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A MORPHOMETRIC AND QUANTITATIVE MICRORADIOGRAPHIC STUDY OF DENTAL TISSUES IN THE HYPOPITUITARY DWARF MOUSE

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Summary—In transverse sections the areas of enamel, dentine and pulp were determined using image analysis morphometry, and the mineralization levels of enamel, dentine and alveolar bone using quantitative microradiography and a microphotometric–microdensitometric technique. There were significant differences in the ratios of dentine to pulp area, and in the enamel to total area in the incisors from dwarf and normal animals. The mean dentine to pulp ratio for dwarf incisors was more than six times greater than in normal incisors. There was also a significantly greater variation in the dentine to pulp ratio in dwarfs, which was attributed to their greater variation in pulp size. Values for mineral density of enamel, dentine and bone were similar to those found by other investigators. For example, for normal specimens, the mean mineral density of outer enamel was 2.84 g/cm^3 (SD = 0.38), percentage mineralization 92%; outer labial dentine was 1.64 g/cm^3 (SD = 0.15), percentage mineralization 52%; and alveolar bone was 1.53 g/cm^3 (SD = 0.13), percentage mineralization 49%. The variation in mineralization within each tissue confirmed previous work in rodents. There was a consistent trend for mineralization levels of all hard tissues in the dwarf sample to be less than in the normal sample but the difference was not statistically significant for any one tissue.

INTRODUCTION

The Snell dwarf mouse is a useful animal model to investigate the effects of hypopituitarism on growth and development. The trait for dwarfism is inherited as an autosomal recessive characteristic dependent on a single gene, which is not sex-linked (Snell, 1929). This causes an absence or marked deficiency of eosinophils and a reduction of basophils in the anterior lobe of the pituitary gland, which explains the deficiency of growth hormone and thyroid stimulating hormone in dwarf animals.

A radiographic study of dental development (Tracey and Roberts, 1985) has demonstrated significant differences in tooth size and eruption rate in hypopituitary dwarf mice compared to their normal litter mates. The length and width of upper and lower incisors were significantly less in the dwarf animals. The mesiodistal dimension of the molar crowns measured at the level of the enamel–cementum junction was also significantly shorter, and molar root length was only 60% of that in normal animals.

A similar reduction in tooth size and retardation of tooth eruption has been demonstrated in hypophysectomized animals. Subjective histological studies of teeth from hypophysectomized animals have also revealed differences in the relative proportions of dental tissues and mineralization levels (Becks, Collins and Simpson, 1946; Baume, Becks and Evans, 1954; Hansson, Stenstrom and Thorngren, 1978).

Our aim now was to establish the relative proportions of dental tissues and mineralization levels in incisors from dwarf mice and their normal litter mate controls using morphometric and quantitative microradiographic techniques.

MATERIALS AND METHODS

Teeth were obtained from skulls used in a longitudinal study (Tracey and Roberts, 1985). The material had not been fixed, but stored in a refrigerator at 4°C; this was satisfactory because hard tissues retain their integrity in the absence of fixation. Upper incisors from the oldest age groups, comprising teeth from 9 dwarf mice (5 were 64 days old; 4 were 80 days old) and 12 normal mice (6 were 64 days old; 6 were 80 days old) were used, where the greatest differences in structure and mineralization between dwarfs and normals could be anticipated (Becks *et al.*, 1946; Baume *et al.*, 1954; Tracey and Roberts, 1985). Ideally, the samples should contain dwarf and normal animals of the same age but, because there are no differences between 64 and 80-day-old mice (Tracey and Roberts, 1985), animals of these age groups were here combined in order to obtain a reasonable sample size. Statistical analysis of our results confirmed this to be valid.

Histological procedures

After removal of adherent soft tissues from each hemiskull, the incisors and their supporting structures were removed from the maxillae using a diamond bur in an air rotor. The upper incisors were fixed in neutral formol saline, dehydrated and then impregnated with methyl methacrylate monomer activated by benzoyl peroxide and heat, using a standardized routine.

Transverse sections were used for microradiography because it is easier to ensure consistency and reproducibility of sectioning in the transverse plane, and this is important if valid comparisons of measurements between specimens are to be made

(Hansson *et al.*, 1978; Rosenberg and Simmons, 1980; Steigman, Weinreb and Michaeli, 1984). The sections were cut perpendicular to the midpoint of a line joining the tip of the incisor on its labial surface and the apex of the incisor on its labial surface. Two sections of approx. 200 μm thickness were cut using an Isomet low-speed saw (Buehler Ltd, Evanston, Ill., U.S.A.) at moderate speed. Plano-parallel sections were then obtained from these using the Logitech precision polishing machine (Logitech, Dunbartonshire, Scotland) to lap to a final thickness of 80–100 μm. These sections were then examined with a Zeiss light microscope, to check thickness and flatness.

Morphometry

A photomicrographic record of one ground section from each specimen was made, giving a final magnification of $\times 100$. The photographs of the transverse ground sections from upper incisors were used for measurement of enamal, dentine and pulp area, using an image analysis computer (Kontron MOP Videoplan) programmed to record areas. A point source of red light was superimposed on the outline to be traced by hand movement of the cursor on a magnetized digitizer. The relative proportions of dental tissues in dwarf and normal samples were compared by calculating the ratios of enamel area to dentine area, dentine area to pulp area, enamel area to total area. A simple comparison of areas of enamel, dentine and pulp would only have reflected the earlier findings of differences in absolute size between the two groups.

Quantitative microradiography

Each specimen (upper incisor ground sections, each section of known thickness, and all between 80 and 100 μm thick) was exposed with an aluminium step wedge (with 9 linear steps, each step 0.5 mm wide, giving a range of thicknesses from 44 to 220 μm) on Eastman Kodak Spectroscopic Plates 649-0. The X-ray source was a Philips PW1720 diffraction generator with a copper anode, nickel filter and beryllium window. The focal area of the tube was 0.4 × 8.0 mm and the focus to film distance 250 mm. The tube was operated at 20 kV and 16 mA. Each specimen was microradiographed twice, once for 40 min and once for 13 min to produce microradiographs suitable for quantitative measurements of enamel and dentine respectively. A constant field was ensured by always locating the specimen and reference system in the same position in the base of the camera, with the camera always orientated in a consistent position with respect to the tube. Good contact of the specimen and step wedge with the emulsion was ensured by evacuating the air between a polythene covering sheet and the emulsion with a vacuum pump. The plates were developed in Kodak D19 at 20°C ($\pm$1°C) for 6 min and the developing tank was kept immersed an a thermostat-controlled water bath, which was agitated mechanically. The plates were rinsed in distilled water for 10 s and then fixed in Ilford Hyram Fixer for 2 min. They were washed in filtered tap water at 20°C ($\pm$3°C) for 60 min. To prevent dust accumulating or scratches occurring, each microradiograph was dried and then mounted with a coverslip and DPX.

A Leitz MPV Compact Photometer was used with a Leitz Orthoplan microscope to measure the density of the microradiograph using transmitted light. A measuring area of 25 μm diameter was used at selected points along transverse scans across the specimen and step wedge on each microradiograph. Readings were made using the 0.8 mm photometer-measuring diaphragm and $\times 25$ objective. Two neutral density filters (ND8 and ND22) were used to increase the darkness of the background field when measurements were made. Each measurement was taken with the photometer set to show a reading averaged over a 2.5 s period. Since photometer readings on each specimen image were compared with readings from the step wedge image on the same slide, consistency in the adjustment of the instrument between microradiographs was not important.

The following readings were made from each microradiograph taken to measure enamel mineralization. For outer enamel (E_o), three readings were taken approx. 10 μm from the outer surface. The central reading was halfway between mesial and distal edges of enamel, and the other two readings on either side of this (Fig. 1). A series of at least four readings was made across relevant steps of the reference system. For inner enamel (E_i), three readings were taken approx. 10 μm from the amelodentinal junction. The central reading was directly below the central reading for outer enamel, and the other two readings on either side of this (Fig. 1). Corresponding readings were made across the reference system. There is a radiolucent band between the outer and inner enamel which has been described by Suga (1979, 1983). This area was not included in photometer readings in our study.

Another microradiograph was taken to measure dentine and bone mineralization. Separate assessments of alveolar bone and the outer and inner areas of both labial and lingual dentine were made (Fig. 2). The long axis of the pulp chamber was used as a guide to the central measurement at each level in dentine and three readings were made of each of the following. For outer labial dentine ($Dlab_o$), the readings were approx. 10 μm from the amelodentinal junction and for inner labial dentine ($Dlab_i$), the readings were approx. 10 μm from the pulp chamber. For inner lingual dentine ($Dling_i$), the readings were approx. 10 μm from the pulp chamber and for outer

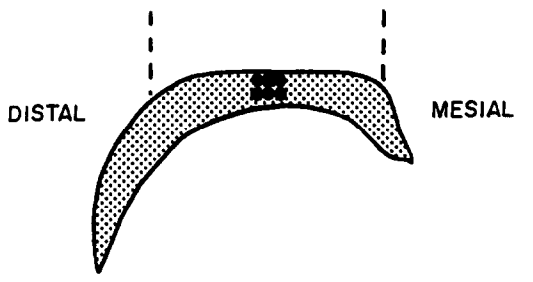

Fig. 1. Measured areas in enamel.

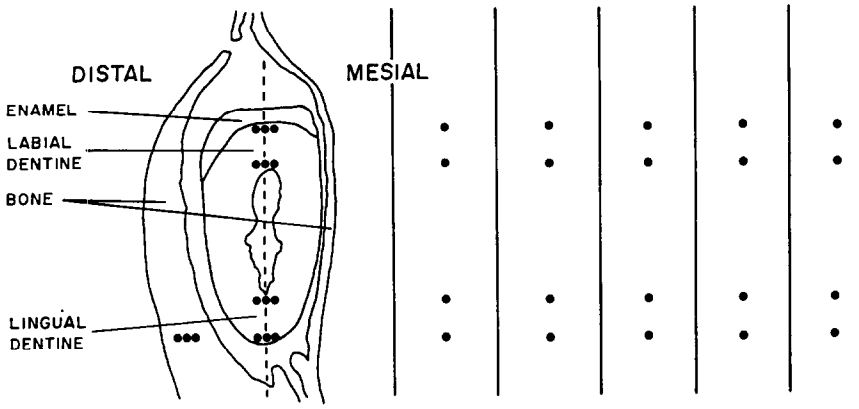

Fig. 2. Measured areas in dentine, bone and step-wedge.

lingual dentine ($Dling_o$), the readings were approx. 10 μm from the dentine–cementum junction. At each level, step-wedge readings were also taken. Alveolar bone (B) was measured on the distal surface in three areas at the same level as outer lingual dentine.

Transmission of light through steps on the wedge was plotted as a function of the number of steps, to provide a calibration curve for each level of each microradiograph, i.e. four curves for each microradiograph measuring dentine and bone, and two curves for each microradiograph measuring enamel. The three photometer values at each level were averaged and then converted to equivalent thickness of aluminium from the appropriate calibration curve. This, together with the thickness of the specimen, provided sufficient information to calculate the density of hydroxyapatite (Ericsson, 1965).

A scanning method for quantitative microradiography was also employed as this avoids the use of a photographic emulsion to record the image by using a narrow X-ray beam with two-dimensional translation of the specimen, and measuring directly the transmitted X-ray intensity with a scintillation counter. This technique allows the whole specimen to be scanned accurately in 15 × 15 μm steps with a reproducibility of $\pm 1.5\%$. A two-dimensional contour map is drawn up (Elliott, Dowker and Knight, 1981). However, the method is time-consuming and therefore can only be used for a small number of specimens. In our study, one section from an 80-day normal incisor and one section from an 80-day dwarf incisor were subjected to this technique. Initially a line scan was performed through the section, along the long axis of the pulp chamber of each specimen. Area plots (1 × 1 mm) were taken from each specimen and then three-dimensional plots constructed from these.

Statistical methods

The F-statistic was used to test differences in variance and the two tailed t-test was used for differences in means for the area ratios and mineralization levels in the two groups. Further analysis of the differences in variance was by coefficient of variation. A t-test designed to test differences between correlated pairs of means was used to compare mineralization levels within each tissue, e.g. comparisons between inner and outer enamel and between different zones in dentine for each specimen. Results were considered significantly different if $P < 0.05$. Left and right teeth were treated separately for all measurements. This provided a limited form of replication, by demonstrating if the results were stable to random errors.

RESULTS

Area ratios for dental tissues in dwarf and normal samples (Table 1)

The differences in area ratios for upper incisors between dwarf and normal samples are given in Table 1.

The mean enamel to total area ratio (ETR) was significantly greater in the dwarf sample than the normal sample for left and right teeth. There was no significant difference in the variance. The mean dentine area to pulp area ratio (DPR) in upper incisors from dwarf specimens was almost six times greater

Table 1. Means and standard deviations for area ratios in upper incisors from dwarf and normal mice

	Dwarf	Normal	$p(F$-test)	$p(t$-test)
Left upper incisors				
Enamel: dentine ratio	$0.14 \pm 0.02\,(n)$	$0.14 \pm 0.03\,(n)$	NS	NS
Dentine: pulp ratio	$46.6 \pm 38.4\,(n)$	$7.4 \pm 3.12\,(n)$	<0.001	<0.05
Enamel: total area ratio	$0.12 \pm 0.01\,(n)$	$0.10 \pm 0.01\,(n)$	NS	<0.05
Right upper incisors				
Enamel: dentine ratio	$0.14 \pm 0.01\,(n)$	$0.13 \pm 0.02\,(n)$	NS	NS
Dentine: pulp ratio	$46.1 \pm 30.9\,(n)$	$8.8 \pm 3.50\,(n)$	<0.001	<0.01
Enamel: total area ratio	$0.12 \pm 0.01(n)$	$0.10 \pm 0.01\,(n)$	NS	<0.01

Table 2. Mineralization levels expressed as enamel density and equivalent per cent mineralization in upper incisors from dwarf and normal mice (data from 64-day and 80-day animals combined)

	Mean g/cm³	Standard deviation	Mean % by volume	Mean g/cm³	Standard deviation	Mean % by volume
	Left incisors ($n = 7$)			Right incisors ($n = 9$)		
E_o	2.84	0.26	90.2	2.77	0.38	87.9
E_i	2.65	0.25	84.1	2.57	0.38	81.6
$Dlab_o$	1.60	0.14	50.8	1.56	0.22	49.5
$Dlab_i$	1.33	0.11	42.2	1.34	0.21	42.5
$Dling_i$	1.25	0.09	39.7	1.35	0.19	42.8
$Dling_o$	1.38	0.14	43.8	1.43	0.22	45.4
B	1.45	0.13	46.0	1.44	0.21	45.7
	Left incisors ($n = 10$)			Right incisors ($n = 11$)		
Normals						
E_o	2.87	0.28	91.1	2.84	0.38	91.7
E_i	2.68	0.27	85.1	2.68	0.36	85.1
$Dlab_o$	1.71	0.10	54.3	1.64	0.15	52.1
$Dlab_i$	1.46	0.17	46.3	1.41	0.16	44.8
$Dling_i$	1.33	0.13	42.2	1.28	0.15	40.6
$Dling_o$	1.49	0.18	47.3	1.46	0.16	46.3
B	1.54	0.11	48.9	1.53	0.13	48.6

than the ratio for normal specimens for both left and right teeth. This difference was statistically significant.

There was also a large difference in the variance for the DPR between dwarf and normal samples for both left and right teeth. Because this greater variance in dwarfs was associated with a significant difference in mean values, the differences in variation were explored further using the coefficient of variation. This showed that even after taking into account differences in the absolute size of the DPR in the two groups, there was twice as much variation in the dwarf sample. This was attributable to a greater variation in pulp size rather than dentine area.

There was no significant difference in the mean or variance for enamel area:dentine area ratio (EDR) between the two groups. All these results were consistent for left and right teeth.

An estimate of measurement error for morphometry was made from the results of six tracings made of the same photograph of an upper incisor from a normal specimen and 95% confidence limits were assessed from the standard error viz. $EDR = 0.12 \pm 0.002$, $DPR = 5.96 \pm 0.28$, $ETR = 0.095 \pm 0.002$. This range indicates the scale of the measurement error inherent in the method.

Mineralization levels

The means and standard deviations for mineralization levels, viz E_o, E_i, $Dlab_o$, $Dlab_i$, $Dling_o$, $Dling_i$, B, were calculated for dwarf and normal samples (Table 2). As density of hydroxyapatite is often quoted as percentage by volume in the literature the results are also given in this form. There was no significant difference between dwarf and normal samples in the mineralization levels at any of the sites measured. However, there was a consistent trend (13 out of 14 samples) across all sites for the dwarf sample to show lower mineralization levels.

An estimate of measurement error for quantitative microradiography was made by calculating mineralization levels from five separate microradiographs taken of the same section from an upper incisor from a normal specimen and 95% confidence limits were assessed from the standard error viz. $Dlab_i = 1.41\ g/cm^3 \pm 0.07$, $Dling_i = 1.29\ g/cm^3 \pm 0.09$, $Dling_o = 1.44\ g/cm^3 \pm 0.17$, $B = 1.69\ g/cm^3 \pm 0.05$. This range indicates the scale of the measurement error inherent in the method.

Comparisons were made between outer and inner enamel (E_o/E_i), between outer and inner labial dentine ($Dlab_o/Dlab_i$), between outer and inner lingual dentine ($Dling_o/Dling_i$), and between the average value for labial dentine and the average value for lingual dentine (Dlab/Dling). This was assessed using a t-test designed to measure differences between correlated pairs of means. It can be seen from Table 1 that $E_o > E_i$, $Dlab_o > Dlab_i$, $Dling_o > Dling_i$, $Dlab > Dling$. These differences were all significant in dwarf and normal samples and in right and left teeth ($p < 0.01$).

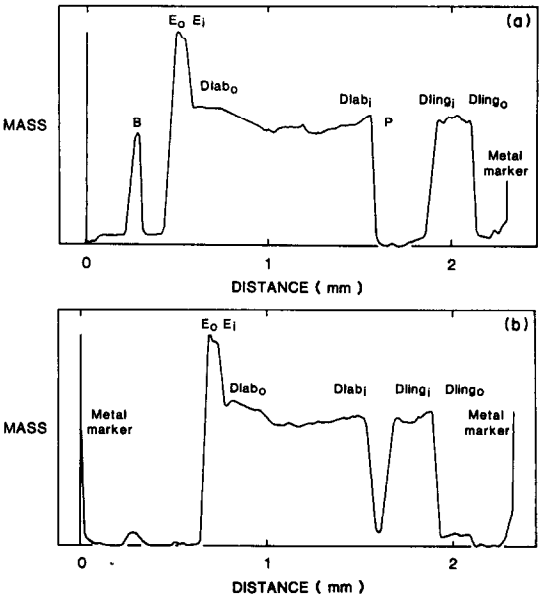

Fig. 3. (a) Line scan for section from 80-day normal animal. (b) Line scan for section from 80-day dwarf animal.

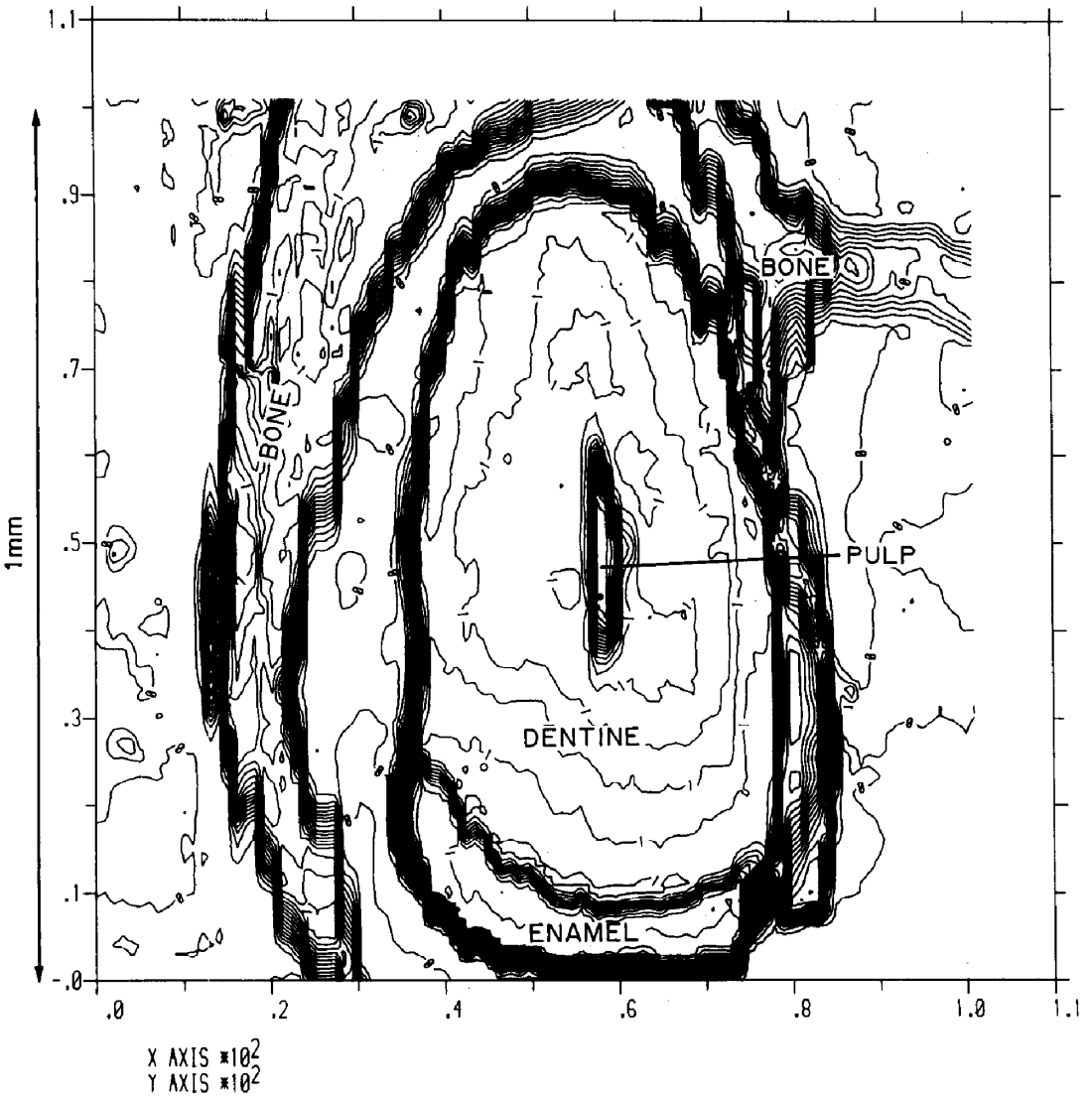

Fig. 4. Area scan 1 × 1 mm for dwarf upper incisor.

Using the scanning method for microradiography, a line scan was performed along the long axis of the pulp chamber of each specimen and the intensity of transmitted X-rays from each specimen was recorded. Graphs of density of hydroxyapatite (Y) against distance (X) were produced for these line scans (Fig. 3a and b). The Y-axis was not calibrated, but it is a linear scale representing mineral density. The relative levels for enamel and dentine mineralization are consistent with the results from the microphotometric–microdensitometric method. The line scans from both dwarf and normal specimens showed broadly the same pattern.

The area scans from dwarf and normal specimens again confirmed the main findings, viz. a decrease in mineralization from outer to inner enamel, and from the outer to the inner region in both labial and lingual dentine. The area scan for a dwarf specimen is illustrated in Fig. 4. The contour lines connecting regions of equal mineralization confirm that the areas chosen for photometric measurement were a suitable choice because the mineralization changes in the vicinity of each group of three readings were small.

Three-dimensional representations were also produced from the area (1 × 1 mm) scans (Fig. 5a and b). The x and y scales represent the area of the scan 1 × 1 mm and the vertical axis was not calibrated, but is a linear scale representing mineral density. These three-dimensional representations of mineralization levels illustrate the relative mineral density both between enamel, dentine and bone and within each of these tissues, confirming the results from the microphotometric–microdensitometric technique.

DISCUSSION

Changes in the proportions of dental hard tissues in dwarf animals were quantified using morphometric techniques to measure areas in sections from upper incisors in dwarf and normal samples. This showed that relatively more dentine was present in sections from dwarf animals. The pulp chambers in sections

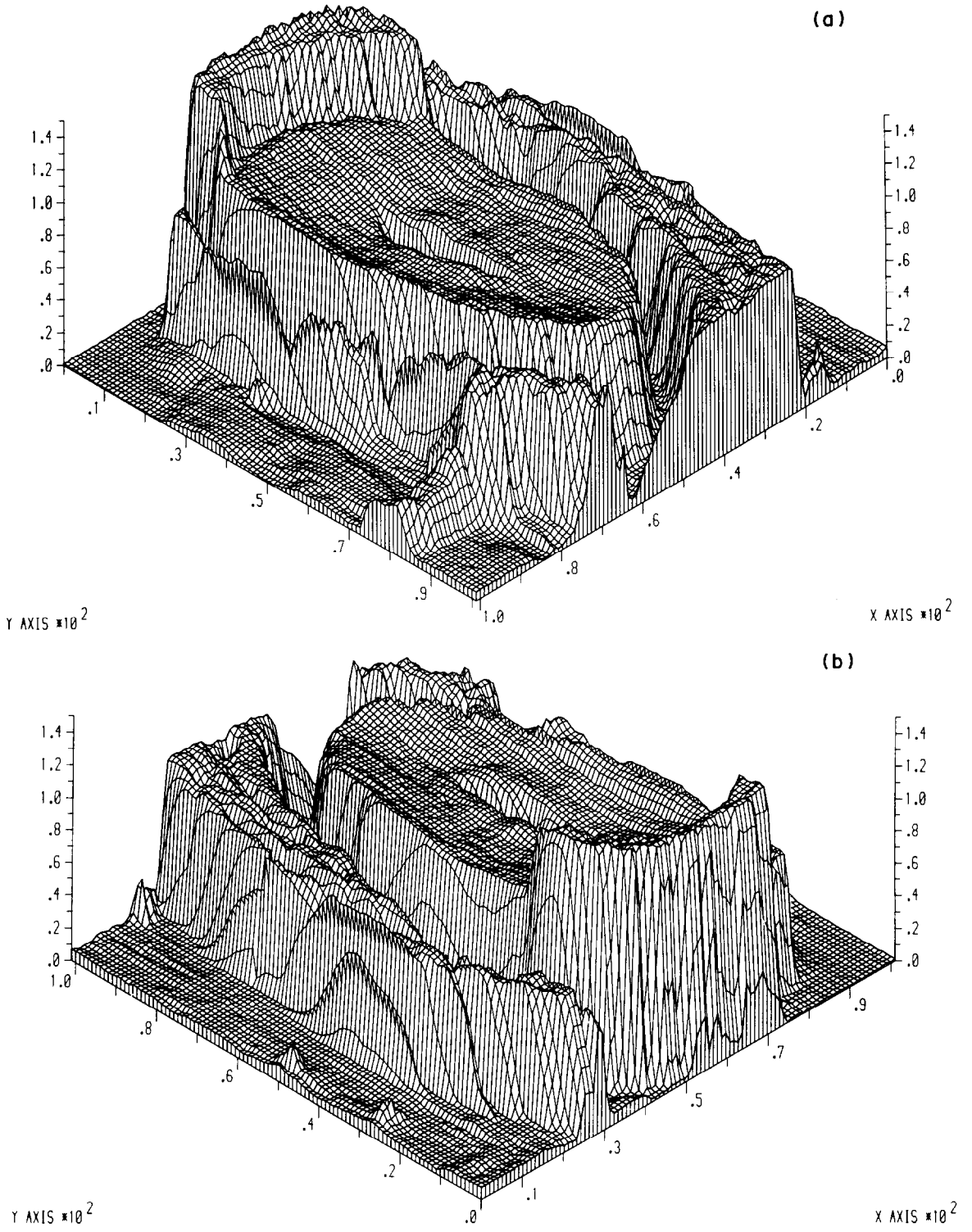

Fig. 5. (a, b) Three-dimensional representation of different mineralization levels within the 80-day dwarf specimen.

from dwarf animals were much smaller and more variable in size than those from normal animals. These results resemble those found in studies on the effects of hypophysectomy on the structure of the rat incisor (Becks *et al.*, 1946; Baume *et al.*, 1954; Hansson *et al.*, 1978). We demonstrate statistically significant differences in the mean and variation for dentine area to pulp area ratio (DPR) between dwarf and normal mice. The most likely explanation for the greater DPR in dwarfs is that dentine continues to form at a reduced rate but in a tooth with a substantially reduced eruption rate. This explanation is sup-

ported by evidence of a greatly reduced eruption rate in hypopituitary dwarf mice, which is most marked in the upper incisors (Tracey and Roberts, 1985). Earlier studies have also demonstrated a reduction in dentine deposition in hypophysectomized rats (Hansson *et al.*, 1978), but longitudinal dentine formation is more severely retarded than appositional formation (Yonaga, 1969). The DPR showed twice as much variation in the dwarf sample as in the normal sample and this variation was attributable to a greater variation in pulp size amongst dwarfs. This is not unexpected as any incremental deposit of dentine will have a relatively small effect on the large area of dentine and a relatively large effect on the small area of pulp. The greater variance in pulp size in dwarfs could be explained if the retardation in eruption rate in dwarfs is not closely correlated with the retardation in dentine formation—lack of synchronization between formation of tissue and continuous eruption could lead to a variable pattern of pulp size.

There was a significantly greater mean enamel area:total area ratio (ETR) in the dwarf sample. It has been shown that the size of the incisors is significantly reduced in dwarf animals (Tracey and Roberts, 1985). However, the difference in ETR between our two groups would tend to suggest that enamel production is less affected by hypopituitarism than is the overall size of the tooth.

The mineralization levels calculated for enamel in our normal mice confirm the values calculated by others for mineralization levels in unerupted rodent enamel (Selvig and Halse, 1972). The mineralization levels calculated for bone in our normal mice were higher than those quoted by Rowland, Jowsey and Marshall (1959), but they did not specify the site of bone sampling, which could influence the exact value. No published figures have been found for mineralization levels in rodent incisal dentine, and because there is variation between species, it would be inappropriate to compare our values with those from another animal.

Differences in mineralization levels within tissues also confirmed previous work on rodent dental tissues. Suga (1979) demonstrated, in a subjective microradiographic study, that at the late stage of rat enamel maturation the outer layer is more highly mineralized than the inner layer. Our plane of sectioning corresponded to the area of late maturation of the ameloblast cells. In this position, outer enamel was significantly more mineralized than inner enamel in both dwarf and normal mice. The outer zone was also more mineralized than the inner zone in both labial and lingual dentine. These results agree with those of Rosenberg and Simmons (1980), who used electron probe microanalysis in transverse sections of incisors from the rabbit. They attributed differences between labial and lingual, and outer and inner dentine to differential growth rates on either side of the spiral axis, the labial side growing faster than the lingual side. This explanation may also apply to the mouse incisor.

We found no significant difference in the mineralization levels between dwarf and normal samples at any of the sites measured. However, there was a consistent trend across all sites for the dwarf sample to have lower mineralization levels.

It is interesting to compare our results and those of the related study (Tracey and Roberts, 1985) with those of Glick and Rowe (1981a, b), who examined the effects of protein deficiency on skeletal and dental development of young rats. Like dwarf mice, these protein deficient rats had retarded growth and reduced body size and weight. The dimensions of individual bones were reduced in experimental rats and there was a marked disturbance in epiphyseal cartilage growth and endochondral bone formation (Glick and Rowe, 1981a). These findings are similar to the effects of hypopituitarism on the skeletal development of dwarf mice (Tracey and Roberts, 1985). Glick and Rowe (1981b) also reported that the eruption rate was reduced and teeth were smaller in experimental animals, again as in dwarf mice (Tracey and Roberts, 1985), but no differences in the morphology of dental tissues were reported and no differences in mineralization levels were established in these rats. Although these studies seem to suggest that many of the effects of hypopituitarism on the skeleton and dentition resemble the effects of protein deficiency, the altered morphology of dental tissues and mineralization levels we observed in the incisors of hypopituitary dwarf mice were not found in protein deficient rats. The reasons for these differences may warrant further investigation.

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REFERENCES

Baume L. J., Becks H. and Evans H. M. (1954) Hormonal control of tooth eruption. *J. dent. Res.* **33,** 80–114.

Becks H., Collins D. A. and Simpson M. E. (1946) Changes in the central incisors of hypophysectomised female rats after different post-operative periods. *Arch. Path.* **41,** 457–475.

Elliott J. C., Dowker S. E. P. and Knight R. D. (1981) Scanning x-ray microradiography of a section of a carious lesion in dental enamal. *J. Microsc.* **123,** 89–92.

Ericsson S. G. (1965) Quantitative microradiography of cementum and abraded dentine. A methodological and biological study. *Acta radiol.* Suppl. 246.

Glick P. L. and Rowe D. J. (1981a) Effects of chronic protein deficiency on skeletal development of young rats. *Calc. Tiss. Int.* **33,** 223–231.

Glick P. L. and Rowe D. J. (1981b) Effects of chronic protein deficiency on the formation of the rat incisor teeth. *Archs oral Biol.* **26,** 459–466.

Hansson L. I., Stenstrom A. and Thorngren K. G. (1978) Effect of hypophysectomy on dentine production in maxillary incisors in the rat. *Scand. J. dent. Res.* **86,** 72–79.

Rosenberg G. D. and Simmons D. J. (1980) Rhythmic dentinogenesis in the rabbit incisor, circadian, ultradian and infradian periods. *Calc. Tiss. Int.* **32,** 29–44.

Rowland R. E., Jowsey J. and Marshall J. H. (1959) Microscopic metabolism of calcium in bone III. Microradiographic measurement of mineral density. *Radiat. Res.* **10,** 234–242.

Selvig K. A. and Halse A. (1972) Mineral content and crystal size in mature rat enamel. A correlated electron probe and electron microscope study. *J. Ultrastructure Res.* **40,** 527–531.

Snell G. D. (1929) Dwarf. A new Mendelian recessive character of the house mouse. *Proc. natn. Acad. Sci. U.S.A.* **15,** 733–734.

Steigman S., Weinreb M. M. and Michaeli Y. (1984) Histomorphic evaluation of the dimensional changes in rat incisor tissues following histological processing and embedding in paraffin wax and glycol methacrylate. *Archs oral Biol.* **29,** 395–398.

Suga S. (1979) Comparative histology of progressive mineralization pattern of developing incisor enamel of rodents. *J. dent. Res.* **58,** 1025–1026.

Suga S. (1983) Comparative histology of progressive mineralization pattern of developing enamel. In: *Mechanisms of Tooth Enamel Formation* (Edited by Suga S.) Part III, pp. 167–203. Quintessence, Tokyo.

Tracey C. and Roberts G. J. (1985) A radiographic study of dental development in the hypopituitary dwarf mouse. *Archs oral Biol.* **30,** 805–811.

Yonaga T. (1969) Quantitative studies of the effect of hypophysectomy on the growth of bone, teeth and hair and their relationship to each other. *Bull. Tokyo med. Dent. Univ.* **16,** 73–84.