Cell sizes and apposition of dental hard tissues in rats

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The daily apposition of the enamel and dentin and the sizes of ameloblasts and odontoblasts in the first upper molar of 2- to 9-day-old and of 12-day-old rats were examined by light microscopy of freeze-dried sections. The formation of enamel in the first molar started at the age of 2 days and was completed in most of the analyzed areas at the age of 9 days. On each day the enamel on the mesial sides of the cusps was about twice as thick as on the distal sides. The differences in thickness of dentin between the mesial and distal sides of the cusps were not as marked as was seen for the enamel. However, the rapid formation of dentin seemed to continue longer on the mesial sides than on the distal sides. The height of the ameloblasts and odontoblasts showed similar differences between the mesial and distal sides as was seen in the formation of enamel and dentin. In addition, these cells showed a gradual increase and decrease in height during the observation period, with a maximal height in the analyzed area at the age of 4–6 days. □ *Ameloblast; dentin; enamel; growth rate; odontoblast*

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Disturbances of the enamel formation seem to be localized to special parts within the dentition. Thus, the prevalence of idiopathic enamel opacities is more frequent on the upper first molars and on the upper central incisors than in the premolars and the canines (1). Furthermore, the buccal surfaces of the teeth seem to be more affected than the lingual and occlusal ones (1). The buccal surfaces also seem to be more severely affected than the lingual surfaces in certain types of amelogenesis imperfecta (2) and in dental fluorosis (3–5). The mechanism behind these preferential localizations is essentially unknown. The localization of dental fluorosis has been suggested to be related to the thickness of the enamel (3). A recent study by Gohdo (6) may support this assumption. He found that the buccal surfaces of the central incisors were thicker than the lingual sides.

The rat incisor is the most commonly used model in investigations of the development of dental tissue. It grows continuously, which means that the entire sequence of enamel and dentin development and the whole life cycle of the ameloblasts and odontoblasts may be studied in one tooth (7–14). In contrast to the rat incisor, the rat molar has a limited growth and is in this respect more similar to the human tooth. The orientation of the outer prism layer is also more like the human tooth in the molar tooth than in the incisor (15). There are few studies concerning growth of the rat molar (16–20). As part of an experimental study of developmental disturbances of rat molar teeth we therefore found it of interest to analyze the growth pattern of the molar tooth.

Materials and methods

Five litters of Sprague-Dawley rats were used for the experiment. One rat, taken at random from each litter, was weighed and killed at 2, 3, 4, 5, 6, 7, 8, 9, and 12 days of age. All rats were killed by decapitation at the same time of day. The heads of the rats were immediately embedded in a mixture of carboxymethyl cellulose and water on a microtome stage and rapidly frozen by immersion in hexane cooled with solid CO_2 (−75°C). At the level of the first upper molar, 20-μm-thick (20–40 μm apart) sagittal sections through the heads of each rat were taken in accordance with the method of Ullberg (21, 22). The sections were stabilized during sectioning by using Scotch tape (3M No 437/38). The use of frozen sections attached to an adhesive tape should minimize the shrinkage artefacts.

The rat molar has three major cusps and an enamel-free area at the tip of each cusp. To make a meaningful measurement of the growth of the dental hard tissues in the rat molar, it is of ultimate importance to measure reproducibly in defined areas. Thus, care was taken to obtain sections from the central parts of the teeth. The 'enamel-free' areas at the tips of the cusps were utilized for the orientation, and the sections included the second molar as well. The errors induced by oblique sectioning in the sagittal direction should thus be minimal.

After being freeze-dried, the sections were brought to room temperature in an air-tight box to prevent moisture from condensing on the sections. From every rat five freeze-dried sections were selected and projected onto a screen by means of a microprojector. The projection was arranged so that the magnification was 100 times. On the projected picture a line was drawn through the central part of the 'enamel-free' areas of the mesial and distal cusps and a second line through the dentino-enamel junction at the bottom of the two fissures (Fig. 1A). A third line was drawn between these two lines and at equal distance from each of them. At the points where the third line crossed the dentino-enamel junction separate lines were drawn perpendicular to the dentino-enamel junction. The thickness of enamel and dentin and the height of the ameloblast and odontoblast layers were measured on these lines (Fig. 1B).

The results obtained of the cell sizes are affected by deviation in cell direction from this line. The deviation of the ameloblasts or odontoblasts was estimated. It never deviated more than about 20° in the occluso-cervical direction. Deviation perpendicular to the sectioning plane was minimized by selecting only those sections in which the ameloblast nuclei appeared as a narrow line. No correction was made for any deviation of the cells, and thus the values obtained may be regarded as relative values. The measurements were made with a vernier callipers. The border lines of the enamel, dentin, and ameloblasts were nearly always clearly distinguishable. The odontoblasts sometimes had a more undefined boundary, which may have impaired the measurements. The daily apposition rates were calculated from the daily figures obtained on the thickness of enamel and dentin.

The mean values of the measurements from the five sections from each rat at 2–9 and 12 days of age were used for statistical analysis. The method used was an analysis of variance four-way ANOVA with the fix factors cusp (medial, mesial, and distal), side of cusp (mesial, distal), age, and the random factor litter. The variables enamel, dentin, ameloblast, and odontoblast were analyzed separately. The statistical method has been

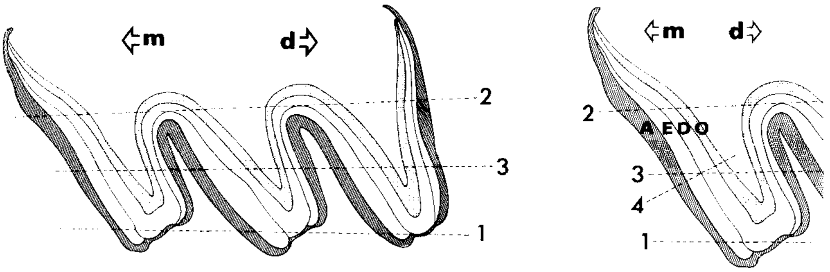

Fig. 1A. Sketch of the first upper molar. Line 1 is drawn through the center of the enamel-free areas of the mesial and distal cusps. Line 2 is drawn through the dentino-enamel junction at the deepest part of the fissures. Line 3 is drawn with equal distances to lines 1 and 2. m = mesial; d = distal. Fig. 1B. Sketch of the mesial cusp. Line 4 is drawn perpendicular to the dentino-enamel junction through the point where line 3 intersects this junction. The measurements of the thickness of enamel and dentin and the heights of the cells are made along line 4 and the corresponding lines on the other cusps. A = ameloblast; E = enamel; D = dentin; O = odontoblast.

described by Kirk (23). The values in Tables 1–4 are mean values ± SD of rats from the five litters.

Results

The enamel formation in the first upper molar started at the age of about 2 days. The apposition of the enamel on the mesial sides of the upper molar started slightly earlier than that on the distal sides, and the apposition rate of enamel varied from day to day, but it was nearly always higher on the mesial sides than on the distal sides (Figs. 2 and 3, Table 1). The highest measured daily apposition on the mesial sides was 41 μm and on the distal sides 23 μm. At every age and on all three cusps the differences between enamel thickness on the mesial and distal sides were statistically significant ($p < 0.001$). However, the magnitude of these side differences varied among the different cusps. At 9 days of age the enamel had almost reached its final thickness in the analyzed area, and there was a slight increase in thickness between 9 and 12 days of age. The differences between the mesial and distal sides of the cusps still remained at the last observation occasion, and the last recorded thickness of the enamel on the mesial sides was about twice that of the distal sides. There was no obvious difference in the duration of growth time between the enamel of the mesial and distal sides.

The sizes of the ameloblasts were in general related to the apposition of the enamel (Fig. 3, Table 2). The ameloblasts were short at the start of enamel formation and increased in size when the secretion of enamel matrix increased. When the enamel formation decreased, there was also a decrease in the height of the ameloblasts. The ameloblasts on the distal sides were only about half the height of those on the mesial sides. The largest measured height was 109 μm and occurred on the mesial side medial cusp at 4 days of age. The corresponding value on the distal side of the same cusp was 60 μm. The differences between mesial and distal sides ($p < 0.001$) were present on all the three cusps. As for the enamel, these side differences varied among the three cusps. At the end of the enamel secretion the ameloblasts on the mesial sides were reduced to 50–60% of their maximum height, but they seem to increase again, and at the age of 12 days the ameloblasts in the analyzed area were about 70% of the maximum height.

At 2 days of age the dentin formation had started on both the mesial and distal sides of the cusps of the first upper molar. The dentin increased more in thickness on the mesial sides than on the distal sides ($p < 0.001$) (Figs. 4 and 5, Table 3). However, the apposition rate varied from day to day, and there was no obvious culmination of dentin formation. It continued for a longer period on the mesial sides of the cusps than on the distal sides, and consequently the differences in dentin thickness were more pronounced at the end than at the start of the observation period.

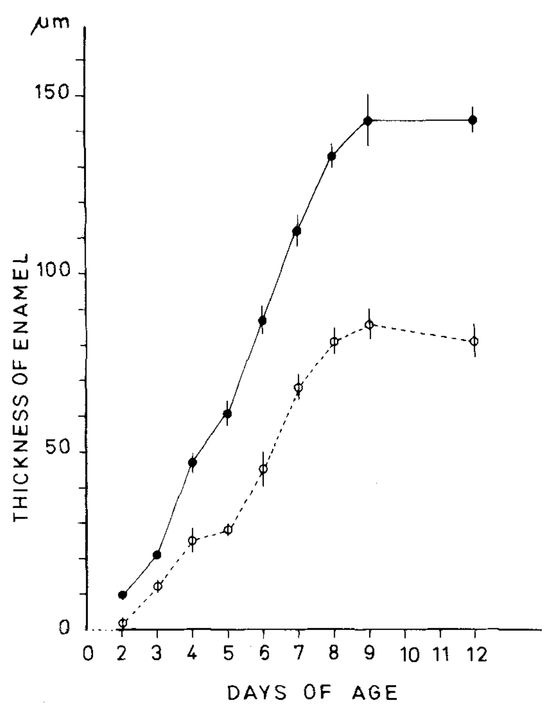

Fig. 2. Thickness of the enamel on the mesial (—●—) and distal (– –○– –) sides of the medial cusps of the first upper molar of rats 2–12 days old. The values are mean values from Table 1.

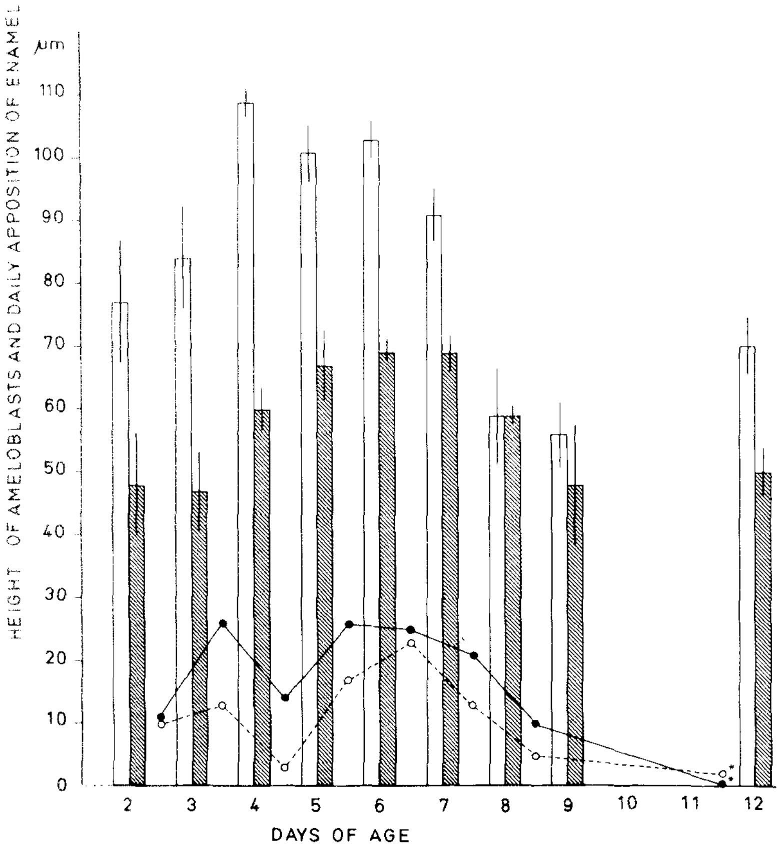

Fig. 3. Histogram showing height of the ameloblasts on the mesial (open bars) and distal (hatched bars) side of the medial cusp of the first upper molar from rats 2–12 days of age (Table 2). The curves show the daily apposition on the mesial (—●—) and distal (- -○- -) sides of the cusp (calculated from Table 1). (*The difference in apposition between days 9 and 12 was calculated and divided by three.)

The sizes of the odontoblasts showed a relation to the dentin formation, since they on all cusps were taller on the mesial sides than on the distal sides ($p < 0.001$) (Fig. 5, Table 4). The odontoblasts were short at the start of the dentin formation and increased in size during the observation period. At 7 days of age the maximum height of the odontoblasts, 97 µm, was observed on the mesial side distal cusp. The corresponding value on the distal side was 63 µm.

There were differences in weight between the rats at the same age (Table 5), but these differences were not related to the differences in enamel and dentin thickness that existed between the litters.

Discussion

Our knowledge about the development of teeth with limited growth is meager. This is mainly because instead of the rat molar the rat incisor with its continuous growth has been the most commonly used model in investigations about odontogenesis (7–14). In the present study the first part of the postnatal molar odontogenesis was examined. There are several difficulties in measuring the hard tissue formation and the cell height of the rat molar tooth. Our efforts to minimize the errors have been described in Materials and methods. In addition, the results may have been influenced by variation among the rats in the prenatal period. This period may vary from 22 to 23 days (24).

In the present study the enamel formation in the first upper molar started around 2 days after birth, and during the observation period, 2–9 days of age, the ameloblasts in the area studied were mainly in their secretory stage. At 12 days of age the ameloblasts

Table 1. Thickness of the enamel (in μm) on the mesial and distal sides of the three cusps on the first upper molar of rats 2–12 days of age. Each value represents the mean value ± SD of five rats

Cusp	Side of cusp	Days of age: 2	3	4	5	6	7	8	9	12
Mesial	Mesial	2 ± 2.3	8 ± 4.9	25 ± 3.2	32 ± 4.3	53 ± 13.0	86 ± 8.0	112 ± 10.3	134 ± 10.3	140 ± 9.6
	Distal	0	2 ± 3.5	7 ± 4.2	13 ± 2.7	24 ± 6.9	40 ± 5.5	58 ± 4.7	63 ± 6.3	62 ± 4.5
Medial	Mesial	10 ± 1.5	21 ± 1.7	47 ± 6.2	61 ± 7.2	87 ± 8.3	112 ± 9.8	133 ± 7.1	143 ± 13.8	143 ± 6.8
	Distal	2 ± 2.5	12 ± 4.6	25 ± 6.9	28 ± 3.2	45 ± 9.9	68 ± 7.6	81 ± 7.4	86 ± 8.9	81 ± 9.3
Distal	Mesial	8 ± 2.9	19 ± 7.2	39 ± 3.4	53 ± 13.4	82 ± 14.2	123 ± 8.2	129 ± 8.1	137 ± 12.0	131 ± 7.1
	Distal	0	3 ± 3.0	13 ± 9.8	17 ± 4.0	30 ± 7.3	44 ± 2.9	55 ± 8.6	56 ± 6.2	67 ± 11.7

Table 2. Height of ameloblasts (in μm) on the mesial and distal sides of the three cusps of the first upper molar of rats 2–12 days of age. Each value represents the mean value ± SD of five rats

Cusp	Side of cusp	Days of age: 2	3	4	5	6	7	8	9	12
Mesial	Mesial	60 ± 22.3	68 ± 6.2	83 ± 6.4	87 ± 13.0	101 ± 9.6	98 ± 12.8	81 ± 9.4	74 ± 16.4	71 ± 6.6
	Distal	41 ± 16.9	35 ± 6.2	47 ± 5.8	52 ± 16.2	55 ± 7.5	55 ± 10.0	48 ± 6.4	40 ± 12.4	47 ± 5.3
Medial	Mesial	77 ± 19.0	84 ± 16.6	109 ± 4.5	101 ± 8.7	103 ± 5.8	91 ± 8.4	59 ± 15.2	56 ± 10.3	70 ± 9.2
	Distal	48 ± 15.7	47 ± 12.6	60 ± 6.4	67 ± 10.8	69 ± 3.8	69 ± 5.1	59 ± 2.7	48 ± 19.0	50 ± 7.6
Distal	Mesial	88 ± 19.6	90 ± 8.8	95 ± 7.2	106 ± 10.2	103 ± 6.8	90 ± 18.0	59 ± 11.7	54 ± 13.6	71 ± 11.6
	Distal	38 ± 8.0	36 ± 13.0	45 ± 10.8	43 ± 20.0	48 ± 6.0	49 ± 14.5	42 ± 9.1	42 ± 16.6	51 ± 3.5

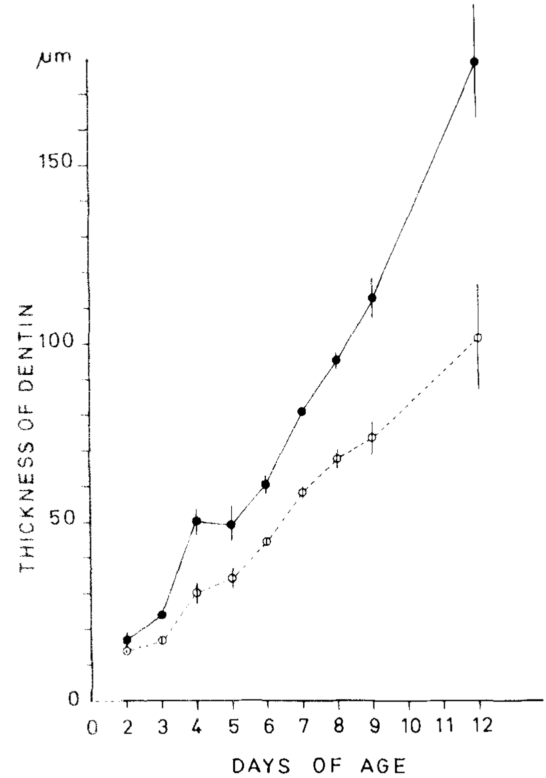

Fig. 4. Thickness of the dentin on the mesial (—●—) and distal (- -○- -) sides of the medial cusp of the first upper molar from rats 2–12 days old. The values are mean values from Table 3.

had reached the maturation phase. The enamel secretion was reduced from 8 days of age, and the thickness of enamel at 9 days of age was about the same as at 12 days of age. The observations of Kurahashi et al. (18) on the first upper molar revealed a similar development, with start of enamel formation at day 2 after birth and a completion of enamel matrix formation at 9–11 days of age. The duration of the enamel secretion period in the continuously growing rat incisor seems to be of about the same length (8–9 days) (13).

There are no studies of the enamel apposition rate in the molar, but the apposition of enamel in the rat incisor has in earlier studies been found to be 16 μm/day (11). In our study a similar average apposition rate (19 μm) on the mesial sides of all cusps was found in rats at 2–9 days of age, but on the distal sides the average daily rate was only 10 μm. However, it should be kept in mind that there were great variations from day to day. At 12 days of age the thickness of the enamel was slightly less than at 9 days of age. This may be due to individual variations, or there may be an actual decrease in enamel thickness. It has recently been shown that the postsecretory modulation of the ameloblasts is associated with changes of the enamel surface (25, 26).

In several studies the different functional stages of the ameloblasts were shown to be correlated with differences in their size (9, 10, 12–14, 20, 27). The two major stages of ameloblast function are usually described as 'tall, secretory' ameloblasts and 'short, postsecretory' ameloblasts. The findings of the present investigation are essentially in accordance with these previous observations. However, it also showed that there are marked changes in cell size within these two main functional stages. Thus, the secretory ameloblasts at 6 days of age were about twice as high as the ameloblasts at the start of matrix formation. These changes seemed to be associated with changes in secretory rate. The height of ameloblasts also varied during the postsecretory stage. Thus, they were lower immediately after the completion of matrix formation than 3 days later. This resulted in the appearance of postsecretory ameloblasts that were taller than some secretory ameloblasts. A possible explanation for the different sizes of postsecretory ameloblasts may be two different appearances of these cells during the enamel maturation stage (8, 9, 14, 27, 28).

The dentin formation had started before our observation period began at 2 days of age. Earlier studies have shown a beginning dentin apposition in the first molar perinatally (17, 18). The dentin apposition in our study continued for a longer time than the enamel matrix secretion and was not completed at 12 days of age.

The results of the present study showed that the average daily dentin apposition at the mesial sides of the cusp was 14 μm in rats 2–12 days of age. The distal sides had a lower mean apposition rate (9 μm), but it should be kept in mind that considerable variations

Table 3. Thickness of dentin (in µm) on the mesial and distal sides of the three cusps on the first upper molar of rats 2–12 days of age. Each value represents the mean value ± SD of five rats

Cusp	Side of cusp	Days of age: 2	3	4	5	6	7	8	9	12
Mesial	Mesial	13 ± 2.3	15 ± 2.3	30 ± 4.8	35 ± 7.0	48 ± 10.8	69 ± 7.6	92 ± 18.0	104 ± 21.3	149 ± 29.0
	Distal	10 ± 4.4	16 ± 1.9	16 ± 9.2	25 ± 0.9	32 ± 4.9	45 ± 10.8	62 ± 7.8	68 ± 12.0	95 ± 23.4
Medial	Mesial	17 ± 4.2	24 ± 2.6	50 ± 6.6	49 ± 9.9	60 ± 4.8	80 ± 2.5	95 ± 5.6	112 ± 12.2	178 ± 33.4
	Distal	14 ± 1.3	17 ± 2.6	30 ± 5.2	34 ± 6.3	44 ± 2.4	58 ± 3.6	68 ± 6.2	73 ± 10.9	101 ± 30.0
Distal	Mesial	16 ± 4.0	21 ± 6.8	42 ± 3.8	48 ± 9.8	66 ± 8.2	79 ± 12.6	96 ± 7.2	105 ± 13.0	162 ± 12.7
	Distal	10 ± 4.7	14 ± 1.5	23 ± 2.5	30 ± 9.2	41 ± 6.4	53 ± 12.4	63 ± 8.8	78 ± 16.0	93 ± 11.9

Table 4. Height of odontoblasts (in µm) on the mesial and distal sides of the three cusps of the first upper molar of rats 2–12 days of age. Each value represents the mean value ± SD of five rats

Cusp	Side of cusp	Days of age: 2	3	4	5	6	7	8	9	12
Mesial	Mesial	46 ± 17.0	65 ± 19.8	79 ± 6.5	71 ± 8.8	78 ± 15.5	93 ± 17.8	87 ± 5.0	75 ± 16.5	70 ± 9.0
	Distal	45 ± 18.0	44 ± 10.4	59 ± 4.8	59 ± 6.5	51 ± 5.7	59 ± 4.9	53 ± 7.8	48 ± 8.4	52 ± 5.4
Medial	Mesial	59 ± 22.0	76 ± 17.6	78 ± 5.4	86 ± 9.3	91 ± 8.7	96 ± 7.8	96 ± 6.3	89 ± 25	96 ± 13.6
	Distal	52 ± 13.9	46 ± 10.8	54 ± 5.4	52 ± 6.9	44 ± 4.8	62 ± 14.2	51 ± 9.2	48 ± 8.3	53 ± 8.3
Distal	Mesial	59 ± 16.2	69 ± 16.4	90 ± 9.0	89 ± 12.6	93 ± 11.4	97 ± 18.4	93 ± 17.3	75 ± 27.0	93 ± 19.0
	Distal	43 ± 8.9	52 ± 14.7	55 ± 5.5	60 ± 11.5	56 ± 6.6	63 ± 5.9	59 ± 5.0	49 ± 8.7	56 ± 7.0

Table 5. The body weights (g) of the rats 2–12 days of age. Each value represents the mean value ± SD of five rats

Days of age: 2	3	4	5	6	7	8	9	12
7.8 ± 0.60	9.4 ± 0.45	10.3 ± 0.50	11.9 ± 1.20	13.8 ± 1.47	16.2 ± 0.60	17.5 ± 1.74	20.8 ± 2.21	26.0 ± 4.25

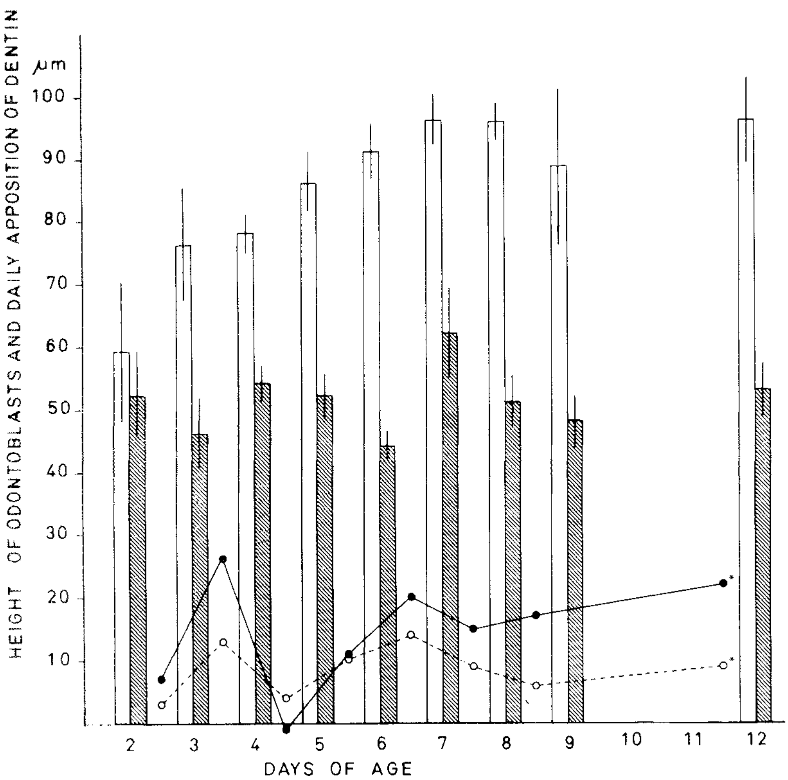

Fig. 5. Histogram showing the height of the odontoblasts on the mesial (open bars) and distal (hatched bars) sides of the medial cusp of the first upper molar from rats 2–12 days old. The curves show daily apposition on the mesial (—●—) and distal (- -○- -) sides of the cusp (calculated from Table 3). (*The difference in apposition between days 9 and 12 was calculated and divided by three.)

in daily apposition were always present. In earlier studies the average daily rate of dentin apposition was 10 µm in the molar (16, 17) and 16 µm in the incisor (11). The differences in dentin apposition and the relation to the odontoblast height found in this study have also been noticed in a study of the human tooth in which the odontoblasts were taller and the dentin was thicker on the buccal surface than on the lingual surface (29).

No functional explanation of these regional differences has been put forward, but it is reasonable to assume that the large cells are able to produce more hard tissue precursors than the short cells. The enamel formation on the mesial sides of the rat upper molar cusps has been found to be sensitive to high doses of fluoride (30). The mechanism behind a higher apposition rate and an increased sensitivity to fluoride remains to be explained and also whether this is valid for other toxic agents.

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