# Section of Odontology

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## Paper

## The Development of Enamel Structure

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These studies examine the relationship between the cells which secrete enamel and the pattern of orientation of the elements in the formed tissue (Boyde 1964).

Enamel consists of myriads of uniformly wide, fairly well oriented crystals of hydroxyapatite packed into an organic matrix. A repetitive pattern of change in the orientation of its constituent elements is responsible for its division into so-called prisms and interprismatic regions.

The discovery that the whole of enamel developed in an extracellular location (Fearnhead 1960, Watson 1960) posed the problem of explaining the origin of prisms, interprismatic regions and prism sheaths in one and the same secretion. Previous explanations for the development of enamel structure had invoked a double siting, as, for example, 'interprismatic substance' from terminal bars and 'prism substance' from the conversion of ameloblast cytoplasm.

Previous electron microscope (EM) studies of amelogenesis have been done on rodent and primate teeth, presumably on the one hand because of the general availability of laboratory rodents and on the other because of the applicability of the results to man. Severe limitations are imposed by restricting such a study to these two orders.

*Materials and Methods*
Developing teeth from the following mammals have been studied: *Primates*, man, rhesus monkey; *Proboscidea*, African elephant; *Sirenia*, manatee; *Cetacea*, bottle-nosed dolphin; *Carnivora*, dog, cat; *Artiodactyla*, cow, sheep, goat, pig; *Perissodactyla*, horse; *Rodentia*, mouse, rat, jird, hamster, squirrel, coypu, guinea-pig; *Lagomorpha*, rabbit; *Insectivora*, hedgehog, mole; *Marsupialia*, rat-tailed opossum, common opossum, Bennett's wallaby, ring-tailed phalanger, Kerr's opossum. One reptile, *Caiman sclerops*, was also studied.

Material for transmission electron microscopy was fixed in Palade's or Dalton's fixatives and embedded in 4 : 1 butyl : methyl methacrylate, sectioned at *c.* 500 Å on a Porter Blum ultramicrotome and photographed at 60kV on a Siemens Elmiskop 1. The relationship of the ameloblasts and the prisms to the topography of the developing enamel surface and the crystallite orientation patterns in developing enamel were studied in electron micrographs of sections cut in many different planes. Confirmation of the latter findings was obtained from the study of stereo-pair micrographs, when the crystallite orientation could be seen directly.

The topography of the developing enamel surface was also studied by several techniques: (1) Via the production of wax reconstructions from large-scale projection drawings made of the profile of the developing enamel surface in 0·5 μ thick sections of the EM blocks. (2) By scanning electron microscopy of the surface resulting when the enamel organ is stripped from the surface of formalin-fixed tooth germs. (3) By a single-stage carbon replica technique (Boyde 1967) of the same type of preparation. (4) By stereo-photogrammetric techniques for the analysis of the stereo-pair images resulting from (2) and (3) (Boyde 1967).

*Results*
The observation of Fearnhead (1960) and Watson (1960) that enamel develops in an extracellular location, that is as a secretion of the ameloblasts (Figs 1, 4, 8), has been confirmed for all the mammalian species so far studied.

The first layer of enamel deposited on the surface of the dentine at the future enamel-dentine junction was found to present a relatively flat surface (Fig 1). The crystallites developing within it were found to stand parallel to each other and perpendicular to a flat mineralizing front. Rela-

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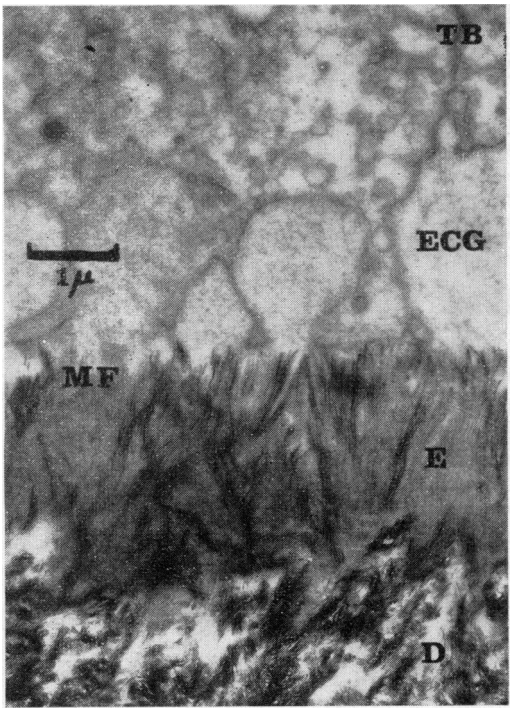


Fig 1 *The first 'prism free' layer of enamel* (E) *at the enamel-dentine junction. Rat-tailed opossum. Note the orientation of the crystallites perpendicular to the mineralizing front* (MF), *the amorphous organic matrix 'extracellular granules'* (ECG) *and the level of the inner terminal bar apparatus* (TB). D, *dentine*

tively larger masses of extracellular granular material (the amorphous organic matrix secretion of the ameloblasts) were found to separate the inner ends of the ameloblasts from the mineralizing front at this earliest stage. During later stages there was usually only a thin seam (*c.* 500 Å thick) into which the underlying hydroxyapatite crystallites had not already grown. Thus the 'mineralizing front' was usually parallel to the 'surface of the developing enamel' and for many practical purposes we may regard the two terms as synonymous. A relatively flat mineralizing front with perpendicular crystallite orientation was found to persist throughout enamel development in the one reptile species studied.

During the formation of the bulk of the enamel its surface was found to present a very regular series of depressions (Figs 2, 3, 9, 11). These minute pits are caused by the ameloblasts pushing out a projection (the Tomes' process) into the matrix which they are secreting and each was found to be occupied by a single cell process in life. Formalin fixation was found to cause the ameloblasts to cleave away from the surface of the developing enamel: this effect was used to

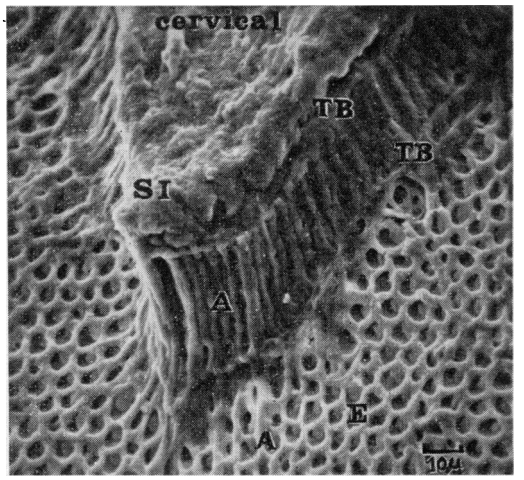


Fig 2 *A developing enamel surface* (E) *with some of the enamel organ cells still in place. Dog deciduous canine.* A, *ameloblasts.* SI, *stratum intermedium.* TB, *outer and inner terminal bar apparatus of the ameloblasts*

remove them and allow direct examination of the surface in the scanning electron microscope or by a carbon replica technique (Boyde 1966) for the transmission electron microscope.

The shape of these pits in the surface of the developing enamel was found to vary in a characteristic way amongst members of the different mammalian orders studied. Simple cylindrical pits with a flat floor meeting a complete circumferential wall at a sharp angle were the type most commonly found in the *Insectivora*, *Cetacea*, and *Sirenia*. An organization into rows in the longitudinal direction of the tooth, with inter-row

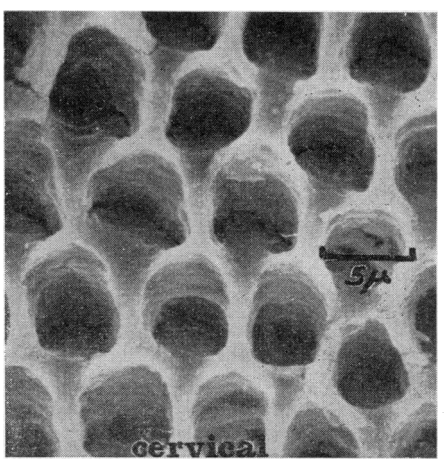


Fig 3 *Pits in the developing enamel surface caused and occupied by ameloblast Tomes' processes. Cracks at site of developing prism sheaths. Human deciduous molar*

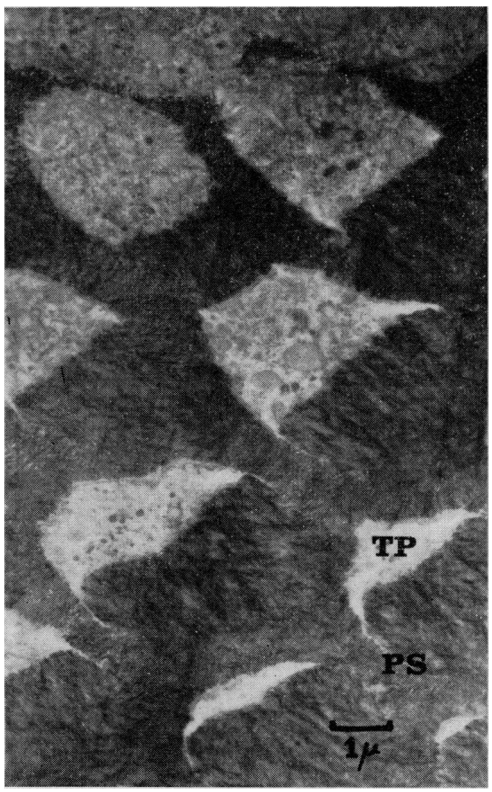


Fig 4 *Tangential section developing enamel surface. Tomes' processes showing depressions filling in from one side. Cat deciduous molar.* TP *Tomes' process.* PS, *prism sheath*

ridges projecting somewhat beyond the level reached by the bridges dividing up the rows (Fig 5 and 11) was found to be characteristic of the *Perissodactyla*, *Artiodactyla*, and *Marsupialia* and was also found in the rhesus monkey and to some extent in man. The cervical sides of the depressions in this second pattern also form their floors, meeting the remaining lateral and cuspal walls at a sharp angle. The underlying hexagonal packing is a prominent feature of the third pattern found in *Carnivora*, *Proboscidea* and *Primates*, including man. One side of each depression, facing a corner of the hexagon, again forms the floor (Figs 2, 3, 4, 9).

The shape of the mineralizing front deduced from these studies can be seen in Fig 9 (Pattern 3) and Fig 11 (Pattern 2). The surface was found to present three characteristic profiles according to the plane of section. Thus tangential sections (Fig 4) presented a 'honeycomb' appearance, with the 'cells' in the honeycomb filling in progressively and predominantly from one side as the plane of section enters deeper into the enamel.

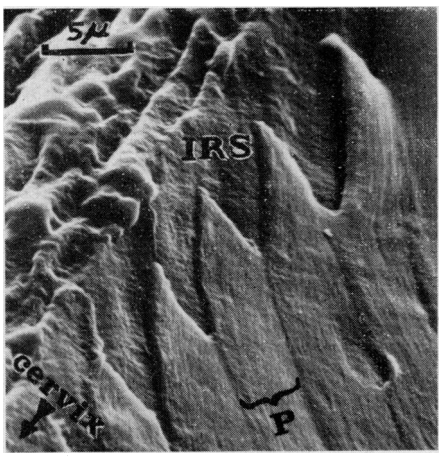


Fig 5 *Longitudinal fracture. Developing enamel surface showing inter-row ridges and ameloblast remnants at top left. 'Picket fence' fracture profile across centre of field.* P, *prism.* IRS, *inter-row sheet. Calf deciduous molar*

Longitudinal sections (Fig 5, 8; Fig 9 left and Fig 11 right) revealed a characteristic 'picket fence' or 'saw tooth' profile and transverse sections a 'battlements' or repetitive box-like profile (Figs 6, 7; bottom of Figs 9 and 11).

The crystallite orientation patterns and their relationship to the shape of the mineralizing front are also shown diagrammatically in Figs 9 and 11. The crystallites tend to grow perpendicular to this front in the cervical floors of the depressions but may grow more nearly parallel with it in the lateral and cuspal walls.

The sharp concavities in the plane of the mineralizing front (at the boundary between the

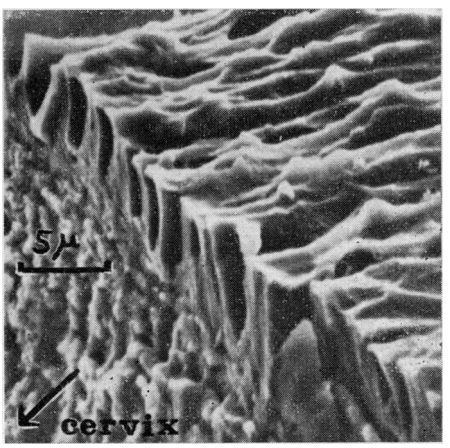


Fig 6 *Developing enamel surface top right, transverse cut surface lower left, showing battlements profile along the line of the cut. Pig deciduous molar*

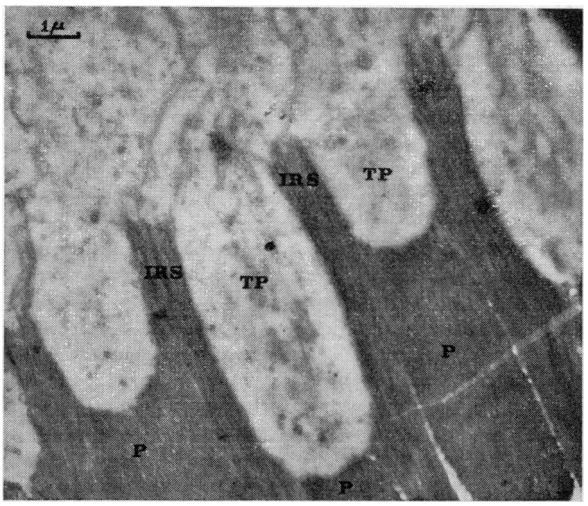


Fig 7 *Transverse 'battlements' section of developing enamel surface showing the almost parallel alignment of the crystallites and the lateral margins of the Tomes' processes.* TP, *Tomes' process.* P, *prism.* IRS, *inter-row sheet. Rhesus monkey deciduous molar*

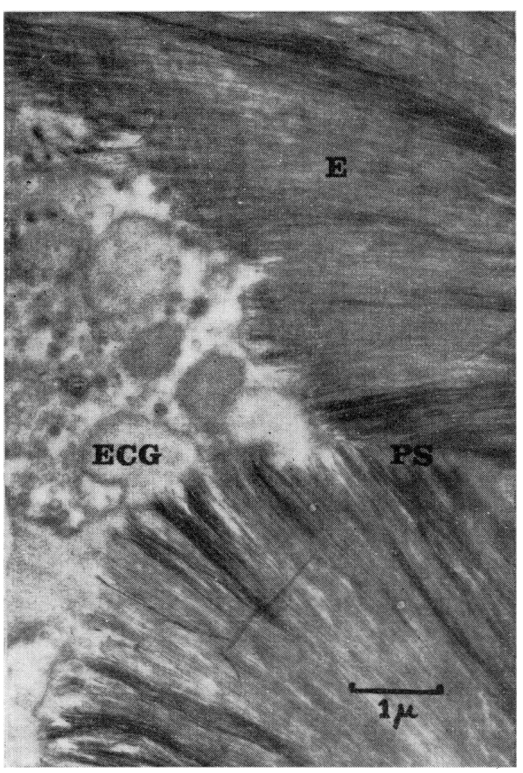


Fig 8 *Longitudinal 'picket fence' section showing the pointed end of one Tomes' process in relation to a developing prism boundary* (PS). E, *enamel.* ECG, *extracellular granules. Rat-tailed opossum*

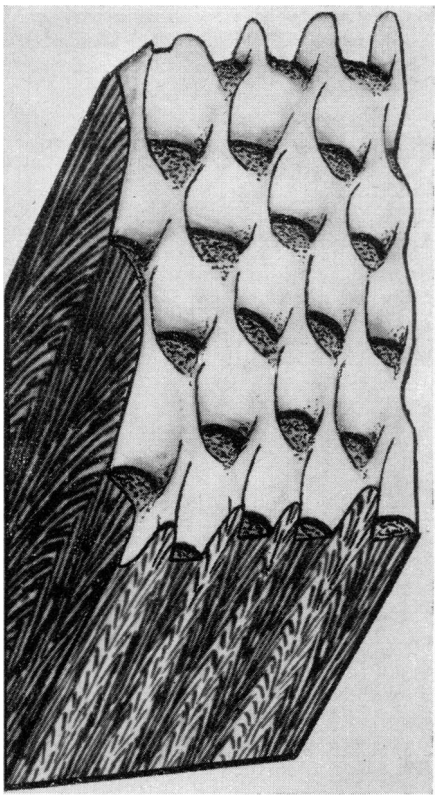

Fig 9 *3D model of human enamel development. The basis for this model was a true scale model made from stereo-pair scanning electron micrographs using the Stereosketch (Boyde 1967). The crystallite orientation patterns have been interpolated from the ultra-thin section results.*

continuously curved lateral-cuspal-lateral wall and the cervical floor) cause abrupt changes in the orientation of the crystallites developing underneath. These planes at which crystallite orientation changes abruptly are the 'prism-sheaths' of classical dental histology. They may be seen in Fig 3 as cracks in the mineralizing front caused by shrinkage during drying.

'Domains' in which the crystallite orientation changes only gradually can be seen to be related to single projections (convexities) of the surface in both the 'picket fence' and 'battlements' planes and each projection is related to two ameloblasts. In the 'picket fence' plane 'domains' represent *prisms*, which thus get contributions from two ameloblasts (in Pattern 2: four in Pattern 3, *see below*). In the 'battlements' plane they represent the *inter-row sheet* (interprismatic) regions between longitudinal rows of Pattern 2 prisms (Fig 11) or the winged processes ('tail' region of Meckel *et al.* 1965) of Pattern 3 prisms. Thus Pattern 3 prisms are related to two ameloblasts in

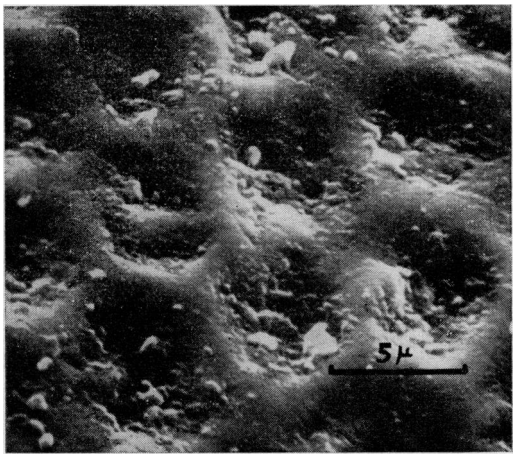


Fig 10 *Adult enamel surface, unworn and unwashed, showing the pits in the surface originally caused by the Tomes' processes. Human premolar*

both planes of section, making a total of four ameloblasts per prism and four prisms per ameloblast: we define all the enamel as belonging to prism domains in this Pattern.

The type of disturbance which causes the incremental lines in the enamel known as the brown striæ of Retzius can apparently also cause the early demise of secretory activity on the part of those ameloblasts which were in any case nearing the end of the secretory phase of their life cycle. The surface of the completed tissue will then show the depressions originally occupied by the Tomes' processes of the ameloblasts, which are also to be regarded as external evidence of the internal extent of the 'prism sheaths' right up to the surface of the tooth. Where enamel formation proceeds to its close in a normal fashion, the ameloblasts lose their Tomes' process projections during the final stages of enamel matrix formation and the mineralizing front therefore tends to become flat once again. The crystallites are then all aligned parallel and perpendicular to the surface and, since there are no changes in orientation, there are also no prisms in this true surface zone.

*Discussion*

The present results confirm the pattern of crystallite orientation and the prism-packing pattern deduced by Meckel *et al.* (1965) for adult human enamel; they also show how this arrangement develops (Fig 9). The most essential feature appears to be the modification of the shape of the mineralizing front of the enamel induced by the projection of the Tomes' process of the ameloblast into its own secretion. The simple reptilian pattern of parallel perpendicular crystallite orientation is thus broken up at concavities in the mineralizing front.

The prism boundaries of the developing enamel do not contain any material more than or different from the surrounding enamel. The higher concentration of organic material at the prism sheaths of adult enamel is presumably acquired during 'maturation', via the remobilization of the organic matrix gel. The pressure gradient causing this movement of thixotropic gel (Eastoe 1964) must force it parallel with the crystallite direction and back towards the ameloblasts. The accumulation of more organic matrix at the prism boundaries is thus simply explained, because this is the only

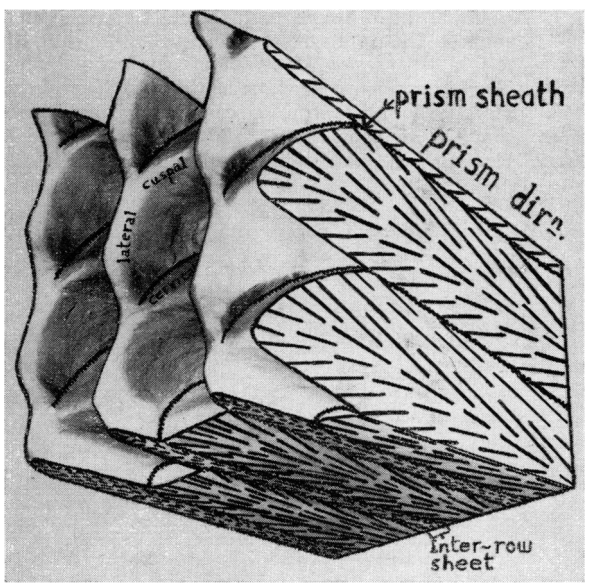


Fig 11 *Model of Pattern 2 enamel development*

region at which crystallites *end* and because the more imperfect packing of crystallites at this plane, where the orientation on either side is different, causes the existence of more spaces which are not filled with crystalline material.

The prism direction represents the fossilized path traced out by the Tomes' processes of the ameloblasts during enamel secretion. A close examination of Figs 9 and 11 will show that the cuspal sides (to be seen in the 'picket fence' profile) and the lateral sides (to be seen in the 'battlements' profile) of the depressions in the mineralizing front are more nearly parallel to the prism direction, whereas the cervical floor sides are in fact more nearly perpendicular to the prism direction. Since Tomes' process is in intimate contact with its own secretion, it can be deduced that their respective surfaces are engaged in a relative shearing movement along the lateral and cuspal sides but must enjoy a more stationary relationship at the cervical floor sides. This shearing, sliding movement in the organic matrix between the cell membrane of Tomes' process and the mineralizing front provides the basis for explaining the divergence of the enamel crystallites in the mineralizing front along the lateral and cuspal sides of the depressions from the general rule of their developing perpendicular to the mineralizing front: this is irrespective of whether the crystallites orient themselves or are oriented by the matrix in which they grow.

The depressions in the surface of the adult enamel at the troughs of the perikymata have long been called the 'enamel rod ends' (Fig 10). The present studies have shown that this name is inappropriate, since every one of these pits is related to more than one prism – a conclusion also reached by Rönnholm (1962). Their greatest significance lies in the absence of the more perfect, prism-like surface zone and the fact that the brown striæ of Retzius reach the surface in these regions, since several authors have touched on the selective involvement of the brown striæ in the caries process.

*Acknowledgments:* Figs 2, 3, 5, 6 and 10 were taken on a Cambridge Instrument Company Stereoscan during my stay at the Department of Medical Physics of the University of Münster/Westf. I am grateful to the Wellcome Trust for their financial support, to Professor G Pfefferkorn for the wonderful facilities he placed at my disposal and to Dr R Blaschke for his friendly assistance during this visit.

The Siemens Elmiskop I was on loan from the Wellcome Trust. The Hilger and Watts Stereometer and Stereosketch used in the photogrammetric analyses were lent by my friend Mr R V Ely.

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*Note re illustrations*

Figs 1, 4, 7 and 8 are transmission electron micrographs of ultra-thin sections; Siemens Elmiskop, 60 kV.

Figs 2, 3, 5, 6 and 10 are scanning electron micrographs of surfaces covered with conducting layers of *c.* 200 Å carbon and 200 Å gold; Cambridge Stereoscan, 10 kV.

Figs 9 and 11 are photographs of three-dimensional models. The front surface of the model shown in Fig 9 is a true scale 3D model of developing human deciduous molar enamel made using the Stereosketch (*see* Boyde 1967) from a scanning electron micrograph (stereo-pair) similar to Fig 3. Fig 11 is based on electron micrographs and wax reconstructions.