

# The diameter and number of dentinal tubules in rat, cat, dog and monkey

## A comparative scanning electron microscopic study

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The aim of the study was to investigate in commonly used experimental animals the pulpal wall and the dentin with special reference to the number and diameter of the dentinal tubules. The coronal dentin of a total of 26 permanent, intact teeth from rat, cat, dog and monkey was fractured experimentally. The calculations were based on the study of about 150 scanning electron microscope (SEM) photographs of fractured surfaces and of the pulp chamber wall. The number of dentinal tubules per unit area was calculated and the tubule diameter was measured at various distances from the pulp. The tubules were found to be widest at the pulpal wall, where the mean diameter ranged from 1.7 to 2.8  $\mu\text{m}$ , the highest value being found in monkey and the lowest in the incisors of rat. In the middle part of the dentin the mean diameter varied from 1.0 to 1.3  $\mu\text{m}$  and at the periphery it was 0.6 to 0.9  $\mu\text{m}$ . The largest number of tubules per unit area was found immediately incisal of 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/ $\text{mm}^2$ , in the middle of the dentin it was between 37,000 and 50,000/ $\text{mm}^2$  and at the periphery it was between 10,000 and 25,000/ $\text{mm}^2$ . In all species examined the dentinal tubules showed a straight course, except in incisors of rat, where local irregularities were seen. A comparison was also made between the species studied and homo with respect to the number and width of the dentinal tubules.

*Key-words:* Dentin; rats; cats; dogs; monkeys

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The dentinal tubules play an important role in transferring stimuli and irritants to the pulp. By this route, and by diffusion in the liquid filling the tubules, various therapeutic agents applied locally to exposed dentin may also reach the pulp. The rate of transport of such agents to

the pulp is dependent *inter alia* on the number and size of the dentinal tubules. Experimental studies on pulp reactions following the application of various agents to dentin are frequently carried out on animals. However, little is known about the number and diameter of the dentinal

tubules in such animals. The aim of the present study was to investigate the pulpal wall and the dentin in rat, cat, dog and monkey, with special reference to the number and diameter of the dentinal tubules.

#### MATERIAL AND METHODS

The material consisted of 26 teeth with completed root development from young adult animals — incisors and molars from rat, permanent canine teeth from cat and dog and upper incisors from monkey. The teeth were extracted immediately after a lethal dose of pentobarbital. Some were placed in 10 per cent formalin whilst awaiting further treatment, others were prepared for fracturing immediately after extraction. In order to produce a longitudinal fracture surface through the centre of the tooth, a groove was cut in the bucco-lingual direction from the lingual surface, leaving the buccal surface intact. The groove was made with an edge-cutting diamond wheel with constant water cooling. A scalpel blade was put in to the groove and the tooth was divided into two by a gentle blow with a hammer. Transverse sections were made in a similar way at different distances from the top of the crown. After longitudinal fracturing, teeth in which the pulpal walls were to be examined were washed for a few seconds in 35 per cent hydrogen peroxide, except for a few specimens which were placed in 10 per cent sodium hypochlorite for 24 hours (monkey, tooth previously fixed) or in 0.5 per cent sodium hypochlorite for 24 hours (rat, cat, dog). All specimens were washed in water and then placed in 10 per cent formalin.

The surface of the fracture was covered in *vacuo* with a film of gold about 200 Å thick and then examined in a Cambridge

scanning electron microscope at an accelerating voltage of 20 kV. Photographs were taken of corresponding areas of the different specimens examined. About 150 pictures were taken, usually at a magnification of about 1,000 to 7,000, of the pulp chamber wall and the fractured dentin at various distances from the pulp. The diameter of the tubules was measured on pictures of transverse sections, using an instrument developed by R. Ivansson which permits measurements to an accuracy of ca 0.1 μm. The mean diameter of 8—20 tubules was calculated from each picture.

In order to minimize the measurement error which may arise when using low magnifications in scanning electron microscopy, the calculations of the diameters were based on observations on pictures with a magnification of over 2,000. Pictures of transversely fractured tubules were also used when calculating the number of tubules per square millimetre. If the magnification revealed a large number of tubules, the calculation was facilitated by covering the photo with a plastic film on which the tubules could be marked off as they were counted. From a knowledge of the magnification, the area photographed was estimated in square millimetres and the number of dentinal tubules/mm<sup>2</sup> in such an area was calculated according to the following formula:

$$X = \frac{1,000,000 \cdot n}{\left(\frac{l}{i}\right)^2}$$

where: X = number of tubules/mm<sup>2</sup>

n = number of tubules on the picture

l = length of side of picture, μm

i = magnification

If the picture side length is kept constant

at 100,000  $\mu\text{m}$  the following simplified formula can be used:

$$X = \frac{i^2 \cdot n}{10,000}$$

The percentage of the area occupied by tubules was calculated using the following formula:

$$\frac{\pi \cdot r^2 \cdot n}{10,000}$$

where:  $n$  = number of tubules/ $\text{mm}^2$   
 $r$  = tubule radius in  $\mu\text{m}$

RESULTS

*Tubule diameter*

In all the teeth studied the largest tubule diameter was found at the pulpal wall. At this location the mean diameters ranged from 1.7 to 2.8  $\mu\text{m}$ , the highest value being found in monkey, the lowest in rat incisor (Figs. 1 & 2). The width of the tubules decreased markedly 50—100  $\mu\text{m}$  from the pulp, which corresponds

to the level of peritubular dentin development. From this level the diameter gradually decreased towards the dentin-enamel junction (Figs. 3—6). Half-way between the pulp and the enamel the mean diameter varied from 1.0 to 1.3  $\mu\text{m}$  and near the enamel it varied from 0.7 to 0.9  $\mu\text{m}$  (Figs. 4—6). The mean diameters and ranges at various distances from the pulp are shown in Table I. In all species examined the greatest variability in tubule diameter was seen at the pulpal wall. The tubules located at the most incisal part of the dentin had a lower average diameter than those in the cervical part and showed a greater mutual variation, especially in areas near the pulp. Further from the pulp the diameter of the tubules was more uniform (Fig. 9), except for the dentin of rat incisors in which ramifications could also be seen extending into the inter-tubular dentin (Fig. 8). However, the structure of the dentin in rat molars did not seem to differ from that of the dentin in the other species examined (Fig. 7).

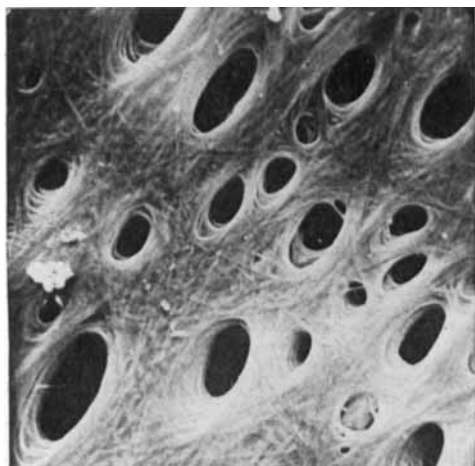


Fig. 1. Rat incisor. Pulpal wall at half the height of the crown. The wall is covered by a membranous structure containing fibres. The number of tubules was calculated to be about 73,000/ $\text{mm}^2$ . Mean diameter: 1.4  $\mu\text{m}$ . Magn.  $\times 3,350$ .

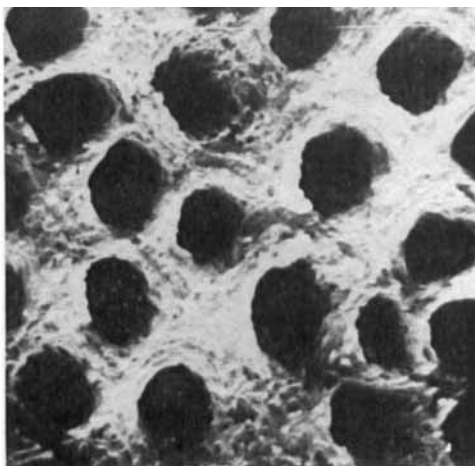


Fig. 2. Monkey incisor. Pulpal wall. The membranous structure present in Fig. 1 has been removed by treatment with 10 % sodium hypochlorite for 24 hours. The number of tubules was calculated to be about 69,000/ $\text{mm}^2$ . Mean diameter: 2.7  $\mu\text{m}$ . Magn.  $\times 3,590$ .

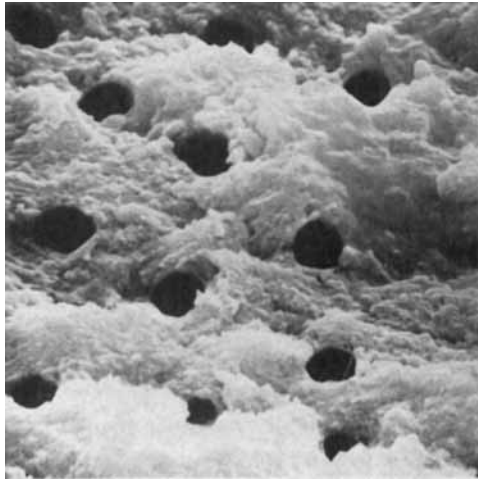


Fig. 3. Cat canine tooth. Cervical third of the crown, about 100  $\mu\text{m}$  from the pulpal wall. Note the uniformity in canal width. The number of tubules was calculated to be about 58,000/ $\text{mm}^2$ . Mean diameter: 1.2  $\mu\text{m}$ . Magn.  $\times$  4,600.

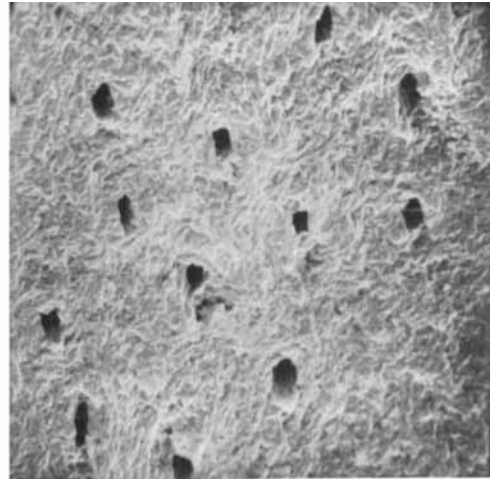


Fig. 4. Cat. Incisal third about half-way between the pulp and the enamel. The number of tubules was calculated to be about 45,000/ $\text{mm}^2$ . Mean diameter: 0.6  $\mu\text{m}$ . Magn.  $\times$  3,810.

Table I. Tubule diameter at various distances from the pulp in the species examined (mean values and ranges)

Distance from pulp	Tubule diameter in $\mu\text{m}$				
	Rat inc	Rat mol	Cat	Dog	Monkey
Pulpal wall	1.7 (0.4—2.7)	2.0 (1.3—2.7)	2.1 (1.6—3.0)	2.1 (1.4—3.6)	2.8 (2.1—3.1)
50—100 $\mu\text{m}$ from the pulp	1.1 (0.8—1.5)	1.3 (0.9—1.5)	1.2 (0.7—1.8)	1.9 (1.3—2.3)	1.7 (1.5—2.1)
Half-way between pulp and enamel	1.0 (0.6—1.5)	1.1 (0.8—1.4)	1.0 (0.5—1.7)	1.3 (0.8—1.9)	1.1 (0.7—1.7)
Inside enamel junction	0.7 (0.5—1.1)	0.8 (0.5—1.3)	0.7 (0.3—1.0)	0.9 (0.5—1.2)	0.9 (0.5—1.4)

Table II. Number of tubules/ $\text{mm}^2$  at various distances from the pulp in the species examined (mean values and ranges)

Distance from pulp	Number of tubules, 1000/ $\text{mm}^2$				
	Rat inc	Rat mol	Cat	Dog	Monkey
Pulpal wall	51 (28—73)	64 (44—78)	50 (35—69)	90 (76—105)	51 (35—69)
50—100 $\mu\text{m}$ from the pulp	53 (47—60)	80 (52—104)	59 (53—87)	74 (71—77)	47 (45—50)
Half-way between pulp and enamel	50 (45—55)	49 (39—62)	41 (37—45)	43 (35—56)	37 (24—50)
Inside enamel junction	16 (14—19)	25 (25—26)	25	24	10

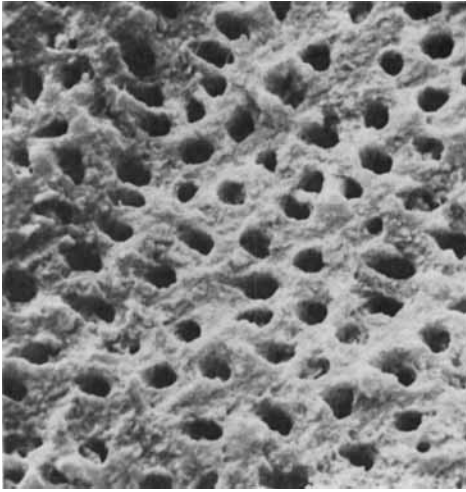


Fig. 5. Dog. Incisal part of the crown. About 300  $\mu\text{m}$  from the pulpal wall (total distance pulp-enamel 1.8 mm). The number of tubules was calculated to be about 77,000/ $\text{mm}^2$ . Mean diameter: 1  $\mu\text{m}$ . Magn.  $\times 1,830$ .



Fig. 6. Dog. Incisal part of the crown near the dentin-enamel junction. The number of tubules was calculated to be about 24,000/ $\text{mm}^2$ . Mean diameter: 0.9  $\mu\text{m}$ . Magn.  $\times 1,740$ .

*Number of tubules*

The largest number of tubules per unit area was found in the incisal part of the crown in the region of the pulpal wall and the smallest number was found cervically, near the enamel (Figs. 5 & 6). This was

true for all teeth except rat incisors, in which the number of tubules in the incisal dentin was slightly smaller than in the dentin of the middle part of the crown. In all teeth examined the number of dentinal tubules per square millimetre was

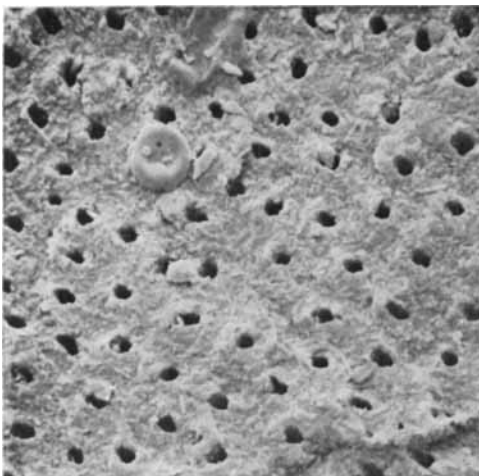


Fig. 7. Rat molar. About half-way between the pulp and the enamel. Notice the erythrocyte in the upper left quadrant. The number of tubules was calculated to be about 50,000/ $\text{mm}^2$ . Mean diameter: 1.2  $\mu\text{m}$ . Magn.  $\times 1,575$ .



Fig. 8. Rat incisor. Middle part of the crown. Longitudinally fractured tubules with ramifications. Magn.  $\times 1,130$ .

Table III. *Tubule diameter, number of tubules/mm<sup>2</sup> and percentage of the area occupied by tubules in different species — mean values half-way between the pulp and the enamel. The human values are from unpublished data of Garberoglio and Brännström*

Type of tooth	Diameter $\mu\text{m}$	Number/mm <sup>2</sup>	Percentage of the area occupied by tubules
Rat, inc	1.0	50,000	3.9
Rat, mol	1.1	49,000	4.6
Cat	1.0	41,000	3.2
Dog	1.3	43,000	5.7
Monkey	1.1	37,000	3.5
Human, young	1.4	30,000	4.6
Human, old	1.1	29,000	2.8

highest and showed the greatest variation at the pulpal wall or immediately inside the predentinal zone. Half-way between the pulp and the enamel the mean number of tubules varied from 37,000 to 50,000/mm<sup>2</sup> and close to the enamel it varied from 10,000 to 25,000/mm<sup>2</sup> (Table II). The dentinal tubules of the cat, dog, monkey and rat molar seemed to have a fairly straight course, while the tubules in the dentin of rat incisors exhibited local irregularities (Figs. 8 & 9).

#### *Comparison between present material and human values*

It was of interest to compare the values from human undecalcified dentin with those obtained in the present study. Table III shows the mean values for the tubule diameter and the number of tubules per unit area, as well as the percentage of the area occupied by tubules half-way between the pulp and the enamel in human dentin and in that of the experimental animals examined.



Fig. 9. Monkey. Middle part of the crown. Longitudinally fractured tubules. Magn.  $\times 1,280$ .

#### DISCUSSION

The data obtained from measurements of this type are subject to certain errors, which may influence the mean values of the number of tubules, as well as the tubule diameter. Some of the errors such as the magnification value and the tilt of the area studied, are due to the SEM procedure, others are due to difficulties in defining precisely the diameter of the tubules in the pictures.

The principal object of the present investigation was to compare the width and number of dentinal tubules in a number of commonly used experimental animals and to relate the findings to the situation in man. Since all the specimens were examined in the same way the errors inherent in the methodology will tend to cancel each other out.

In order to obtain reliable information about the dentinal tubules it is of importance that the examination be performed on dentin unaffected by decalcification. In a study of human decalcified dentin *Hoppe & Stüben* (1965) found

tubule diameters that were larger than those reported for undecalcified dentin (Fromme & Riedel, 1970; Brännström & Garberoglio, 1972; Tronstad, 1973; Garberoglio & Brännström, unpublished data). One reason for this discrepancy seems to be that decalcification causes an almost complete removal of peritubular dentin (Kubota *et al.*, 1969; Isokava, Toda & Kubota, 1970; Garberoglio & Brännström, 1972; Brännström & Johnson, 1974).

It was not surprising that the tubule width varied at the pulpal wall lined with predentin. Cytoplasmic structures and membranes seem to be preserved in freeze-dried specimens (Brännström, Garberoglio & Johnson, 1974). When formalin-fixed specimens are dehydrated *in vacuo* these structures seem to stick to the pulpal wall. Therefore, in some places it was difficult to estimate the diameter of the tubules. When most of the predentin was removed using sodium hypochlorite, the width of the tubules tended to be more uniform and probably gave a more reliable estimate of the diameter of dentinal tubules in this area (Fig. 2). Organic material coating the pulpal wall also interfered when the number of tubules was counted, since some tubules were completely covered.

The human values, shown in Table III, have been obtained from a study by Garberoglio & Brännström (unpublished) in which the method used was essentially the same as that used in the present study. It is obvious that the values for the animal dentin do not differ very much from those for human dentin. Only the rat incisor dentin revealed irregularities and ramifications to an extent that has not been observed in human dentin or in the other species examined in the present study. When ramifications have been observed in human dentin they have been confined

to the area near the enamel (Brännström & Garberoglio, 1972).

The results of the present study thus show that the diameter and number of dentinal tubules in the species investigated and in man are similar. It is well known that the transport of various agents and the liquid bulk flow in dentin is dependent on the existence of patent dentinal tubules. Therefore, the permeability of dentin, which is dependent on the dentinal tubules, may be similar in rat, cat, dog, monkey and man. Thus, the results of physiological and histological experiments concerning the permeability of the dentin and pulp reactions, carried out on these animals might also be valid for human dentin and pulp.

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