

STEREOLOGICAL ANALYSIS OF ORGANELLE DISTRIBUTION WITHIN RAT INCISOR  
ENAMEL ORGAN AT SUCCESSIVE STAGES OF AMELOGENESIS

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INTRODUCTION

Cells of the enamel organ typically undergo several morphological changes during amelogenesis (1). Of interest have been findings that dramatic alterations in cell shape and/or organelle content occur within short times relative to the global life cycle of these cells (2). For example, preameloblasts stop dividing and differentiate into highly polarized, tall columnar secretory cells within about 36 hours. When the enamel layer has been formed these ameloblasts either die (25% of the cells) or they reorganize themselves for transport, and presumably resorptive, activity within about 19 hours. Adjacent cells of the papillary layer also hypertrophy and develop many microvilli at their surface during this postsecretory transition period. Once the enamel has been fully mineralized, the ameloblasts and papillary layer cells eventually regress in about 24 hours to form the reduced enamel organ which covers the completed enamel surface until the tooth erupts (3). Despite considerable improvements that have been made to our understanding of processes such as secretion, modulation, and ionic transport, events that occupy enamel organ cells for much of their life cycle (reviewed in 1, 4-6), it still remains unclear whether the mass of organelles altered during the short transition intervals leading to secretion, maturation and regression constitute a major, or only minor, amount of the internal contents of these cells. The present study was undertaken therefore in an attempt to obtain basic quantitative information about organelle changes using conventional stereological techniques.

MATERIALS AND METHODS

The experimental design for this study was based exclusively on rationale outlined in detail by Weibel (7). Briefly, 10 male Sprague-Dawley rats weighing about 150 grams were perfused with 5% glutaraldehyde in 0.05M sodium cacodylate buffer (pH 7.4). The mandibular jaws were removed and decalcified for 5 days in EDTA, and then small pieces of the enamel organ were dissected from each incisor at 6 locations along the labial aspect corresponding to young enamel organ (presecretion), start and end of the secretory stage,

early and late maturation stage, and regression stage of amelogenesis. After embedding in Epon, thin sections were cut at about 50 nm thickness from each block, and a series of 2 to 4 overlapping micrographs were taken at low magnification (x2,500) on a Philips 400 electron microscope from inner to outer surfaces of the enamel organ in 2 areas selected randomly on each grid. A continuous strip of 4 to 11 abutting, but non-overlapping, micrographs also were taken in a similar fashion at high magnification (x11,500) in 4 separate areas selected randomly on each grid. The low power micrographs were printed at x6,000 final magnification, and the prints from each series were montaged in order to recreate individual "unit samples of enamel organ" as they were seen originally in the electron microscope. The volume fraction of cells, nuclei, cytoplasm and intercellular spaces was estimated from the montaged prints by standard techniques in point counting analysis using a double square lattice test system (7) (1:4 ratio of course points to fine points) which was mounted on a transparent acetate sheet and superimposed over each unit sample. High power micrographs were projected through an enlarger at x30,000 final magnification onto a similar square lattice test system and the number of points hitting organelles such as mitochondria, endoplasmic reticulum, Golgi saccules, lysosomes, etc. within the cytoplasmic compartment of each enamel organ cell was estimated across all micrographs. Point count data from a contiguous strip of micrographs through the same cell type (e.g., ameloblast) were pooled to yield a single estimate of the volume fraction of organelles for a given unit sample of enamel organ (4 per incisor x 2 incisors per animal x 10 animals per location x 6 locations). Point count data were collected, stored and analysed by standard stereological and correction equations (7) using a Hewlett-Packard HP-85 microcomputer and software either written locally in HP Basic (unpublished) or purchased commercially from the HP Series 80 User's Library. Parameters that were estimated included (a) the volume fraction of ameloblasts, stratum intermedium, stellate reticulum, outer dental epithelium (or papillary layer cells at later stages of amelogenesis), intercellular spaces, and gross organelles in unit samples of the enamel organ, (b) the volume fraction of organelles in each cell layer of the enamel organ, and (c) the percentage of organelles derived from ameloblasts. In a separate experiment the absolute volume of organelles within enamel organ cells was estimated in cubic micrometers by first determining nuclear volume from serial semi-thin sections, and then deducing corresponding cell, cytoplasmic, and organelle volumes from the known volume ratios for these components in each cell. Nuclear volumes were computed with the aid of a Zeiss MOP-3 image analyzer by measuring profile areas of nuclear fragments in the serial sections (volume = sum of areas x section thickness taking into account a correction for capping relative to the first and

TABLE 1

## VOLUMETRIC COMPOSITION OF ENAMEL ORGAN AT SUCCESSIVE STAGES OF AMELOGENESIS

	% VOLUME ( $\pm$ S.D.)					
	Presecretion	Secretion		Maturation		Regression
		Start	End	Early	Late	
Ameloblasts	57.6 $\pm$ 6.6	71.5 $\pm$ 4.4	70.3 $\pm$ 2.9	49.8 $\pm$ 6.3	53.9 $\pm$ 5.1	35.1 $\pm$ 11.9
SI/PL1	13.5 $\pm$ 2.3	6.2 $\pm$ 1.2	9.4 $\pm$ 1.4	13.8 $\pm$ 2.7	15.7 $\pm$ 2.7	14.1 $\pm$ 2.5
SR/PL2	15.9 $\pm$ 3.7	10.7 $\pm$ 2.5	1.6 $\pm$ 1.9	-----	-----	21.2 $\pm$ 11.3
ODE/PL3	9.4 $\pm$ 2.6	9.1 $\pm$ 2.1	16.0 $\pm$ 3.2	26.5 $\pm$ 5.5	26.8 $\pm$ 5.4	26.7 $\pm$ 11.6
Spaces	3.2 $\pm$ 1.6	2.4 $\pm$ 1.5	2.5 $\pm$ 1.1	9.7 $\pm$ 2.3	3.1 $\pm$ 0.8	2.7 $\pm$ 1.5

SI=Stratum intermedium SR=Stellate reticulum ODE=Outer dental epithelium  
 PL1, PL2, PL3=Papillary layer cells at base of ameloblasts, intermediate, or  
 abutting outer surface of enamel organ respectively

the last section cut through the nucleus).

## RESULTS

Gross Distribution of Cells and Organelles. Intercellular spaces were detected at all stages and occupied as much as 10% of total enamel organ volume during early maturation (relative to ruffled-ended ameloblasts) but only about 2.5% to 3% of enamel organ volume during other stages of amelogenesis (Table 1). Ameloblasts alone accounted for approximately 60%, 70%, 50% and 35% of total enamel organ volume during the presecretory, secretory, maturation and regression stages respectively. Papillary layer cells occupied about 40% of enamel organ volume during early maturation which was similar to the volume occupied by stratum intermedium, stellate reticulum and outer dental epithelium in the young enamel organ. Otherwise, these auxiliary cells accounted for only about 27% of enamel organ volume during the secretory stage but for slightly more than 60% of enamel organ volume by the regression stage of amelogenesis. Nuclei generally accounted for the highest percentage of organelle volume across all stages of amelogenesis and occupied about 38% of total volume within the young enamel organ but only about 18% of enamel organ volume during the maturation and regression stages. Other organelles tended to increase in relative concentration away from the presecretory stage with the (a) endoplasmic reticulum, Golgi saccules, lysosomes, (b) mitochondria, (c) granules (pigment), (d) vacuoles/autophagic vacuoles, and cytoplasmic filaments reaching their highest volume density of about (a) 20%, 5%, 0.6%, (b) 11%, (c) 6%, (d) 4% and 15% of enamel organ volume by the (a) secretory, (b) early maturation, (c) late maturation, and (d) regression stages of amelogenesis respectively.

Source of Organelles. For the most part the ameloblasts constituted the largest single source of organelles within the enamel organ (Table 2). This was

TABLE 2

SOURCE OF ORGANELLES WITHIN ENAMEL ORGAN AT SUCCESSIVE STAGES OF AMELOGENESIS

	% ORGANELLES DERIVED FROM AMELOBLASTS					Regression
	Presecretion	Secretion		Maturation		
		Start	End	Early	Late	
Nucleus	68	72	65	60	56	39
Mitochondria	55	65	56	36	52	41
ER	67	90	94	76	67	30
Golgi saccules	58	86	90	69	70	29
Lysosomes	67	83	83	80	50	25
G/V/AV	67	80	93	75	96	41
Filaments	20	43	33	42	42	30
Cytoplasm	57	68	64	52	50	37

ER=endoplasmic reticulum G/V/AV=granules, vacuoles, autophagic vacuoles  
 Cytoplasm=unclassified structures and remaining cytoplasmic matrix

TABLE 3

ABSOLUTE CHANGE IN ORGANELLE CONTENT BETWEEN SUCCESSIVE STAGES OF AMELOGENESIS  
 (expressed as multiplication factor between stages compared - see text)

	PS → SS	SS → FS	FS → OB	OB → PG	PG → RE	PS → RE
Nucleus	1.1 -1.3	-1.5 1.2	1.1 -1.1	1 1	-2.8 1.2	-3.8 1
Mitochondria	1.5 1.3	1.6 2.7	1.1 1.7	1 -1.9	-3.8 -1.7	-1.4 1.9
Endoplasmic reticulum	4.0 1.1	1.5 1.2	-1.7 1.8	-2.5 -1.7	-5.1 1.3	-3.6 1.8
Golgi saccules	3.4 1	1.5 1.3	-1.6 1.8	-1.2 -1.7	-8.0 1.3	-3.0 1.6
Lysosomes	3.3 1	1.2 1	1.2 1.4	-2.9 1.4	-3.3 1.9	-2.0 3.3
G/V/AV	2.7 -1.5	7.5 3.4	-3.0 1.7	10.1 1.4	-8.1 4.4	8.5 23.7
Filaments	6.2 1.4	1.3 2.3	1.8 1	5.8 5.1	-1.4 1.7	62.6 28.1
Cytoplasm	1.7 1	-1.1 1.3	1.1 1.6	1 -1.1	-3.1 1.1	-1.8 1.9
Whole Cell	1.7 1	1.1 1.4	-1.1 1.5	1 -1.1	-3.3 1.1	-2.0 2.0

G/V/AV=granules, vacuoles, autophagic vacuoles

Cytoplasm=unclassified structures and remaining cytoplasmic matrix

PS=presecretion SS=start secretion FS=end secretion

OB=early maturation PG=late maturation RE=regression

seen at all stages up to and including late maturation. During the regression stage, however, only about 25% to 40% of organelles were derived from ameloblasts with the majority originating from remnants of the papillary layer. These data further suggested 3 patterns in the distribution of organelles across the stages of amelogenesis from presecretion to late maturation; that is, (a) a group of organelles with a very high percentage derived from ameloblasts (from 70% up to 96%), (b) a group of organelles where the majority derived from ameloblasts (from 50% up to 70% with some fluctuation below 50%), and (c) a single class of organelle derived mostly from auxiliary enamel organ cells. Organelles in the first group included endoplasmic reticulum, Golgi saccules, lysosomes, vacuoles, and autophagic vacuoles. Those comprising the second group included nuclei, "cytoplasm", and mitochondria. Of these, mitochondria consistently showed the lowest percentage derived from ameloblasts being greatest at the start of the secretory stage (65% derived from ameloblasts) and least during early maturation (36% derived from ameloblasts). Cytoplasmic filaments formed the sole organelle of the last group.

Changes in Organelle Content During Amelogenesis. Data comparing changes in the absolute organelle content of ameloblasts and auxiliary enamel organ cells (combined stratum intermedium, stellate reticulum, and outer dental epithelium, or papillary layer at older stages of amelogenesis) are shown in Table 3. These values represent multiplication factors; that is, the values greater than 1 indicate a net gain, those less than 1 a net loss, and those equal to 1 indicate no change in organelle volume between stages that are compared. The upper number for every pair listed in the columns of the table corresponds to results for ameloblasts and the lower number corresponds to results for the auxiliary enamel organ cells. It is evident from this table that there were profound differences between ameloblasts and the auxiliary cells in the pattern of development of organelles between stages as well as sharp differences in the pattern of net gain or loss of organelles in these cells when comparing the youngest to the oldest stages of amelogenesis (data in last column). Ameloblasts clearly showed the greatest net positive gain in cell size (bottom row) and organelle volume during the transition period leading to secretion except in the case of mitochondria which increased noticeably in volume throughout the period from presecretion to the end of the secretory stage (PS $\bar{Z}$ FS). Granules (pigment) and cytoplasmic filaments were plentiful in ameloblasts by the late maturation stage (OB $\bar{Z}$ PG). The auxiliary enamel organ cells in contrast showed their greatest net positive gains in organelle volumes spread over older stages of amelogenesis beginning with the mitochondrial compartment which increased during the secretory stage (SS $\bar{Z}$ FS). Endoplasmic

reticulum, Golgi saccules and "cytoplasm" increased markedly in volume during the transition period leading to maturation (FS $\bar{Z}$ OB), whereas cytoplasmic filaments increased throughout maturation (OB $\bar{Z}$ RE), and lysosomes, vacuoles and autophagic vacuoles showed the greatest increases in volume during the transition period from maturation to regression (PG $\bar{Z}$ RE). In terms of net decline in volume of organelles, ameloblasts showed their greatest loss of organelle volume in the transition period leading from late maturation to regression (PG $\bar{Z}$ RE). Auxiliary enamel organ cells showed no similar tendency for massive decline in organelle volume although there was evidence for a gradual decline in the volume of mitochondria, endoplasmic reticulum, and Golgi saccules between early and late maturation (OB $\bar{Z}$ PG), and between late maturation and regression (PG $\bar{Z}$ RE). In comparing the oldest to the youngest stages (PS $\bar{Z}$ RE), the ameloblasts showed a net loss of cell size and organelle volume for all structures except vacuoles, autophagic vacuoles, and cytoplasmic filaments. Auxiliary enamel organ cells, however, showed a net gain in cell size and organelle volume except in the case of nuclear volume which remained unchanged.

#### DISCUSSION

Many findings in this study are consistent with a view of amelogenesis that has evolved from qualitative observations of cell morphology (reviewed in 1). This view includes the perception that (a) ameloblasts are the dominant cell type within the enamel organ, and they are rich in organelles associated with the synthesis and secretion of protein (endoplasmic reticulum and Golgi saccules), (b) auxiliary enamel organ cells are comparatively poor in organelles associated with synthesis but rich in organelles associated with metabolism/transport (mitochondria), (c) major changes in organelle content of ameloblasts occur during transition periods leading to the secretion, maturation, and regression stages, and (d) auxiliary enamel organ cells hypertrophy and increase their content of mitochondria during the transition period from secretion into maturation. What has not been so clear previously are the findings in the present study that (a) nuclei occupy a significant proportion of enamel organ, and cell, volume throughout amelogenesis, (b) ruffled-ended ameloblasts (early maturation) contain about the same volume of endoplasmic reticulum and Golgi saccules as young secretory ameloblasts (early secretion), (c) papillary layer cells contain about twice the volume of mitochondria and about one-half the volume of endoplasmic reticulum and Golgi saccules as ruffled-ended ameloblasts, (d) the greatest increase in mitochondrial volume occurs during the secretory stage (all cells) whereas the greatest increase in lysosomal volume occurs as preameloblasts differentiate into tall secretory cells, and (e) auxiliary enamel organ cells show a net gain

in the volume of organelles (except nucleus) over the course of amelogenesis whereas ameloblasts show a net loss in volume of all organelles except vacuoles, autophagic vacuoles, and cytoplasmic filaments. In regard to various organelles, the findings for nuclei were somewhat unusual and suggest that there are aspects of amelogenic nuclear activity which distinguish enamel organ cells from other cells. That is, the finding of a net decline in relative nuclear volume between presecretion and early maturation is in keeping with a general phenomenon of nuclear condensation and/or cytoplasmic swelling that typifies differentiation and aging in most tissues. Nuclei in fully differentiated, mature cells generally occupy no more than 10% of cell volume (7-11), which is considerably less than the amount estimated in the present study for differentiated enamel organ cells. This would suggest that the enamel organ cells maintain a greater nuclear mass or they maintain a smaller cytoplasmic mass in relation to the cytoplasm or the nucleus, respectively, as mature cells in other tissues. Another feature which distinguishes the enamel organ from other stratified epithelia, as for example the oral mucosa from which it derives, is that mitochondrial volume appears to increase, rather than decrease gradually, along the renewal axis (presecretion toward regression) (11). This is the case at least up to the maturation stage. Ameloblasts do show a massive decline in mitochondria, however, during transition from maturation into regression with the net result that these cells maintain less mitochondrial volume near the end of amelogenesis as they did within the young enamel organ. Auxiliary enamel organ cells, however, lose about half their existing mitochondrial volume between early and late maturation, and then they lose another half during transition from maturation into regression. In this case, however, the auxiliary cells eventually contain a greater volume of mitochondria than they did in the young enamel organ. Finally, the data in this study have added some support to the idea that lysosomes participate in amelogenesis during both the secretory and maturation stages (1, 12). This is suggested by the fact that ameloblasts rapidly expand their pool of lysosomes in preparation for the secretory stage (Table 3), and then the size of this pool gradually enlarges as ameloblasts progress through the secretory stage and into the maturation stage of amelogenesis. However, these data do not preclude the possibility that lysosomes are utilized most actively during the secretory stage and what we see later during the maturation stage is the collection of terminal secondary lysosomes and/or residual bodies within the cytoplasm of these cells. Studies by Kallenbach (13) and Ozawa et al., (14) suggest that the lysosomes in enamel organ cells remain functionally active, however, at least up to the early maturation stage of amelogenesis.

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## RESUME

### STEREOANALYSE DE LA DISTRIBUTION DES ORGANITES CELLULAIRES DE L'INCISIVE DE RAT DURANT L'AMELOGENESE.

Les cellules de l'organe de l'émail et principalement les adamantoblastes présentent des modifications importantes durant l'amélogénèse. Les implications d'organites cellulaires spécifiques tels que le noyau, les mitochondries, le reticulum endoplasmique, l'appareil de Golgi et les lysosomes, sont peu connues. Dans ce travail la distribution des divers organites est analysée à l'aide de techniques stéréologiques. Les territoires suivants de l'organe de l'émail de l'incisive de rat correspondant à des stades fonctionnels ont été analysés :

- début de la phase de sécrétion - fin de la phase de sécrétion - stade de maturation précoce - stade de maturation tardive - stade de régression. Le stade de référence correspond à des adamantoblastes post-mitotiques avant leur cytodifférenciation.

Il est particulièrement intéressant de souligner que le volume absolu des mitochondries est maximum pendant la phase de sécrétion. Le volume absolu des lysosomes est maximum juste avant et au début de la phase de sécrétion. L'augmentation la plus importante du volume du reticulum endoplasmique et de l'appareil de Golgi, des cellules du stratum intermedium, du reticulum stellaire et de l'épithélium dentaire externe est observée à la fin du stade de sécrétion.

