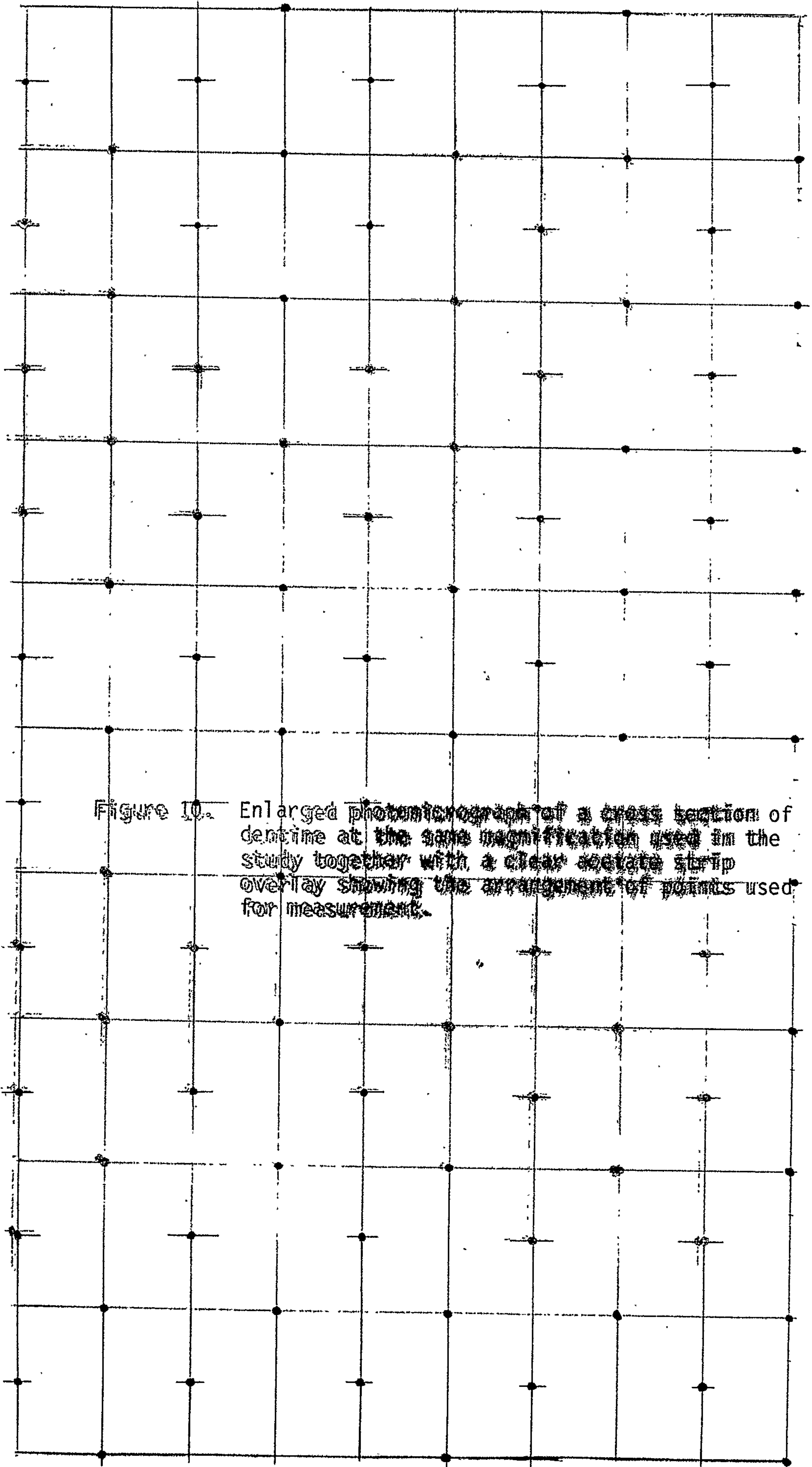
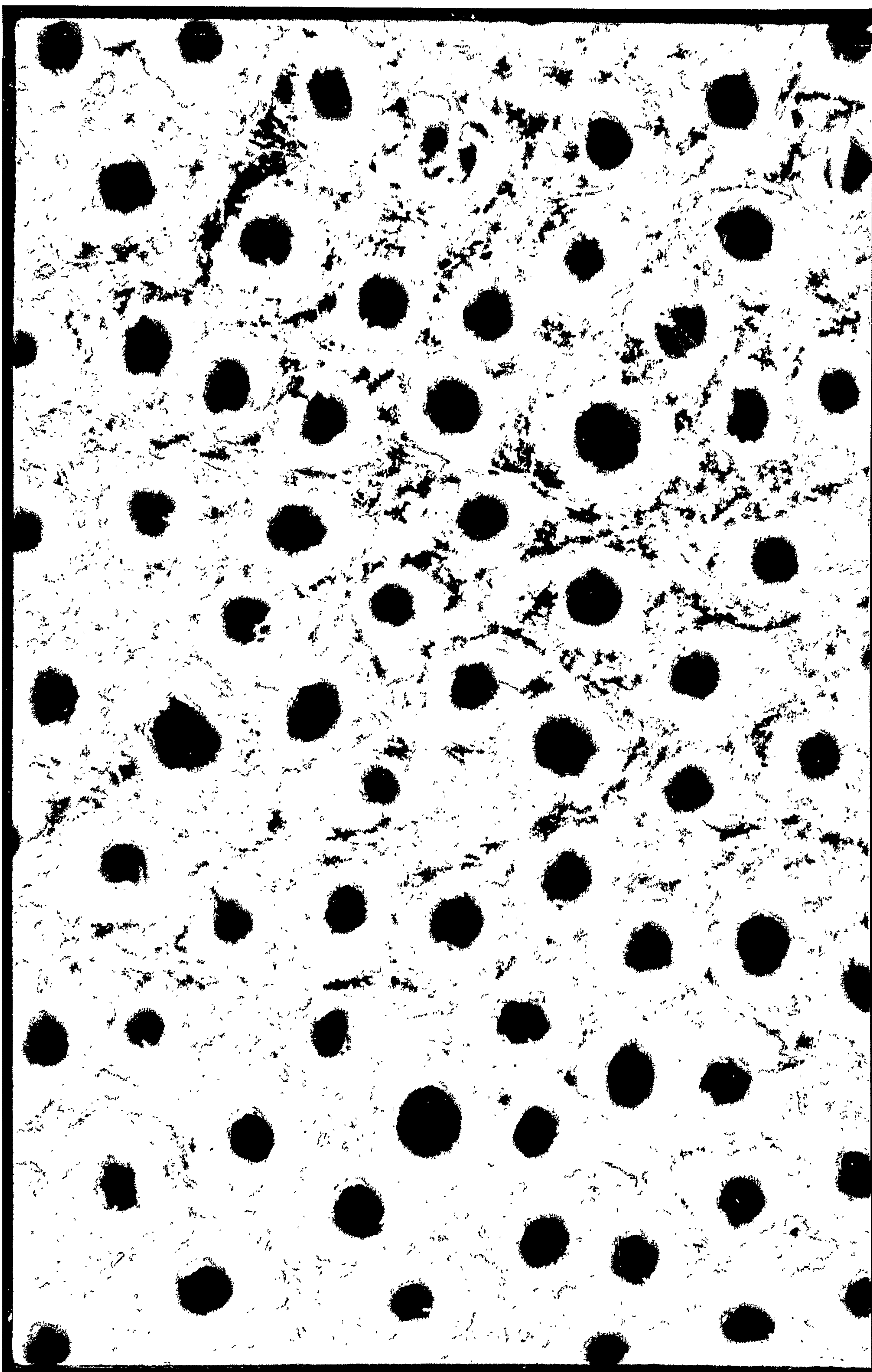


Figure 9. High power photomicrograph of plastic spheres 0.48 micrometers used to calculate the magnification of the photographic print. X21,000.

10KN X3000

10 0n





10411 43000 0353 10 011 M0405

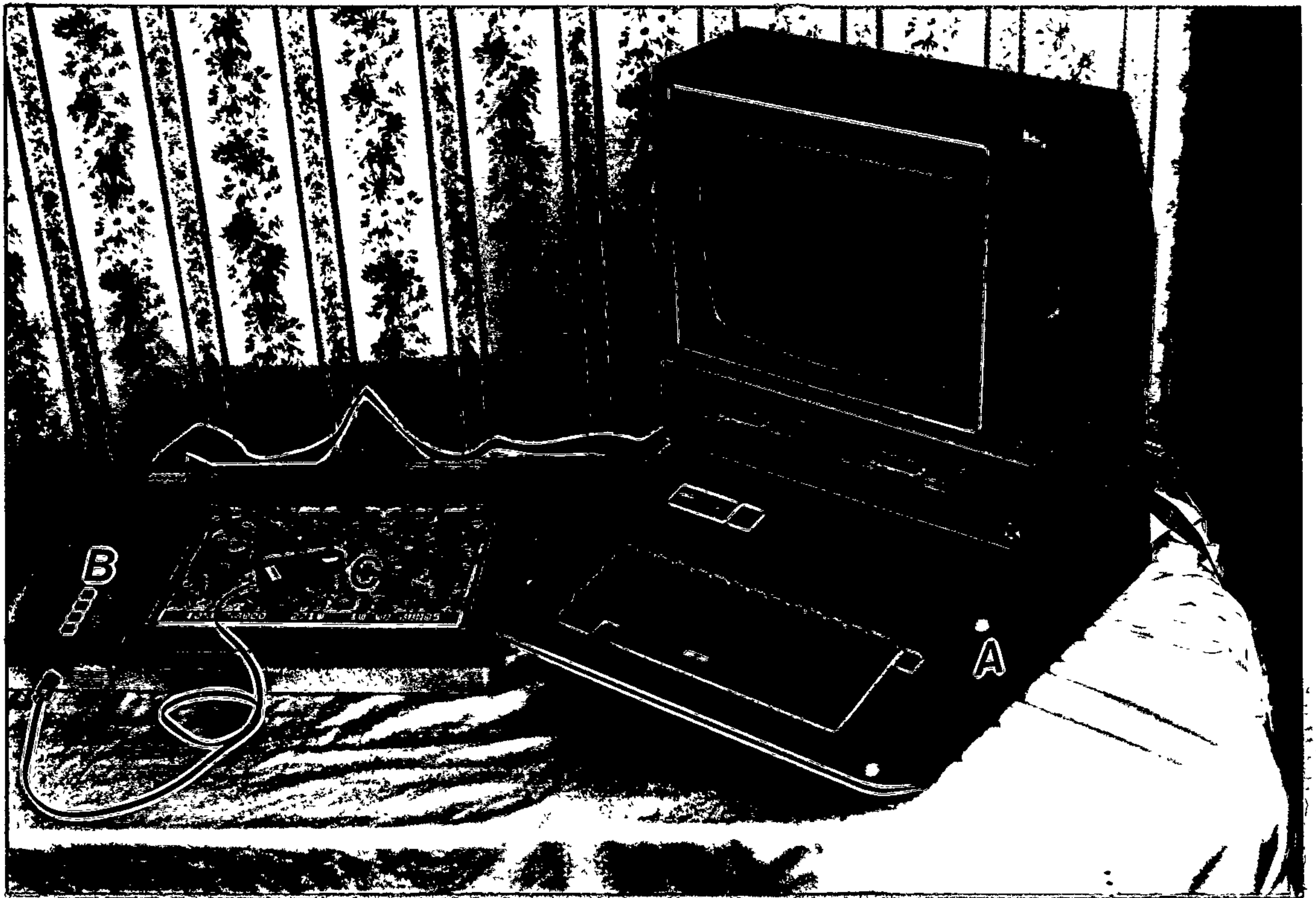


Figure 11. (A) Apple IIe computer, (B) Hipad digitizing tablet and (C) magnifying cursor used in this study.

Figure 12. Photomicrograph of a fractured cross section of dentine 0.3mm from the dentino-enamel junction. At this level tubules were fairly regular in outline and were surrounded by a distinct border of peritubular dentine. X3,400.

Figure 13. Photomicrograph of a fractured cross section of dentine 1.4mm from the dentino-enamel junction. At this level tubules are wider and more numerous than those tubules at the dentino-enamel junction. A broad zone of peritubular dentine is visible. X3,400.

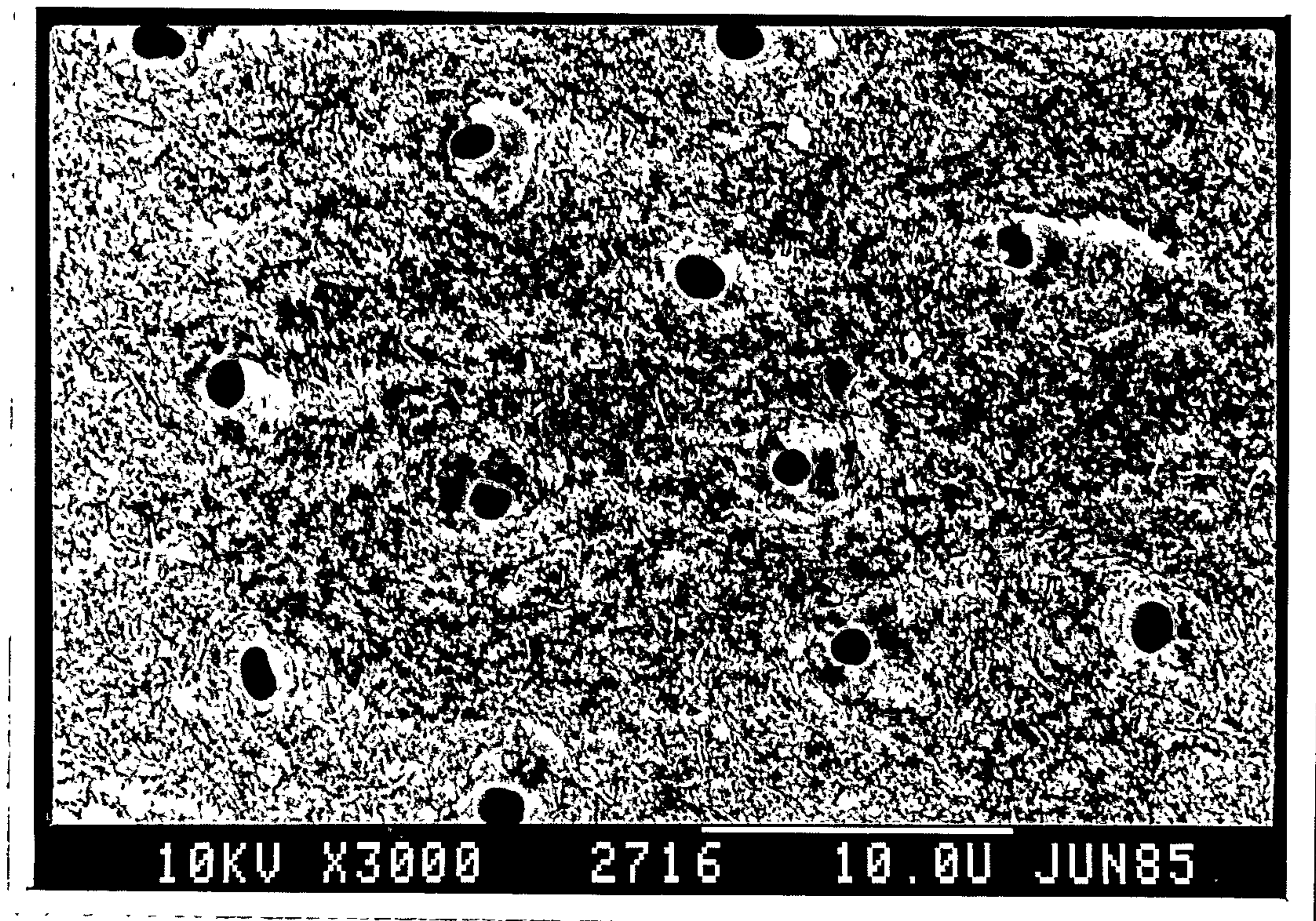


Figure 12.

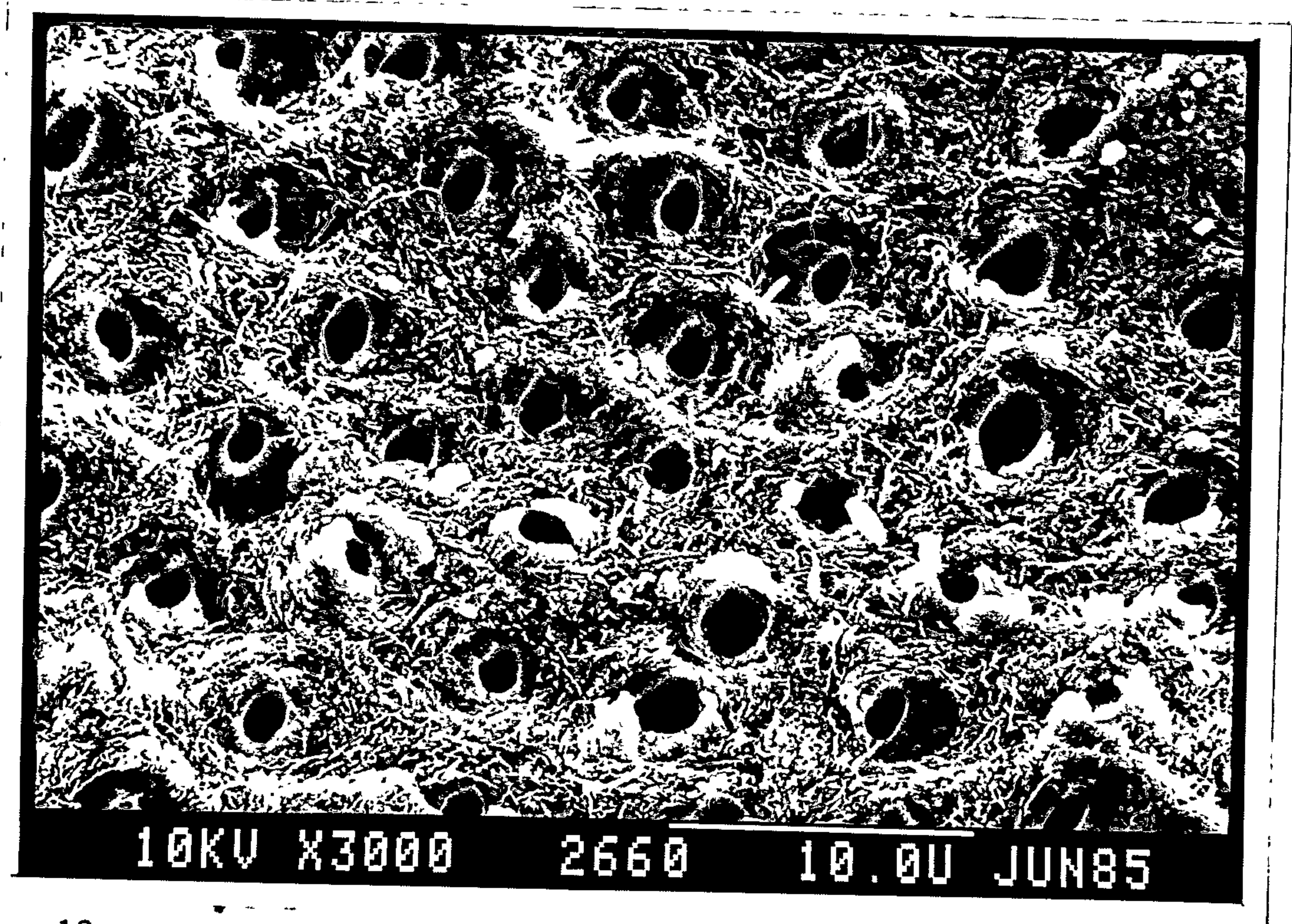


Figure 13.

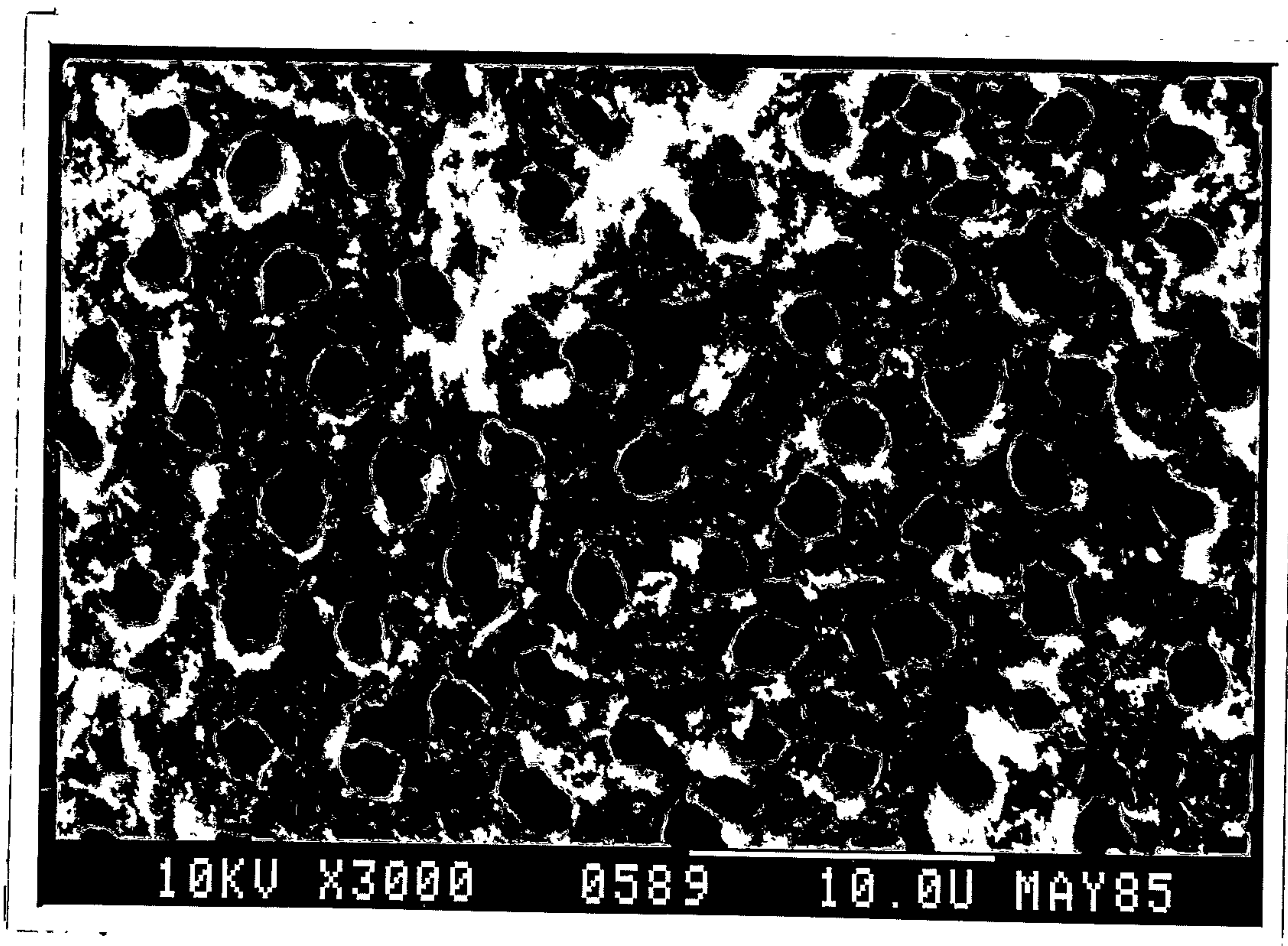


Figure 14. Photomicrograph of a fractured cross section of dentine 2.6mm from the dentino-enamel junction. At this level tubules are large numerous and more irregular in outline. Little if any peritubular dentine can be seen. X3,400.

Figure 15. Photomicrograph of a longitudinal section of dentine just at the dentino-enamel junction. Tubules are narrow and fairly widely spaced. X1,700.

Figure 16. Photomicrograph of a longitudinal section of dentine just above the pulp (P). Tubules are wider and closer together. X1,700.

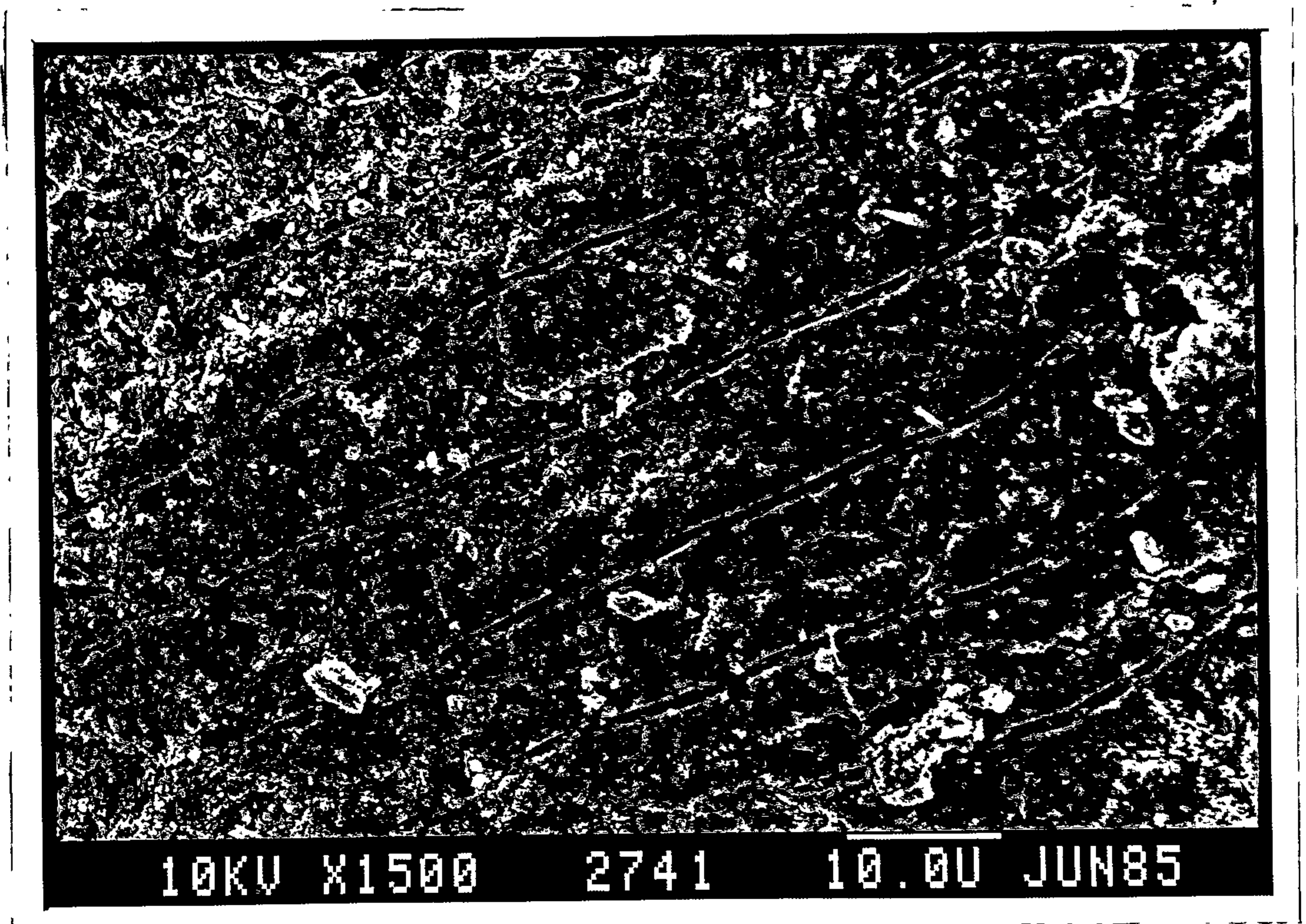


Figure 15.

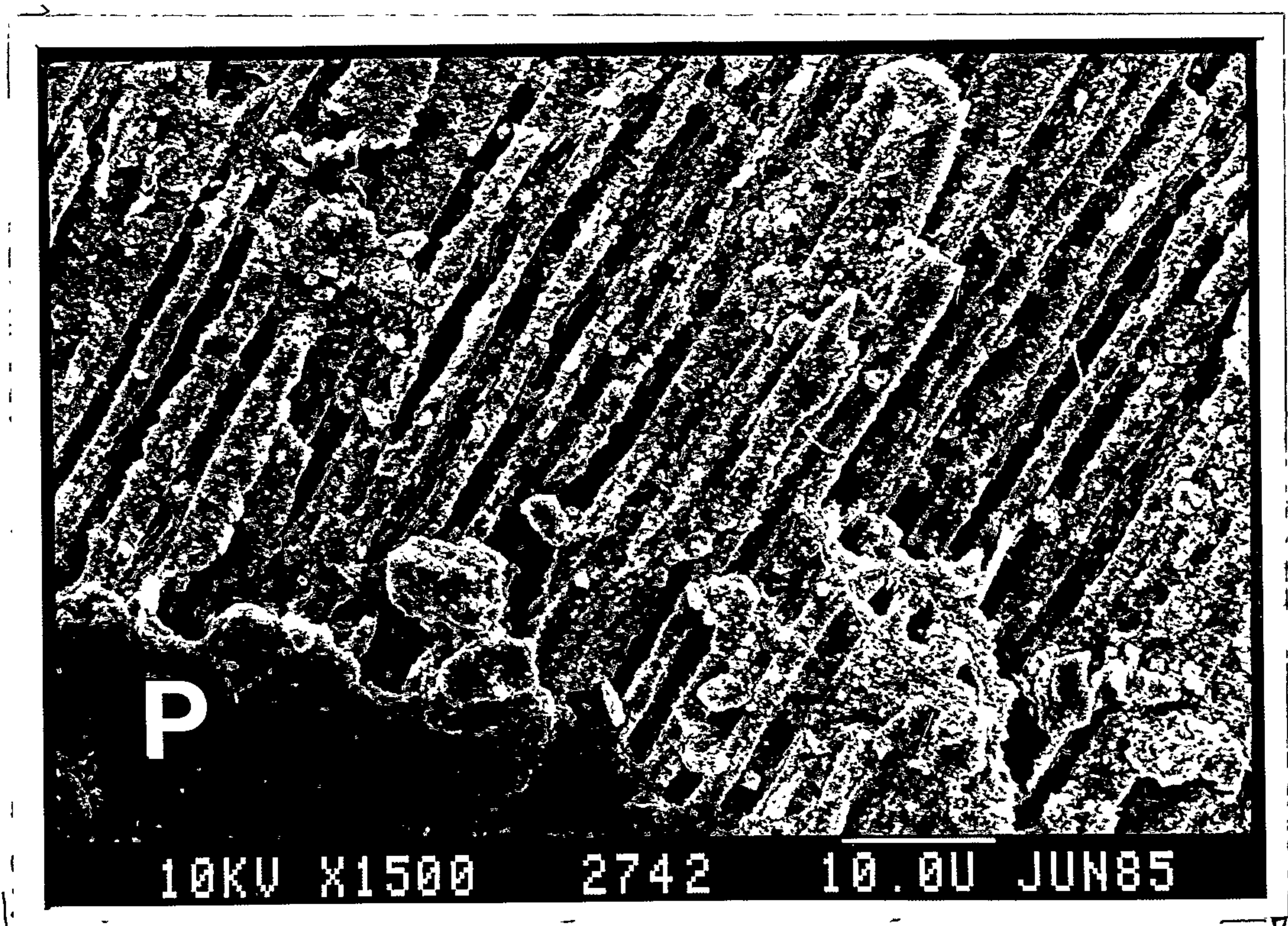


Figure 16.

Figure 17. Photomicrograph of a cross section of dentine 0.3mm from the dentino-enamel junction. A large variation in the diameters of individual tubules can be seen. A very small tubule is arrowed. X3,400.

Figure 18. High power photomicrograph of an individual dentinal tubule from a cross section of dentine 0.3mm from the dentino-enamel junction. A number of lateral branches can be seen radiating out from the main tubule. An opening of a small lateral branch into the main canal can be seen. X19,000.

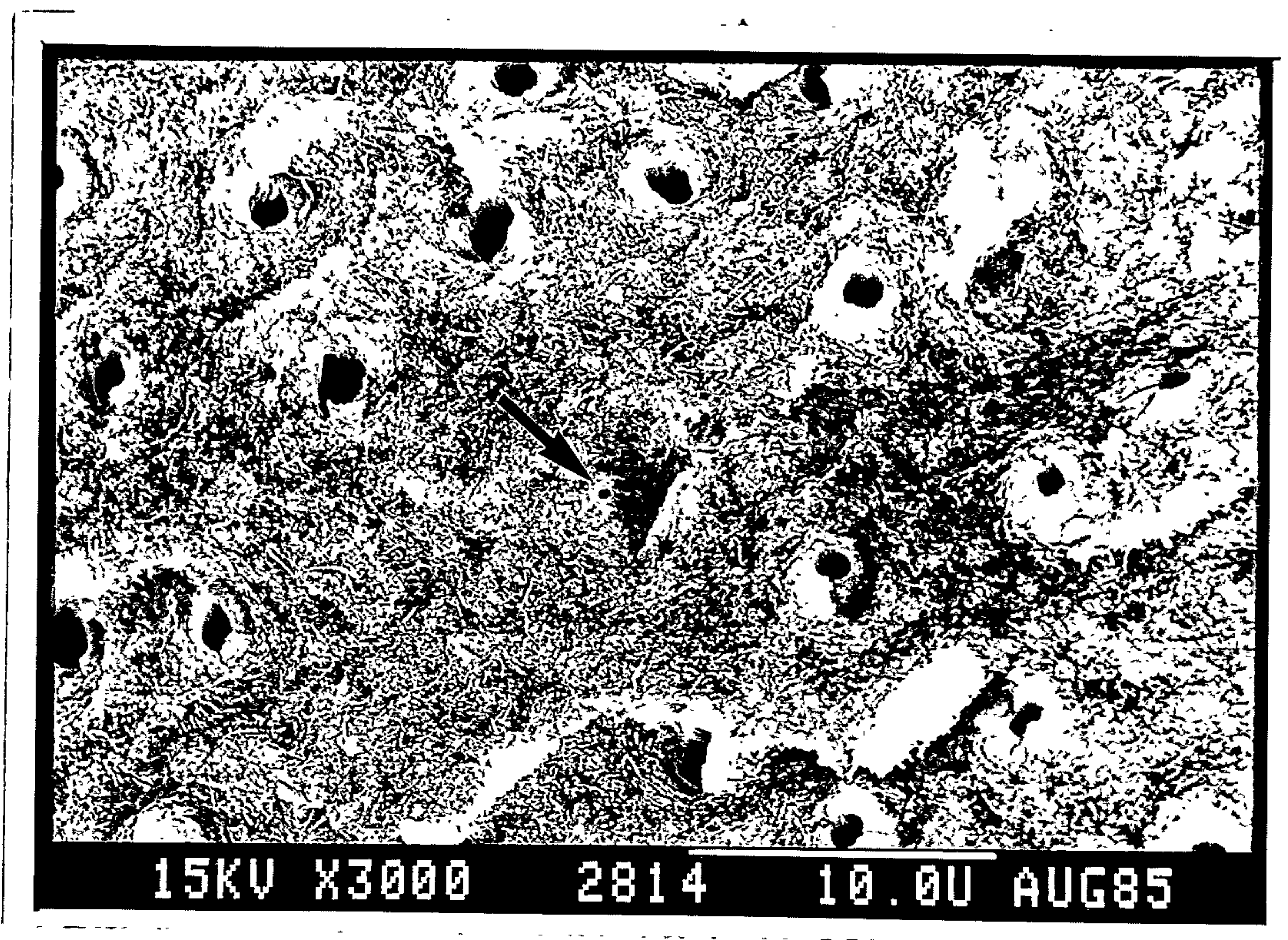


Figure 17.



Figure 18.

Figure 19. Photomicrograph of a cross section of dentine 0.3mm from the dentino-enamel junction. Structures resembling odontoblast processes can be seen protruding from some of the tubular openings. X3,400.

Figure 20. Photomicrograph of a cross section of dentine 0.7mm from the dentino-enamel junction. Structures resembling odontoblast processes can be seen protruding from some tubular openings. X3,400.

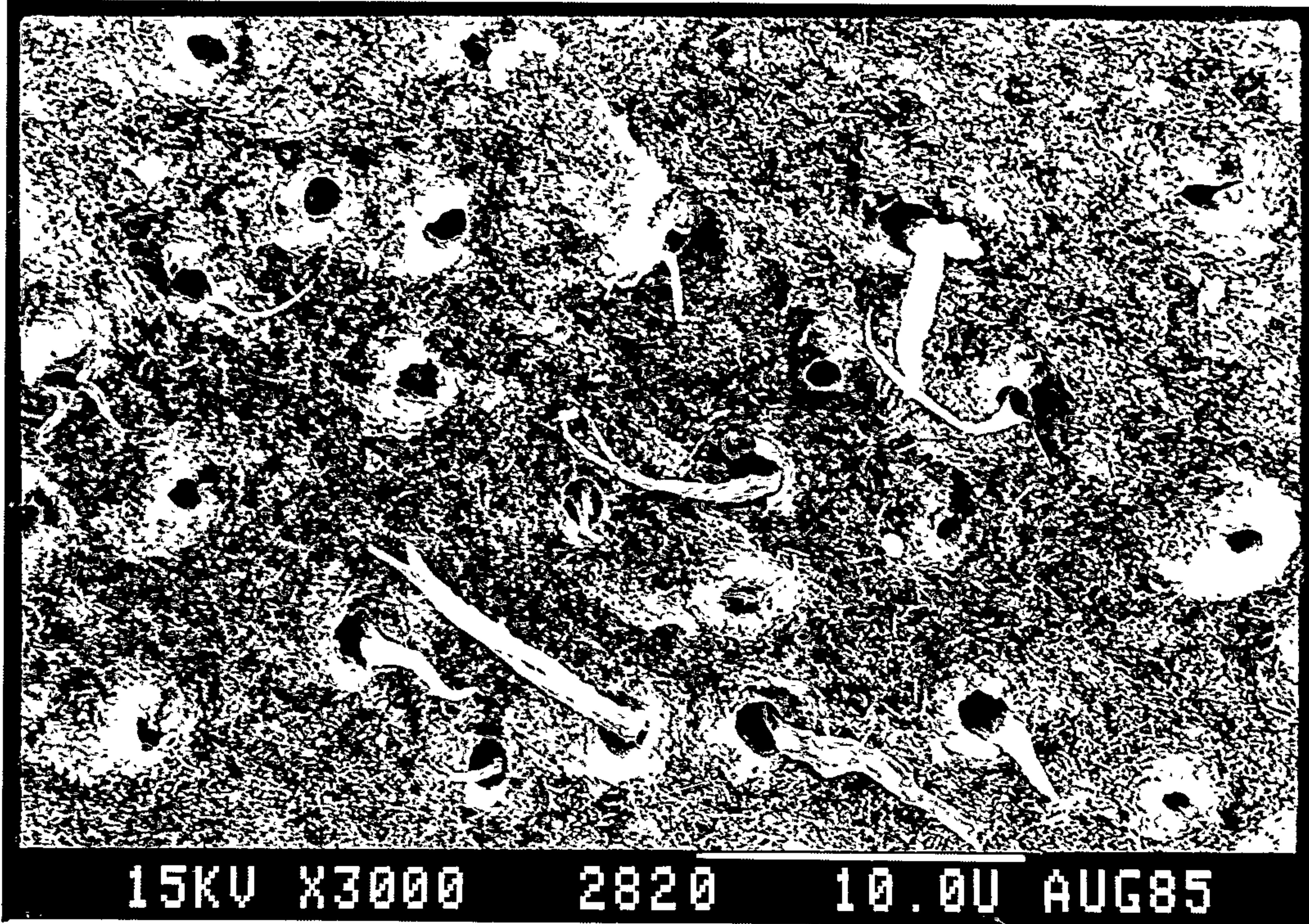


Figure 19.

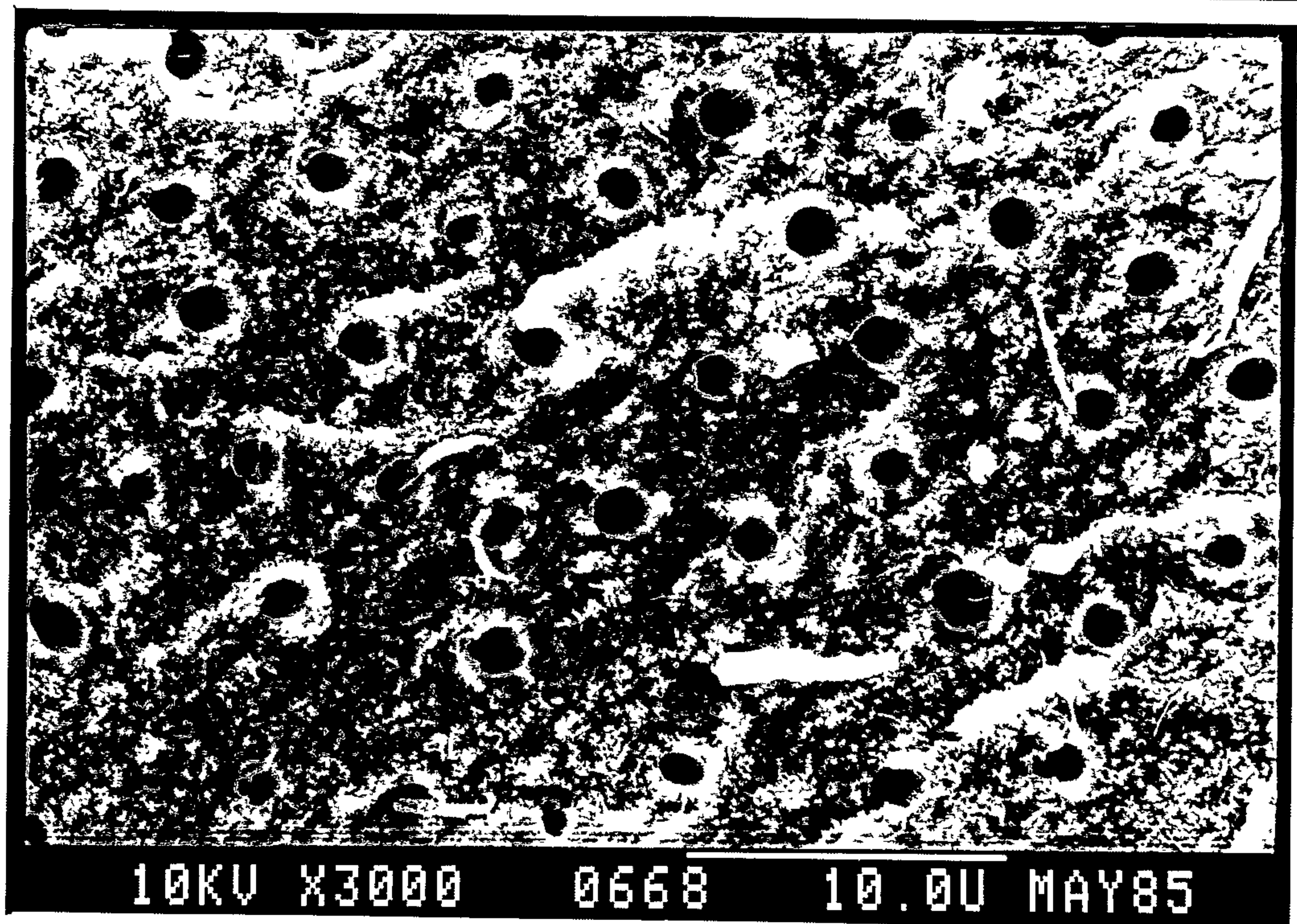


Figure 20.

Figure 21. High power photomicrograph of a cross section of dentine 0.3mm from the dentino-enamel junction showing a cable like structure, possibly an odontoblast process protruding from an open dentinal tubule. Small lateral branches can be seen extending from surface of the process. X12,000.

Figure 22. High power photomicrograph of a cross section of dentine showing a branching process (possibly odontoblast process) protruding from a dentinal tubule 0.7mm from dentino-enamel junction. X21,000.

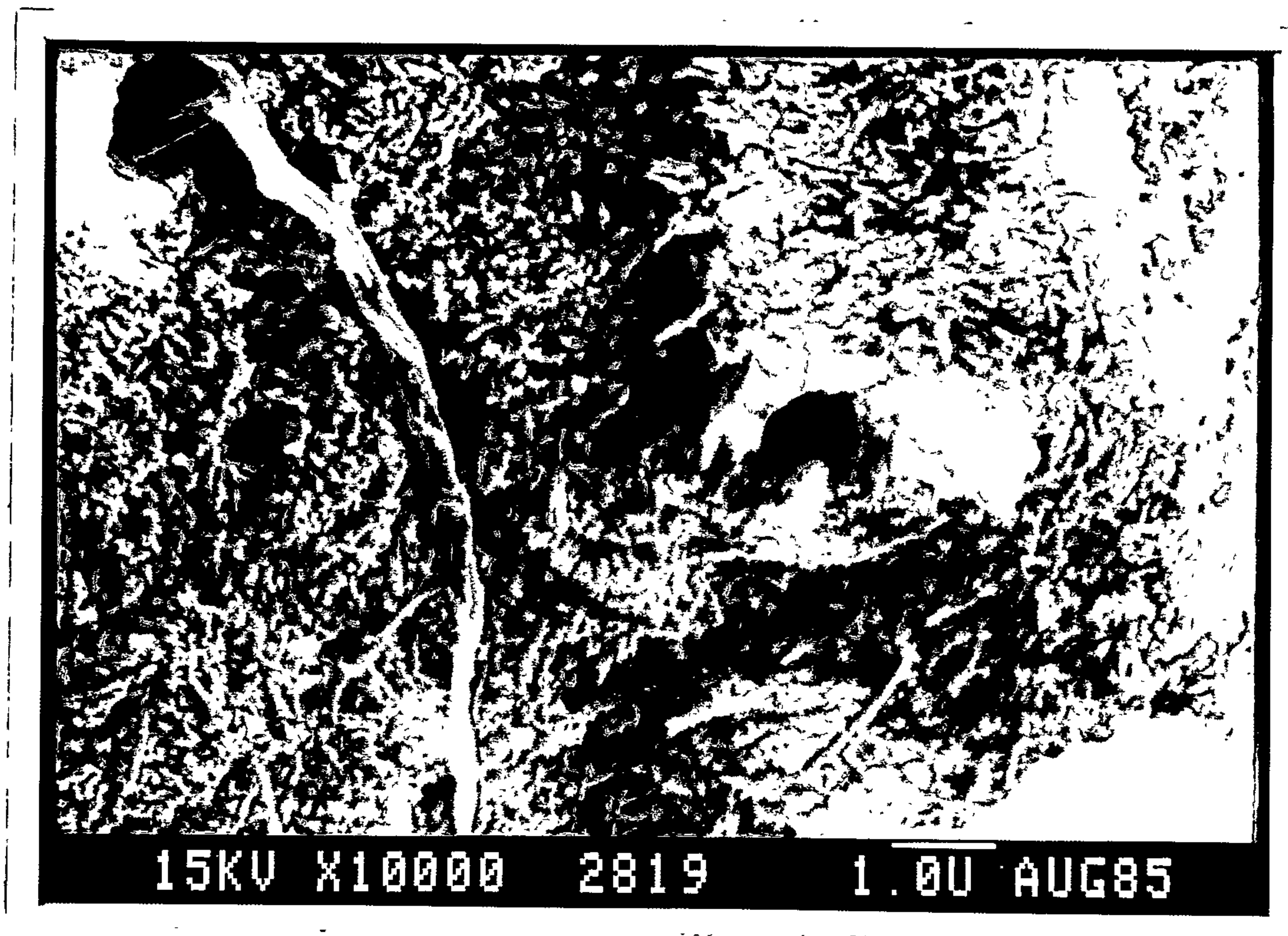


Figure 21.

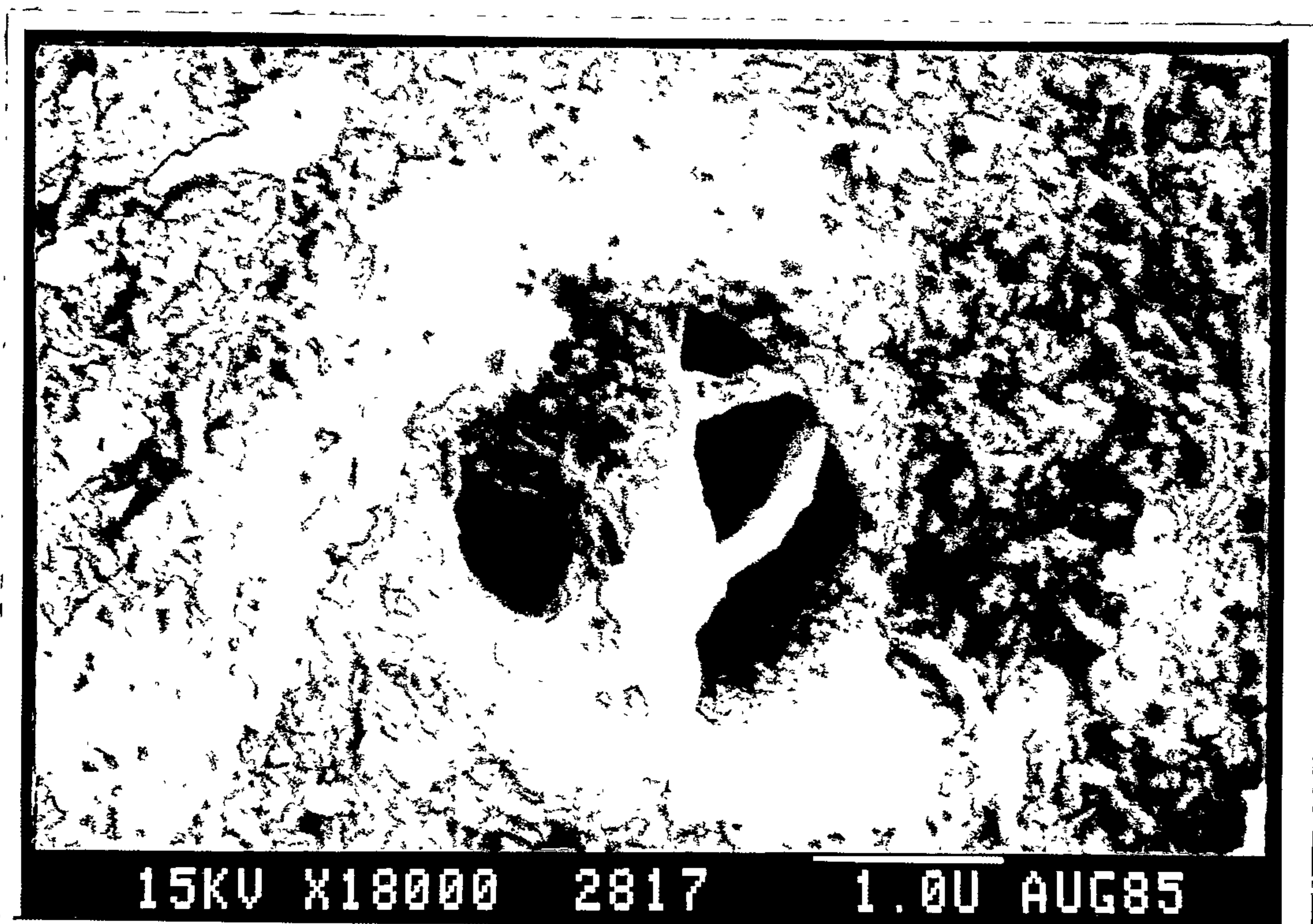


Figure 22.

Figure 23. High power photomicrograph of a cross section of dentine 0.7mm from the dentino-enamel junction showing a cable like structure, protruding from a dentinal tubule. The fibre appears to be attached to the inner surface of the tubule. X21,000.

Figure 24. High power photomicrograph of a cross section of dentine 0.3mm from the dentino-enamel junction showing a twisted membranous structure protruding from a dentinal tubule. X21,000.

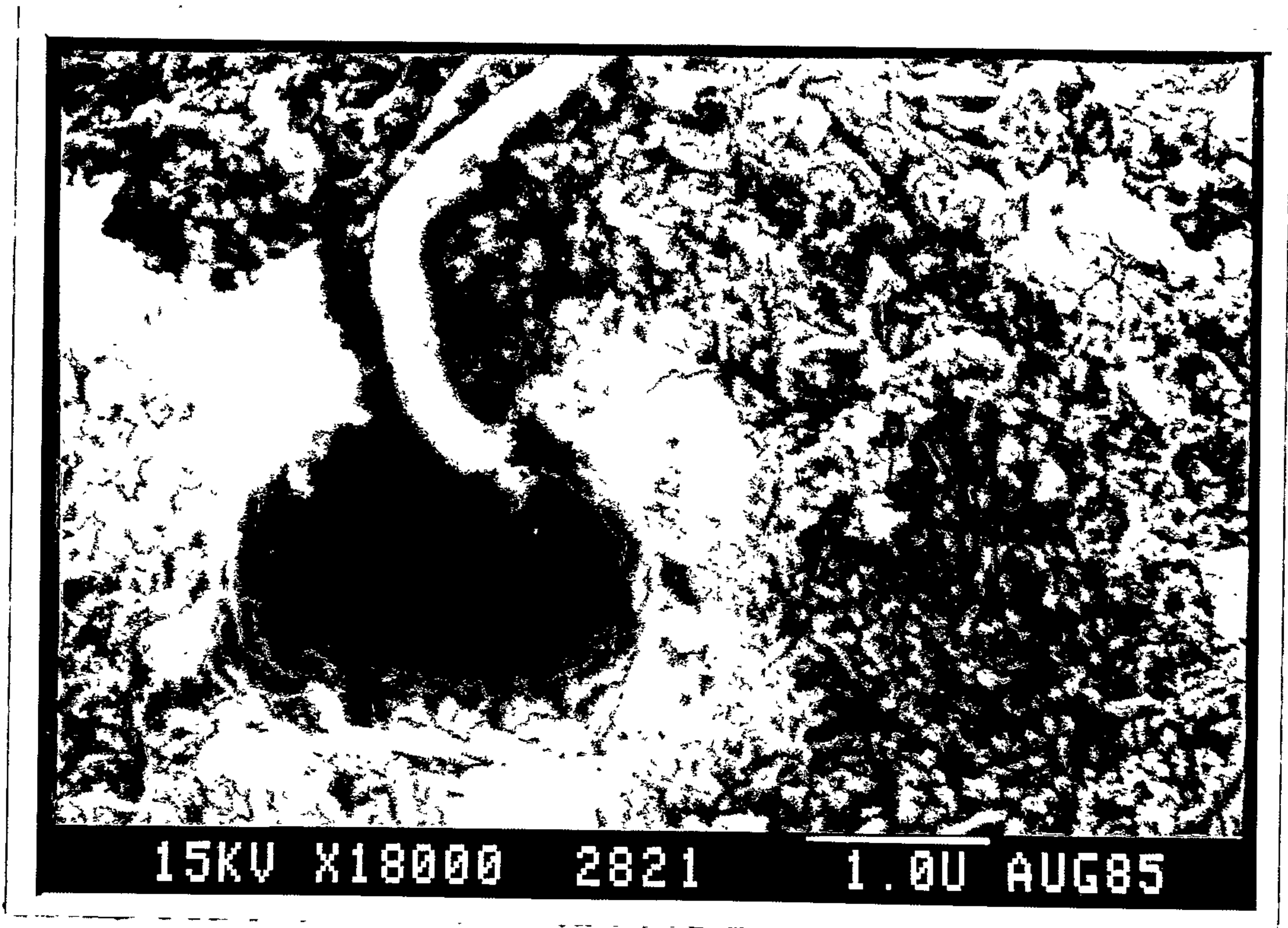


Figure 23.

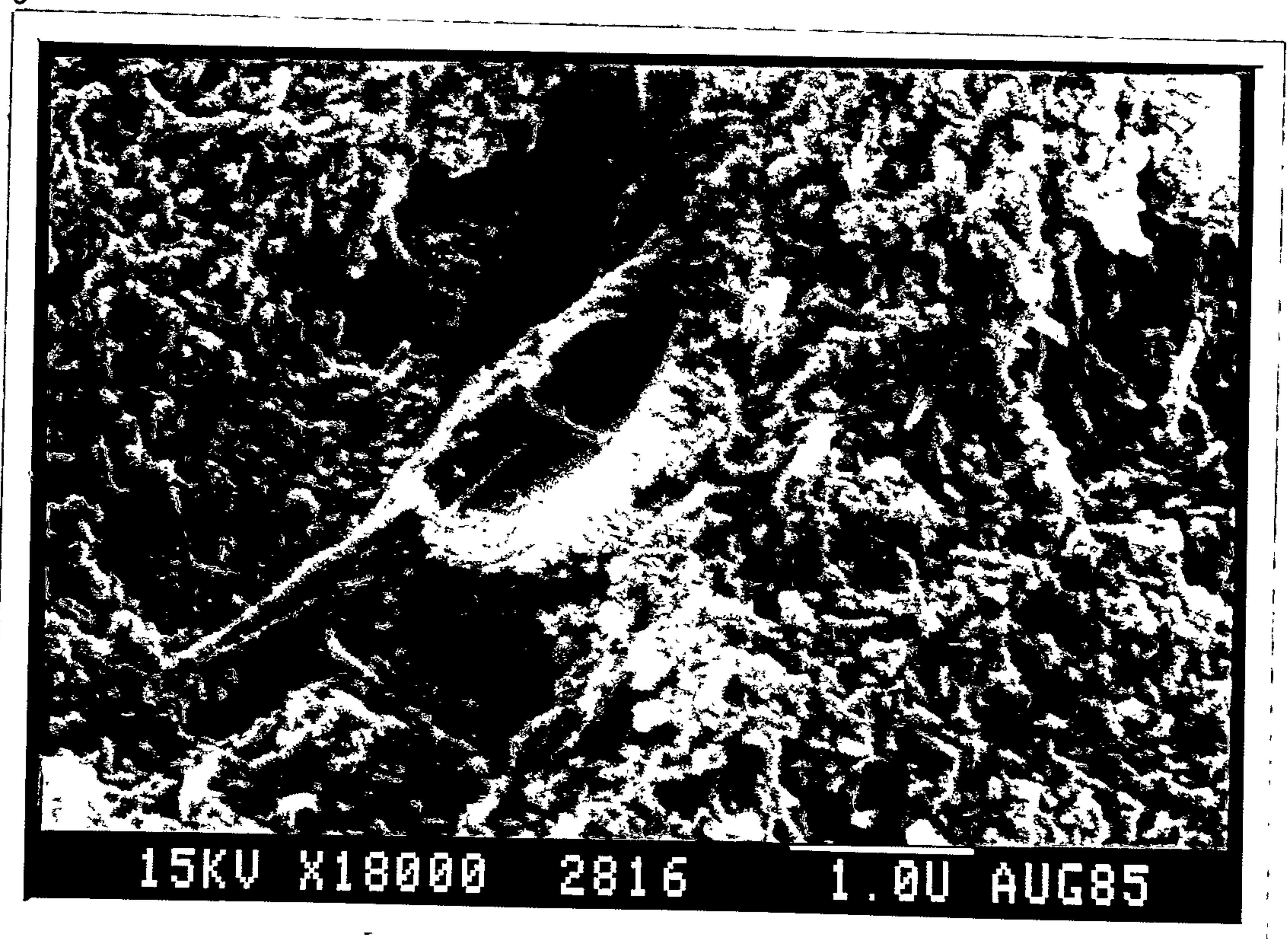


Figure 24.

Figure 25. Photomicrograph of a cross section of dentine 0.3mm from the dentino-enamel junction showing long fibres extending from the orifice of two dentinal tubules. X2,300.

Figure 26. High power photomicrograph of area arrowed in the above illustration showing the point of exit of the fibre from the tubule. X21,000.

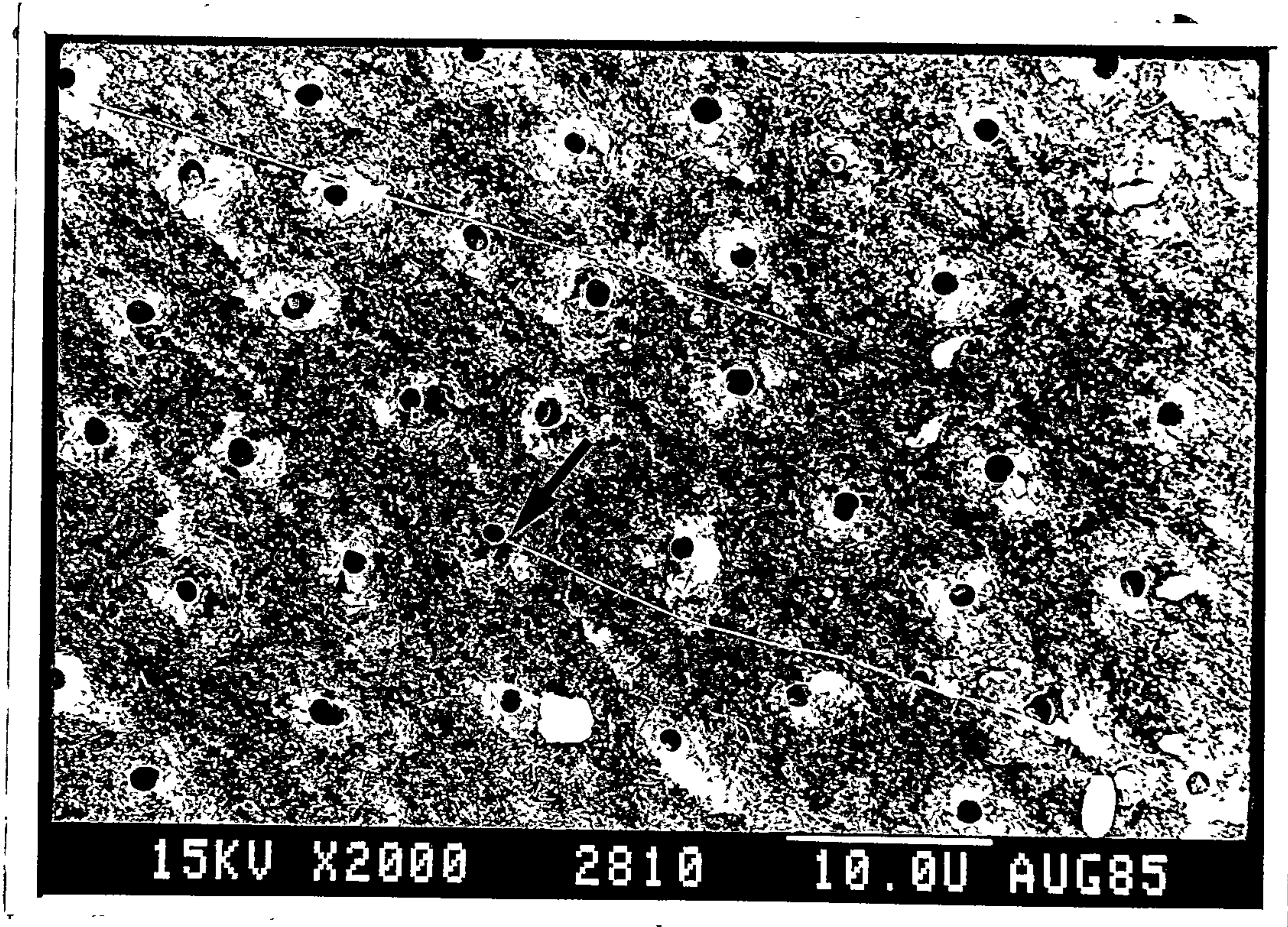


Figure 25.

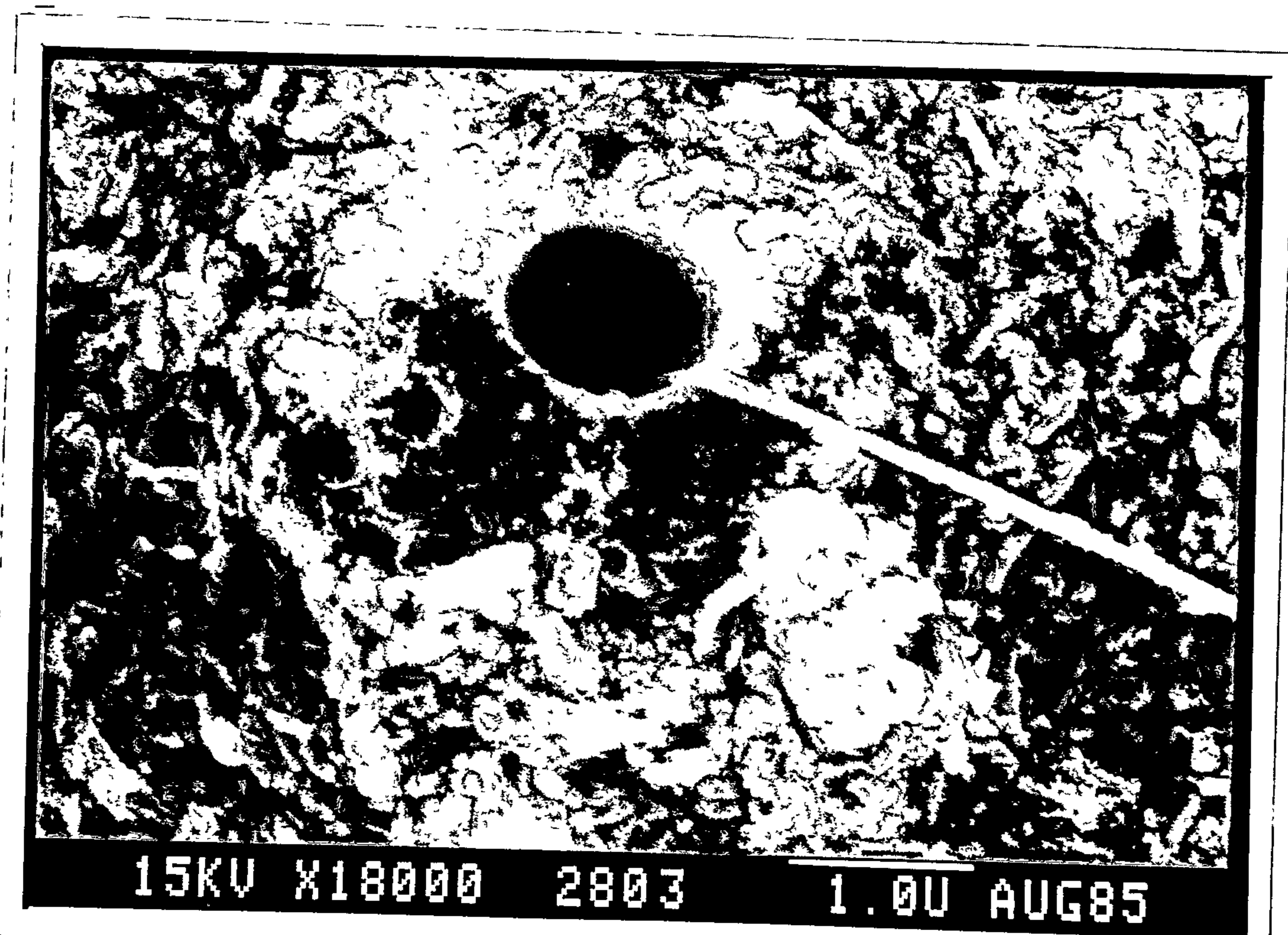


Figure 26.

Figure 27. Photomicrograph of an oblique section of dentine showing a long branching, fibrous extension protruding from a dentinal tubule. X2,700.

Figure 28. High power photomicrograph of the area arrowed in the above illustration. X15,000.

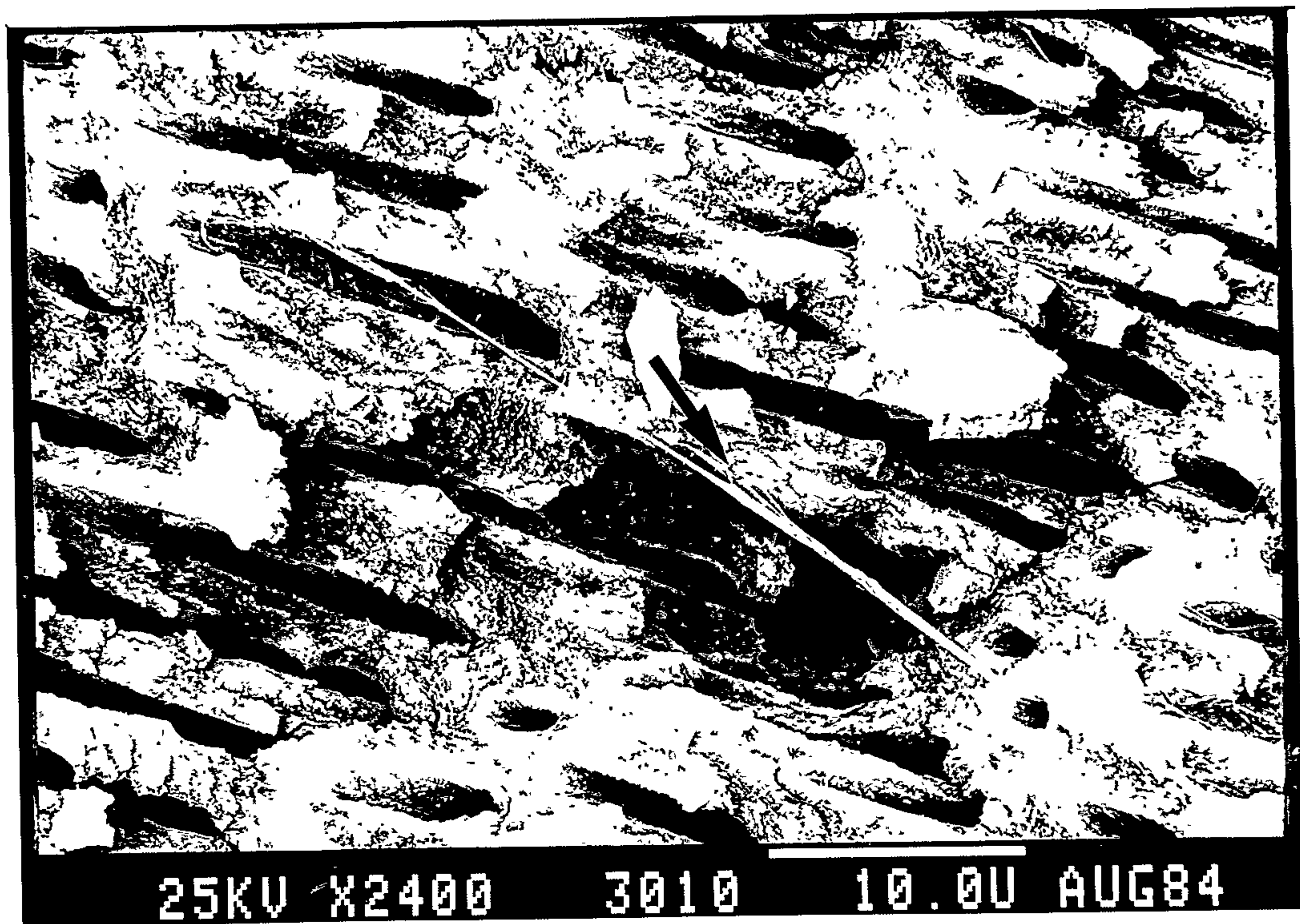


Figure 27.

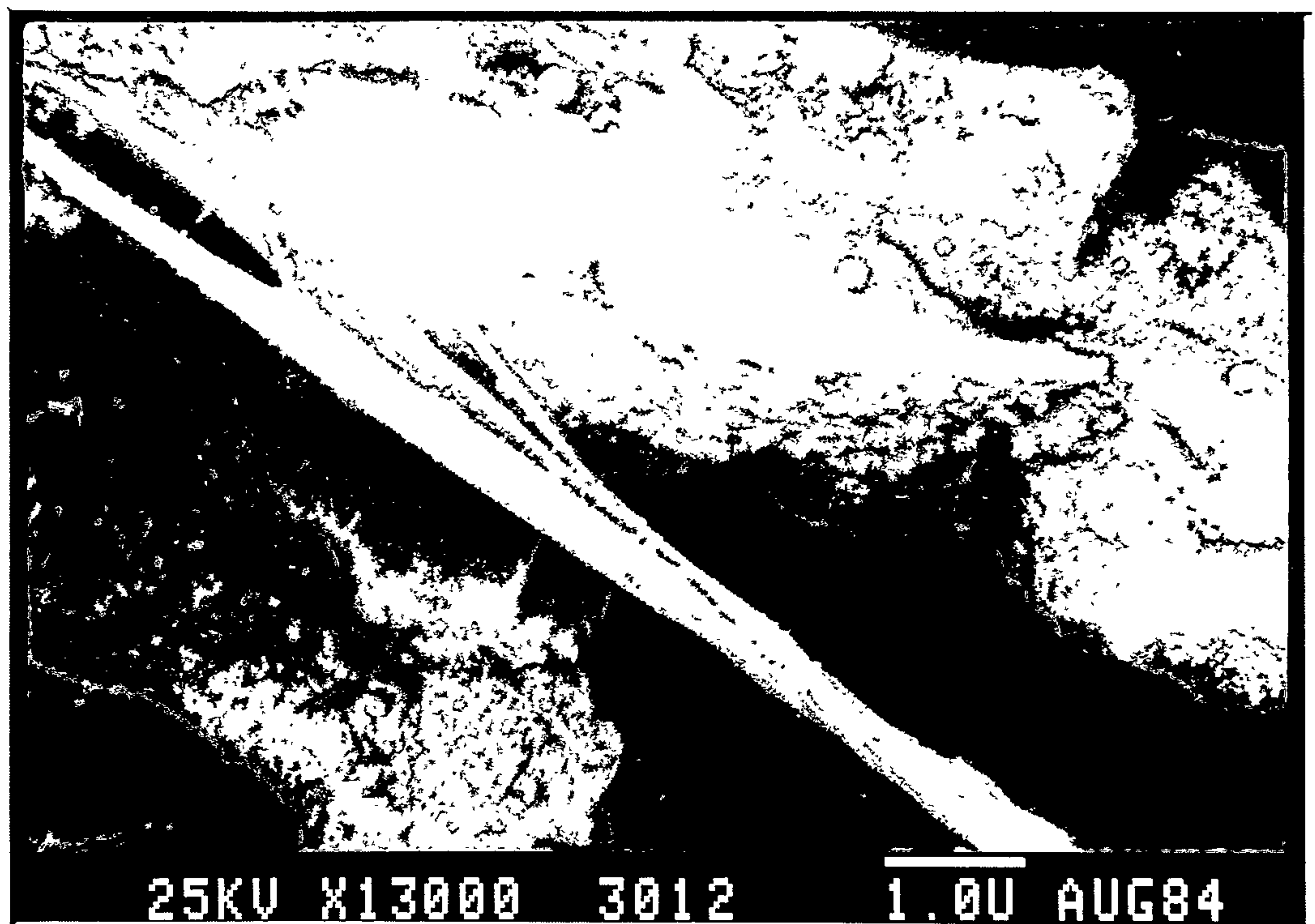


Figure 28.

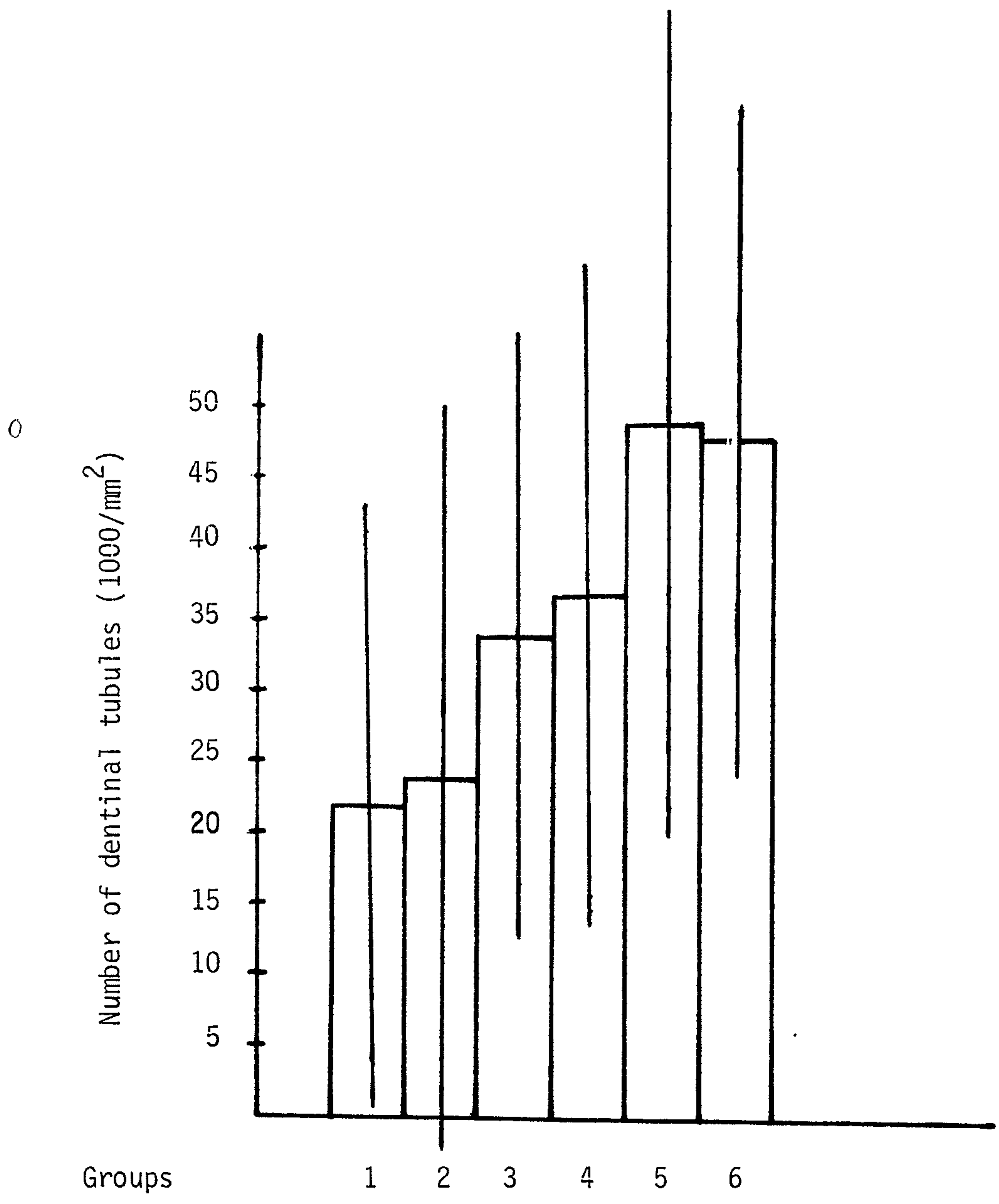


Figure 29. Distribution of number of dentinal tubules/mm² for each of the different groups using Method A.

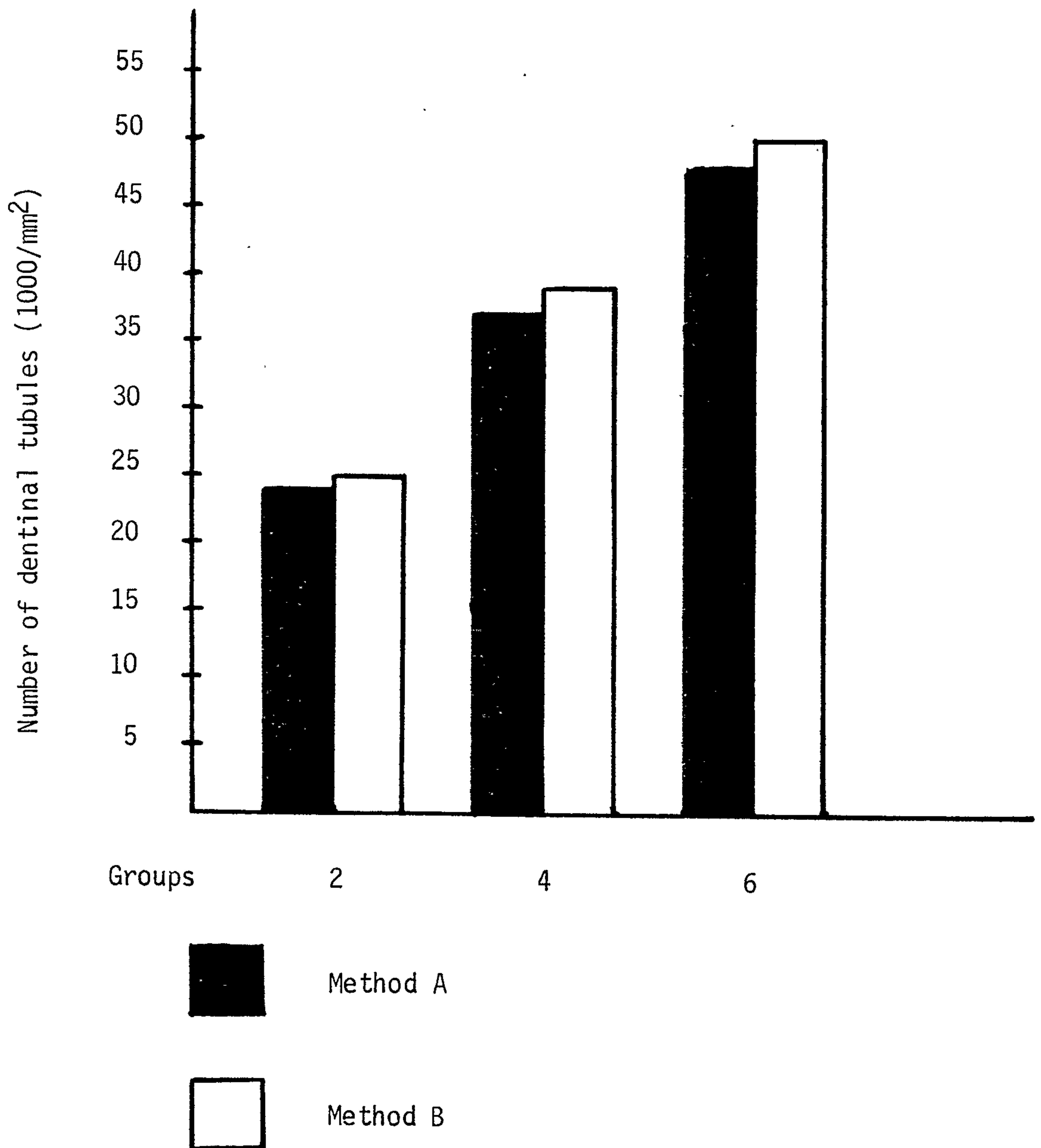


Figure 30. Comparison of number of the dentinal tubules/mm² in Method A and Method B.

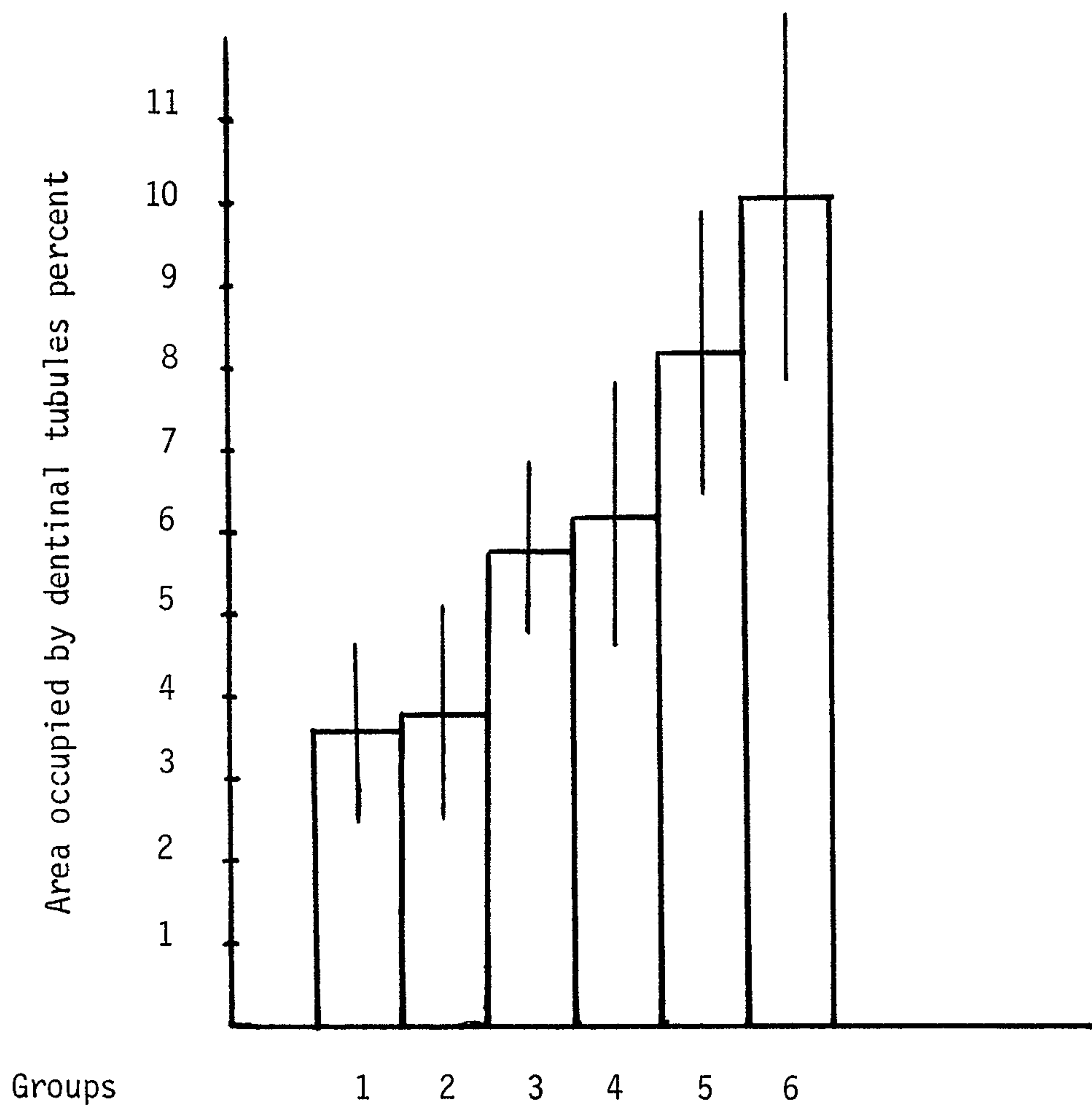


Figure 31. Distribution of percentage area occupied by dentinal tubules in the different groups using Method A.

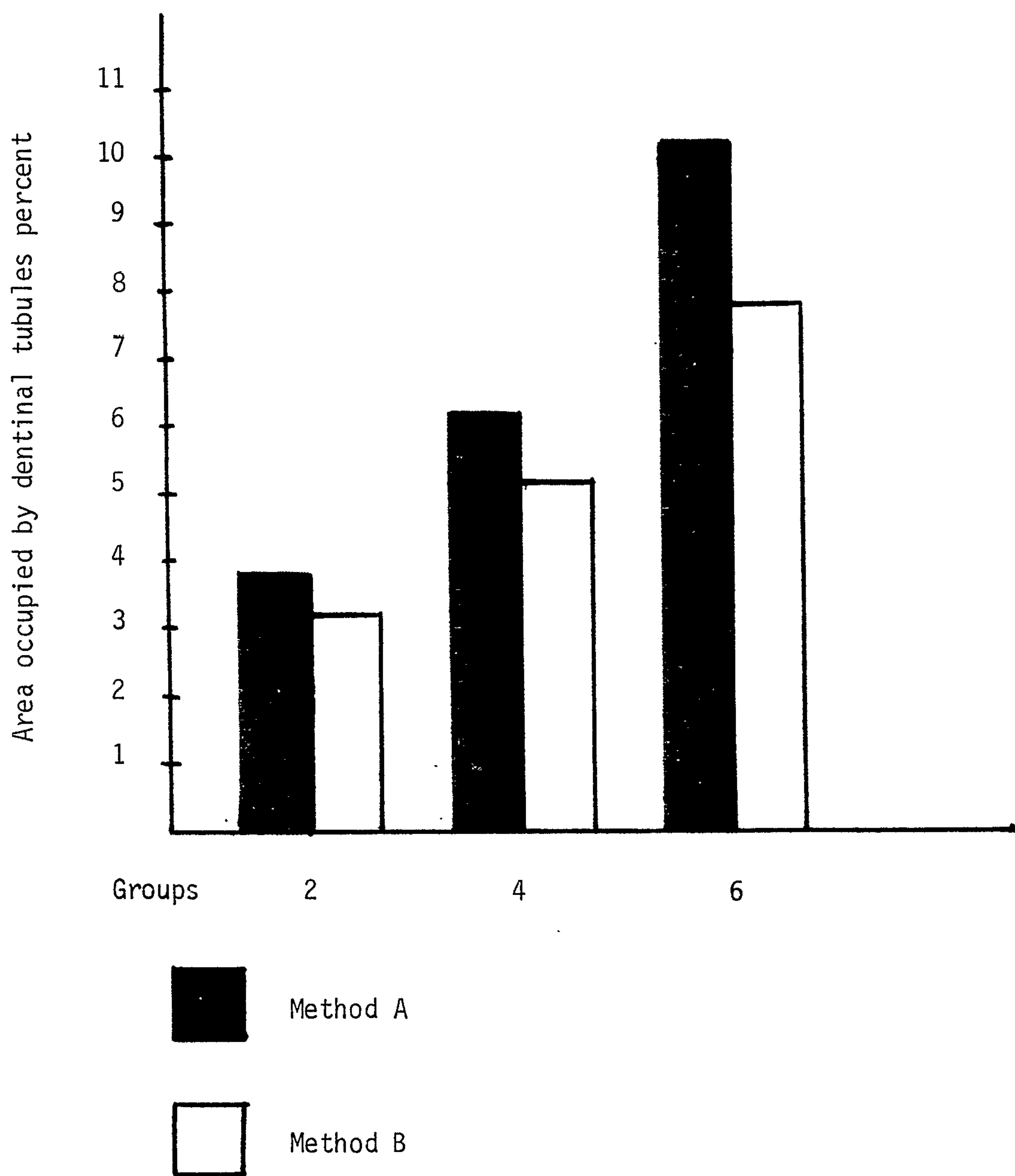


Figure 32. Comparison of percentage area occupied by dentinal tubules in Method A and Method B.

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