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MORPHOLOGICAL STUDY OF CALCOSPHERITES IN RAT AND RABBIT INCISOR DENTIN

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Abstract

Calcospherites from incisors of rats and rabbits were studied, by means of secondary and backscattered electron images, using scanning electron microscopy. Part of each incisor specimen was made anorganic to allow observation of the surface of the mineralization front by scanning electron microscopy; the other part was ground on one side for observation by scanning electron microscopy with a backscattered electron image detector. In both species the labial mineralization front was wavy and the image showed fused calcospherites, whereas the lingual mineralization front appeared to consist of a combination of linear and globular forms. In rat labial dentin the calcospherites were large and globular form, but they did not develop in the lingual dentin and were small and oval. The shape and size of rabbit incisor calcospherites varied from the pulp horn to the root apex. There were great differences between rats and rabbits with respect to the size and shape of the calcospherites at the mineralization front. This may be due to species differences and possibly the differing rates of dentinogenesis in rats and rabbits.

Key Words: Rat, rabbit, incisor, labial dentin, lingual dentin, calcospherites, mineralization front

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Introduction

The scanning electron microscope (SEM) can be used to obtain various signals, such as secondary, Auger and backscattered electrons, cathodoluminescence, characteristic X-rays and continuous X-rays, etc.. Electron signals usually are used to study the morphology of irregular surfaces of samples. Backscattered electron images have been used to study tissues stained by heavy metals [1, 2, 17], immunohistochemistry studies [6, 18, 19] and to investigate calcification pattern of hard tissues [4]. Jones and Boyde [7] reported that the backscattered electron image provided new information about the mineralization front and crystal orientation of calcified tissues. Several attempts have been made to observe the three-dimensional structure of calcospherites, after sodium hypochlorite (NaOCl) treatment [3, 9, 16]. Comparison of the backscattered with the secondary electron image after NaOCl treatment may, therefore, provide new information about the nature of calcospherites.

Mishima *et al.* [12] divided rat incisor dentin into enamel-covered labial dentin and cement-covered lingual dentin. They demonstrated that the histological structure of labial and lingual dentin differed and that the shape of the calcospherites in predentin differed from those in dentin. The incisors of rats and rabbits grow continuously and are enamel-covered on the labial side and cement-covered on the lingual side [15]. However, little has been reported on the morphology of calcospherites in rabbit predentin. This study was designed to characterize the morphology of the mineralization front and the calcospherites of the labial and lingual dentin of rat and rabbit incisors using combined backscattered and secondary electron images.

Material and Methods

Bilateral incisors were extracted from 10 male Wistar-strain rats (200 to 400 g each) and 5 male Japanese white rabbits (2.5 to 3.5 kg each). Each incisor was fixed in 2% glutaraldehyde fixative solution (pH 7.4, 0.1 M phosphate buffer solution) for 24 hours prior to use. Some samples were fixed in 10% neutral formalin for 24 hours.

After fixation, one of each pair of incisor specimen was cut transversely into three portions with a Torx dental turbine (Morita Co. Ltd., Tokyo) equipped with a diamond disc. These portions were divided further, with the same turbine, into separate labial and lingual regions, prior to NaOCl treatment to expose the mineralization front. For this treatment the specimens were kept in fresh 10% NaOCl solution for 30 minutes at room temperature with mild vibration, rinsed well with distilled water and dehydrated with an alcohol series, after which the alcohol was substituted by isoamyl acetate and the specimens were subjected to critical-point drying with liquid carbon dioxide. The pulpal surfaces were gold-coated for morphological examination (secondary electron image) of the mineralization front, which was observed from a direction perpendicular to the tooth's longitudinal axis, with a JSM-T200 SEM (Japan Electron Optics Laboratory Co. Ltd., Tokyo).

The other incisors were halved parallel to their longitudinal axes using an Isomet low-speed saw (Buehler, Co.), after fixation. The cut surface was ground with a whetstone initially and then with diamond paste (0.25 μm particle diameter), after which the specimen were subjected to ultrasonic cleaning for 3 minutes, washed well with distilled water, followed by dehydration and critical-point drying as described above. The polished surfaces were carbon-coated in a TB-500 carbon coater (EMSCOPE Laboratories Ltd.), and backscattered electron images were observed with a JSM-T200 SEM.

Results

Secondary electron images after anorganic treatment

The morphological characteristics of calcospherites at the mineralization front from the incisal pulp horn to the root apex of the incisor were observed. The results presented here are mainly for the calcospherites at the intermediate region between the root apex and the incisal pulp horn.

Rat incisor dentin. Secondary electron images of the mineralization front of anorganically-treated incisors showed that rat labial dentin contained large globular calcospherites, which ranged in size from 7 to 31 μm (Fig. 1), and each contained 2-16 dentinal tubules. The calcospherites in the lingual dentin were oval and smaller (7-20 μm) than those in the labial dentin (Fig. 2), and each in the lingual dentin contained 2-8 dentinal tubules. The calcospherites were arranged with their longitudinal axes parallel to the longitudinal axis of the incisor.

Rabbit incisor dentin. The calcospherites in the mineralization front of rabbit incisor labial dentin were globular (Fig. 3). They were small (8 to 15 μm) compared with those in rat labial dentin and contained 2-9 dentinal tubules. The size of the calcospherites decreased and their shape changed from globular to mulberry-like towards the incisor pulp horn (Fig. 4). In rabbit lingual dentin, the fused calcospherites were ridge-like (Fig. 5) and were smaller (0.9 to 3 μm) than those in rabbit labial dentin and rat lingual dentin. These calcospherites accumulated in the direction of the longitudinal axis of the tooth, their shape changed from ridge-like to oval (Fig. 6), and their size increased (1.5 to 4 μm) towards the incisal pulp horn region. The calcospherites' shapes also varied from the incisal pulp horn to the root apex (Table 1). In the labial dentin the calcospherites changed from globular to mulberry-like and at the incisal pulp horn, moreover, the calcospherites appeared as very small granules (0.3-0.9 μm). In the lingual dentin the calcospherites changed shape from small and granular to oval, and at the incisal pulp horn they resembled small granules.

Observation using backscattered electron images

Rat incisor dentin. Backscattered electron images (BEI) were remarkably similar to microradiography images. The mineralization front in the labial dentin was visualized clearly and had an undulating appearance (Fig. 7), with fused calcospherites. The peritubular dentin was highly calcified, and poorly calcified interglobular dentin occurred sporadically. Globular calcospherites (6 to 20 μm in size) were present in the predentin (Fig. 8), and there were Tomes' fibers (odontoblastic processes) in the dentinal tubules.

The mineralization front of rat lingual dentin appeared to consist of combined linear and globular forms (Fig. 9). The calcospherites in the predentin were flat compared with those in the labial dentin, they were not differentiated clearly from the surrounding tissue, and were arranged parallel to the longitudinal axis of the incisor.

Rabbit incisor dentin. The backscattered electron image showed the mineralization front in the labial dentin of rabbit incisor clearly (Fig. 10). It was wavy, similar to that in rat labial dentin. The calcospherites were slightly smaller than those of rat incisors.

The mineralization front of rabbit lingual dentin was interrupted by calcospherites (Fig. 11). The calcospherites in the predentin were flat, smaller than those of rat lingual and rabbit labial dentin, and were arranged parallel to the longitudinal axis of the incisor.

	Root apex ←			→ Incisal pulp horn
Labial:	large globular ↔	small globular ↔	mulberry-like ↔	very small granules
Lingual:	small granular ↔	ridge-like ↔	oval ↔	very small granules

Table 1. Shape of calcospherites at the mineralization front of rabbits.

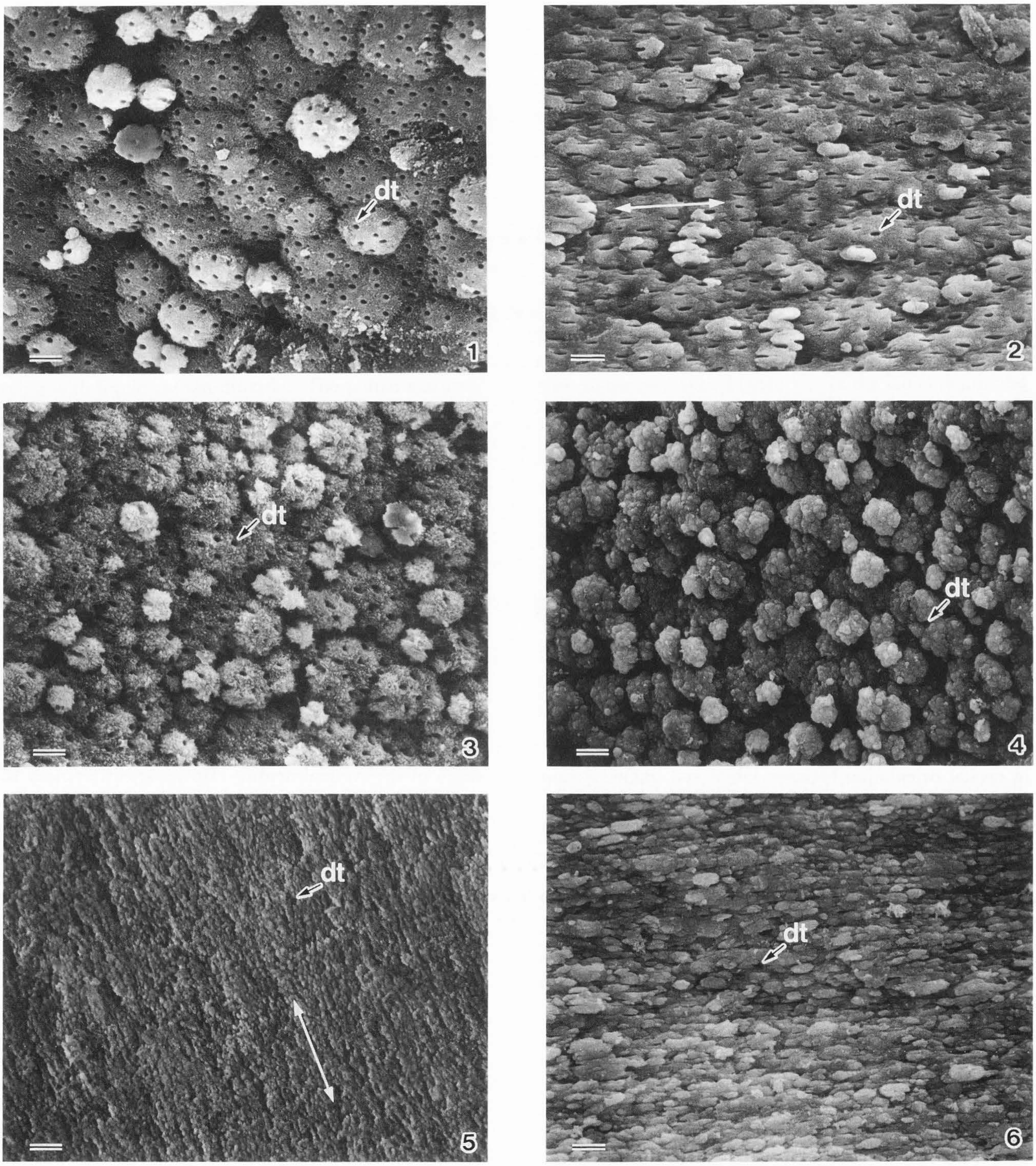
Discussion

Secondary and backscattered electron images of incisor dentin showed highly calcified globular calcospherites in rat labial predentin and a wavy mineralization front. In rat lingual predentin large round calcospherites did not develop; they were small and oval, and the lingual mineralization front appeared to consist of combined linear and globular forms. The shape and size of rabbit incisor calcospherites varied from the incisal pulp horn to the root apex. There were great differences between rats and rabbits with respect to the size and shape of calcospherites at the mineralization front.

Mishima and Sakae [10] reported that the difference in crystal orientation between labial and lingual dentin was due to the different patterns of dentinal calcification, Kawasaki [8] reported that the dentinal mineralization front showed three different configurations: linear, globular and linear/globular, and Fujita [5] stated that globular calcification occurred at sites showing the most rapid growth, whereas linear calcification occurred at sites with the slowest growth. Mishima *et al.* [12, 13] reported that the structures of labial and lingual dentin in the rat incisor differed. In labial dentin, calcospherites and interglobular dentin were observed clearly; the collagen fiber arrangement and the crystal orientation were irregular and calcification was predominantly globular. In lingual dentin, however, incremental lines were well developed, the arrangement of the collagen fibers and the crystal orientation were regular and calcification was predominantly linear. Mishima *et al.* [11] demonstrated the mineral content of rat and rabbit dentin by color images, which were taken from microradiograms of transverse ground sections with a PIAS-II image processor (PIAS Inc., Osaka). In rat and rabbit incisors the mineral content of the labial dentin (40-48 vol%) was 3-9 vol% higher than that of the lingual dentin (31-45 vol%). The mineral content of the calcospherites was 46-48 vol%, and that of the interglobular dentin was 40-45 vol%. Fujita [5] reported that calcospherites, observed by light microscopy, were not present in regions of linear calcification. However, our observation with scanning electron microscopy showed that calcospherites were always present in the calcification front, irrespective of whether the dentinal calcification pattern was globular or linear. It can be assumed, therefore, that there is a correlation between the shape of the mineralization front and the calcospherite size.

Schmidt and Keil [15] examined calcospherites by polarizing microscopy and found that the morphology differed according to animal species, and that the size and shape of calcospherites differed depending on the depth of the coronal dentin. However, differences between calcospherites in labial and lingual dentin of rat and rabbit incisors were not observed in the study. This may have been because labial and lingual dentin were not differentiated and it was assumed that incisor dentin, which shows continuous growth, is a uniform structure. Furthermore, there is little information available about differences between the dentinal structure of rats and rabbits. The results of our study show that there are extreme differences between rats and rabbits with respect to the size and shape of calcospherites in the lingual mineralization front. This may be a species difference and probably is related to the rate of dentinogenesis [7, 14].

The size and shape of predentinal calcospherites differ from region to region in the incisors of rats and rabbits. Interglobular dentin and poorly defined incremental markings are correlated with large calcospherites. The change of size, shape and formation conditions of calcospherites appear to depend on the position and stage of the life cycle of odontoblasts.



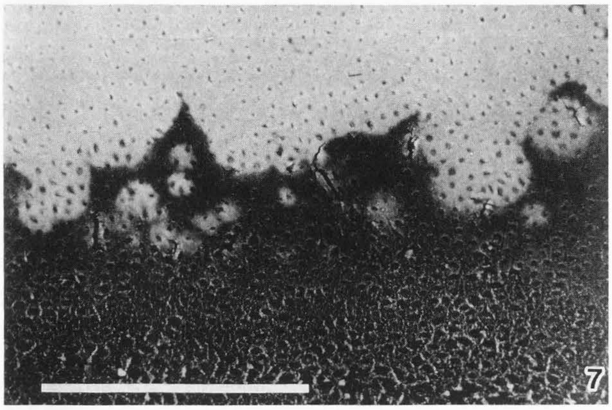
Figs. 1-4 (at left). SEM secondary electron images of anorganic mineralizing front of labial (Figs. 1, 3, 4) and lingual dentin (Fig. 2) from a rat (Figs. 1, 2) and rabbit (Figs. 3, 4); dt: dentinal tubules; bars = 10 μm.

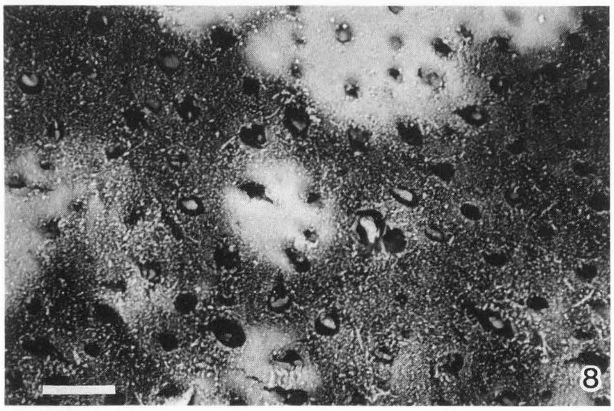
Fig. 1. The calcospherites are large and globular. Intermediate region. **Fig. 2.** The calcospherites are small and oval and arranged parallel to the longitudinal axis of the incisor (arrow: the long axis of the incisor). Intermediate region. **Fig. 3.** The calcospherites are globular and small compared with those in rat labial dentin. Root apex. **Fig. 4.** The calcospherites are mulberry-like. The size of the calcospherites decreased and their shape changed from globular to mulberry-like towards the incisor pulp horn. Incisal pulp horn.

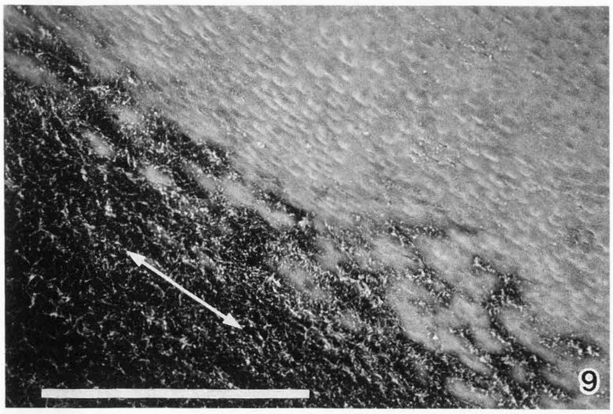
Figs. 5-6 (facing page, bottom). SEM secondary electron images of anorganic mineralizing front of lingual dentin from a rabbit; dt: dentinal tubules, bars = 10 μm.

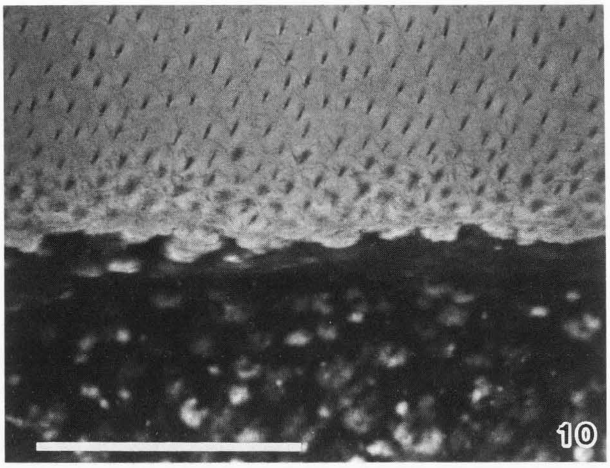
Fig. 5. The fused calcospherites are ridge-like and have accumulated with a parallel arrangement, in the direction of the longitudinal axis of the tooth towards the incisal region (arrow: the longitudinal axis of the tooth). Root apex.

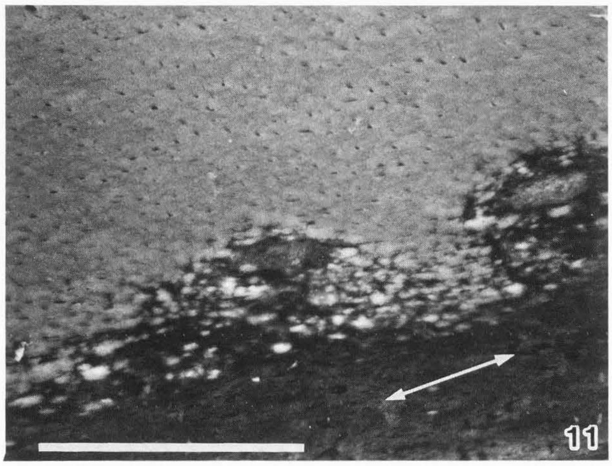
Fig. 6. The calcospherites are oval. The shape of calcospherites changes from ridge-like to oval. Incisal pulp horn.











Figs. 10-11 (above). Labial (Fig. 10) and lingual (Fig. 11) dentin from a rabbit (SEM backscattered electron images). Bars = 100 μm. Intermediate region.
Fig. 10. The mineralization front is wavy with fused calcospherites. **Fig. 11.** The mineralization front is interrupted by calcospherites, which are flat and small and arranged parallel to the longitudinal axis of the incisor (arrow).

Figs. 7-9 (at left). SEM backscattered electron images of labial (Figs. 7, 8) and lingual (Fig. 9) dentin from a rat. Intermediate region. Bars = 100 μm (Figs. 7, 9), and 10 μm (Fig. 8). **Fig. 7.** The mineralization front can be seen clearly and has an undulating appearance. The calcospherites in the predentin are globular and large. **Fig. 8.** Globular calcospherites can be seen in the predentin, and their size ranges from 6 to 20 μm. **Fig. 9.** The mineralization front appears to consist of a combination of linear and globular forms. Flat calcospherites are arranged parallel to the longitudinal axis of the incisor.

Conclusions

In conclusion we have demonstrated:

1. In rats and rabbits the labial mineralization front was wavy and consisted of fused calcospherites, whereas the lingual mineralization front appeared to consist of combined linear and globular forms.

2. The calcospherites in rat labial dentin were large and globular, but they did not develop in the lingual dentin and were small and oval.

3. The shape and size of rabbit incisor calcospherites varied from the incisal pulp horn to the root apex.

4. There were great differences between rats and rabbits with respect to the size and shape of calcospherites at the mineralization front, which may be due to differences between species and differing rates of dentinogenesis.

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Discussion with Reviewers

J.D.B. Featherstone: How did the authors determine the orientation of their specimens in order to make the interpretations about the shape of the calcospherites that they have?

Authors: We observed from a direction perpendicular to the tooth's longitudinal axis. We made sure of the orientation of tooth axis.

J.D.B. Featherstone: How can the authors be sure that the wavy mineralization front that they discuss is in fact

the mineralization front and how can they readily interpret the backscattered electron images?
Authors: Jones and Boyde (1984) reported that a backscattered electron image provided new information about the mineralization front. As compared with secondary electron images, backscattered electron images can clearly show the internal structure of dentin. Backscattered electron images are remarkably similar to microradiography images. The mineralization front in rat labial dentin appeared to be undulated by light microscopy the same as backscattered electron images [12].

J.D.B. Featherstone: How many specimens did the authors look at for each of the conclusions drawn and how can they be sure that the orientation of their specimens were each the same so that the conclusions were valid?
Authors: We observed the incisors extracted from 10 Wistar-strain rats and 5 Japanese white rabbits. We made sure of the orientation of tooth axis and the arrangement of calcospherites with low magnification.

J.D.B. Featherstone: What is the relevance of the present study on rat and rabbit dentin to the human situation? Jones and Boyde [7], for example, compared several species. How does the present study overlap or add to that information?
Authors: It could be argued that labial dentin is comparable to the coronal dentin of the human and lingual dentin, to root dentin. Amelogenesis-free root dentin possibly differs from coronal dentin in the make-up of the organic matrix and calcification formation. Differences between calcospherites in labial and lingual dentin of rat and rabbit incisors were not observed in former studies. Little has been reported on the morphology of calcospherites in rabbit predentin. The results of this study show that the calcospherites in rat labial dentin were large and globular, but they did not develop in the lingual dentin and were small and oval. There were great differences between rats and rabbits with respect to the size and shape of calcospherites at the mineralization front.

R.P. Shellis: By definition, calcospherites are characterized by crystal growth in a radial fashion from a center. What evidence do the authors have that all the particles they observed (especially the elongated ones) have this crystal arrangement and are not simply areas involving only crystal deposition on collagen fibers?
Authors: We have not directly observed the radial crystal arrangement in the calcospherites. We examined the rat incisor dentin by the X-ray micro-Laue method and polarizing light microscopy [10, 12]. In the labial dentin, the crystals showed radial orientation with globular calcification.

D.H. Pashley: The small size of the calcospherites on the lingual surface of the rabbit incisor is remarkable. In most cases, the spherites are smaller than the tubule diameter. Thus, the tubule walls appear to be made up of clusters of 0.9 μm calcospherites. Are the dentinal tubules of the lingual surface of rabbit incisors made up of calcospherite subunits? Does the peritubular dentin of this dentin look different from that of labial incisor dentin?
Authors: Yes, we think that the dentinal tubules of the lingual surface of rabbit incisors are made up of calcospherites subunits. Yes, we think that the peritubular dentin of lingual dentin look different from that of labial dentin. However, we have no data in detail.

D.H. Pashley: Is the difference in calcospherites size between rat and rabbit lingual incisor dentin reflected in the molar dentin of these animals? That is, is it a species difference?
Authors: Yes, we think that the difference in calcospherites size between rat and rabbit lingual incisor dentin is reflected in molar dentin of these animals. It may be due to species differences and possibly the differing rates of dentinogenesis.

D.G.A. Nelson: Do you have any ideas of what causes the nucleation event in the center of the mineralizing calcospherites and how are the nuclei located relative to the positions of the odontoblasts at the time?
Authors: We think that the nucleation event in the center of the mineralizing calcospherites is related to the collagen bound phosphoproteins which Steinfort (1990) reported. We assume that the location of nuclei differed depending on the positions of the odontoblasts.

Additional Reference

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