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The chemistry of enamel development

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ABSTRACT The central problems of enamel biochemistry are the mechanisms concerned with initiation and development of the mineral crystals, together with their architectural arrangement within the tissue. These processes are mediated by the extracellular matrix as well as the composition of the mineral itself. Initial mineral deposition occurs at the dentine surface, nucleated either by dentinal components or early enamel matrix, possibly non-amelogenin molecules. The early crystals are small in size and rich in magnesium and carbonate resulting in relatively poor crystallinity. This is in spite of the fact that fluoride is high at this stage. Crystal development includes a reduction in magnesium, carbonate and fluoride as crystals increase in length following the retreating ameloblasts from the dentine. The matrix acquires increasing concentrations of amelogenin and albumin. Prismatic structure begins to develop together with some growth of crystals in width and thickness. Degradation of amelogenin and non-amelogenin molecules generates a series of specific molecular fragments possibly concerned with modulating crystal growth and morphology and the creation of prismatic and interprismatic structures. Towards the end of secretion, matrix, now almost completely degraded, is replaced by fluid followed by massive crystal growth during maturation. Degradation of albumin also occurs at this stage, probably as a result of comprehensive destruction of molecules which might impair crystal growth. Selective acquisition of magnesium and fluoride at this stage may reflect the hydrated state of the tissue as well as cell changes. Fluid is displaced as crystals grow and the enamel acquires concentrations of mineral characteristic of mature tissue.

KEY WORDS: *enamel, development, matrix, mineral, chemistry*

Introduction

The formation of the most highly mineralized tissue in the mammalian skeletal repertoire poses numerous unique biological problems. These include not only the deposition of mineral *per se* but the modulation of mineral morphology, chemistry and architectural distribution within the tissue. Enamel architecture manifests itself in the mature tissue as many millions of almost identical, highly ordered crystals of calcium hydroxyapatite, arranged into supracrystalline structures which, while following a basic theme, vary from species to species. The formation of these crystals and their arrangement into the prismatic/interprismatic ultrastructure are the central problems of enamel biology.

All enamels are initially secreted as a soft partially mineralized organic matrix comprising, by weight, about 30% mineral and the remainder organic material and water (Deakins, 1942; Robinson *et al.*, 1978). During development this ratio is reversed such that the mature tissue contains up to 90% mineral (Glimcher *et al.*, 1964; Robinson *et al.*, 1971). Unlike mesenchymal skeletal tissues, the organic matrix of enamel does not persist to any great extent into the mature tissue and it is, therefore, presumed that the role of the organic matrix is to form an ephemeral informational support in which the crystals and prismatic structure develop. This is borne out by the simple fact that *in vitro* dissolution of the matrix during the early developmental stages causes complete disintegration of the enamel structure while removal of matrix at the later, so called maturation stage of development, has little effect on the gross integrity of the tissue (Brookes, unpublished results). The development of this complex tissue architecture is most conveniently considered as a series of discrete stages characterized by the appearance of the cells responsible for its formation (ameloblasts) (Reith, 1963; Smith and Warshawsky, 1975), the appearance of the tissue (Robinson and Kirkham, 1985) and more importantly, as far as this chapter is concerned, the chemistry of the tissue itself (Robinson *et al.*, 1981b, 1982; Deutsch and Pe'er, 1982; Robinson and Kirkham, 1984). It should be noted that all of these parameters are related and it is possible to correlate the chemistry of the subadjacent enamel with specific ameloblast morphology (Robinson *et al.*, 1981a).

These stages are: secretion, transition, maturation and mature tissue. Their characteristics are summarized in Fig. 1 (Robinson and Kirkham, 1990).

Abbreviations used in this paper: SDS, sodium dodecyl sulphate; PAGE, polyacrylamide gel electrophoresis.

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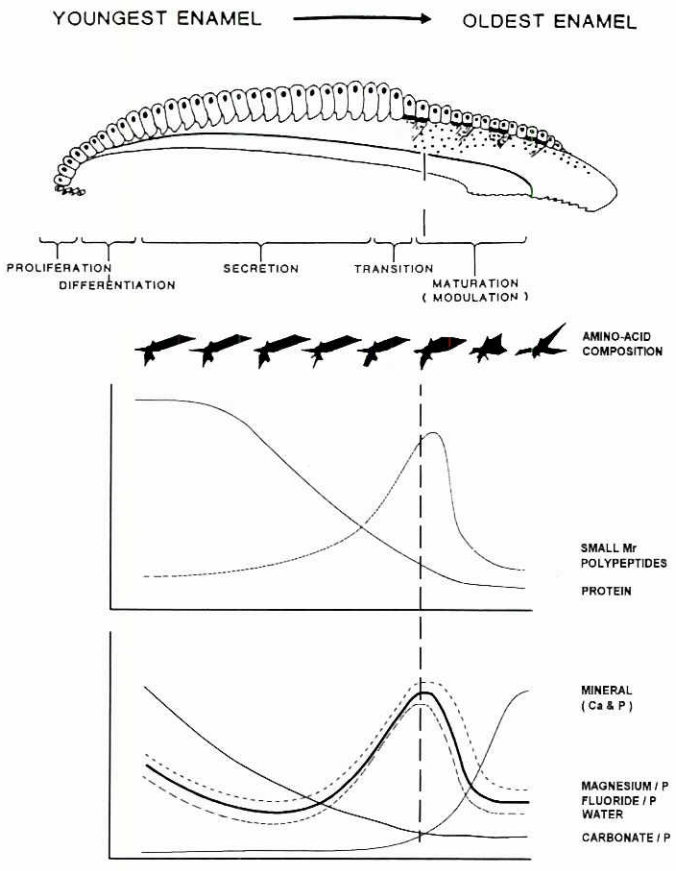

Fig. 1. Summary of chemical changes occurring during enamel development. *Changes in both mineral content and composition are illustrated together with alterations in matrix protein. The amino acid composition of matrix at each stage is shown as rose diagrams. The broken line represents the beginning of maturation. (Based on Robinson and Kirkham, 1990).*

Secretory stage

It is during this stage that secretion by the ameloblast is initiated. Matrix is deposited on the preformed dentine and the ameloblasts themselves acquire their characteristic Tomes processes and columnar morphology. Crystals of hydroxyapatite form almost immediately within the newly secreted matrix although at this, the earliest of stages, they are much less uniform in size and shape and are not organized into prisms (Warshawsky, 1971). Secretion continues as the ameloblasts retreat from the dentinal surface leaving behind a protein matrix in which is embedded the immature mineral crystals.

Mineral phase

The mechanism which results in the initiation of the mineral phase on the surface of the dentine is still a matter for speculation. Most authorities consider this to be the only site of mineral crystal initiation with subsequent crystal growth following the retreating ameloblasts. Growth follows the c-axis direction of the crystals, such that extraordinarily long flat crystals appear, running from the dentine to the enamel surface (Daculsi and Kerebel, 1978). While mature enamel gives well defined apatitic X-ray diffraction patterns (Frazier, 1967; Young and Spooner, 1969), the initial crystals give a much more diffuse pattern (Nylen *et al.*, 1963). The initial phase is thought by many to be of a more acidic character. It has even been suggested that a brushite like phase ($CaHPO_4.2H_2O$) may be present which may hydrolyze to apatite or acquire an apatite-like overgrowth. Octacalcium phosphate ($Ca_8H_2(PO_4)_6.5H_20$) has also been cast in this role, the great similarity of its crystal structure to that of apatite offering the possibility of almost isomorphous overgrowth (Brown *et al.*, 1962; Brown, 1966).

Extraneous mineral components are also acquired by the mineral at this stage which profoundly affect the chemical behavior of the enamel when subsequently exposed to the oral environment. Relatively high concentrations of both magnesium and carbonate have been reported in the earliest enamel i.e. that near the dentine surface (Hiller *et al.*, 1975; Robinson *et al.*, 1984; Aoba and Moreno, 1990). This pattern seems to persist into mature tissue (Robinson *et al.*, 1981c).

Carbonate can be very easily incorporated into the mineral as it transforms from simpler precipitated material into the more stable but more complex hydroxyapatite (Bachra *et al.*, 1965). The reason for preferential uptake of carbonate and magnesium at this developmental stage may be related to the early phases themselves being less well ordered and more capable of including extraneous materials and/or the relatively large surface to mass ratio of the young crystals. Carbonatoapatites are well known in skeletal tissues, with carbonate occupying phosphate or, at higher pCO_2, hydroxyl sites (Rey *et al.*, 1991). Such inclusions would tend to produce a less stable apatite. The location of magnesium is less clear. It is not so easily accommodated into the apatite lattice although distortions due to carbonate would facilitate magnesium uptake.

The chemistry of these early crystal deposits has a further implication in that they form the central core of the crystals of mature tissue which have developed by increasing girth in the

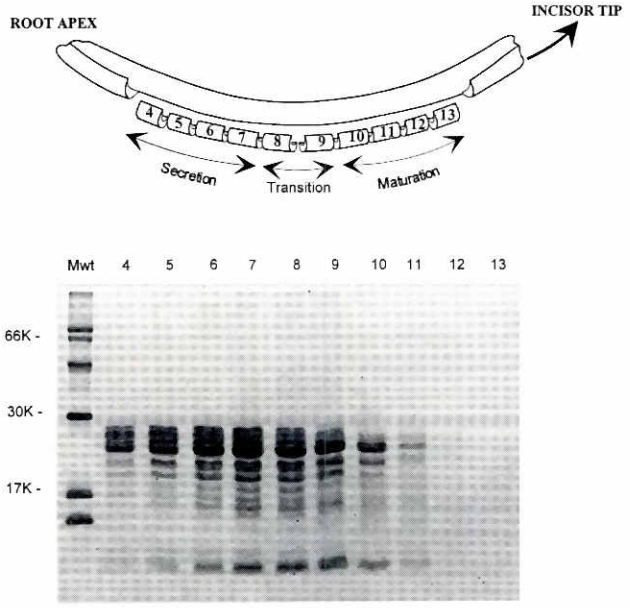

Fig. 2. SDS polyacrylamide gel electrophoretogram of enamel matrix proteins at each developmental stage. *Changes in molecular size distribution of matrix proteins largely result from proteolytic degradation of amelogenin. The major features of amelogenin processing are shown in Figure 3.*

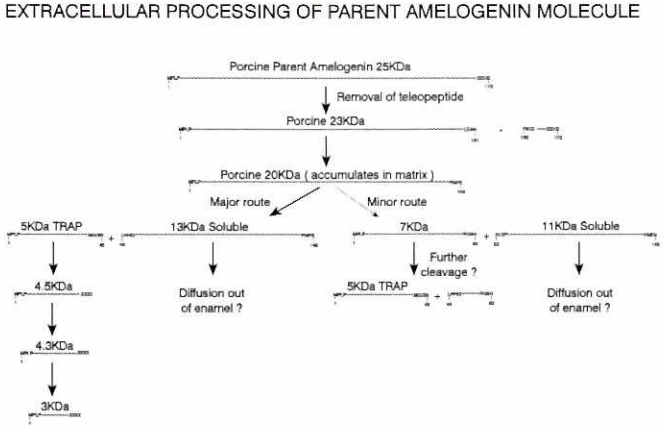

Fig. 3. Major extracellular secretory stage processing pathways for porcine amelogenin. *See Brookes et al., 1995.*

crystallographic a- and b-axis directions. An electron-dense central dark line in such crystals has been attributed to possible retention of early, less well ordered, phases. Preferential dissolution along this line also argues for a less well ordered domain (Johnson, 1967).

The alternative location for both carbonate and magnesium is at the crystal surfaces. The likelihood is that as crystals grow, magnesium and possibly carbonate phases are, to some extent, recrystallized towards the outer surface of the crystal. This has some support in that carious dissolution of mature enamel crystals results in preferential removal of carbonate and magnesium from the surfaces of the crystals as well as from their interior (Hallsworth *et al.*, 1972, 1973; Tohda *et al.*, 1989). This surface location has been explored further in some reports which have suggested that carbonate and magnesium may form part of very small but separate mineral phases — for example, dolomite and whitlockite (Driessens and Verbeeck, 1982, 1985). Both of these would account for much of the carbonate and magnesium present.

Fluoride is also accumulated during the early part of the secretory stage (Weatherell *et al.*, 1975, 1977). Unlike carbonate and magnesium, however, fluoride tends to favor hydroxyapatite formation and the formation of more highly ordered crystalline structure (Weatherell *et al.*, 1975), which apparently conflicts with the higher levels of carbonate and magnesium, and the apparent lower degree of crystalline order in the early enamel mineral. The high concentrations of all three components may, however, simply be related to the relatively large surface area of the young enamel crystals at this stage. Indeed the surface chemistry of such small crystals of the order of only one or two unit cells of apatite in thickness may be the dominant factor determining the properties of early enamel mineral.

As secretion proceeds, the concentrations of carbonate, magnesium and fluoride fall rather steeply towards the transitional stage (Fig. 1). This supports the view that some of these components are located in the crystal interiors and that the overall concentrations fall as magnesium-, carbonate- and fluoride-poor mineral is added to the growing crystals. It must also be said, however, that we may be also looking at a surface phenomenon, where the surface to mass ratio of the crystal falls, giving rise to apparently lower concentrations of these ions. Another possible location must also be considered in that some of these extraneous ions may exist in the matrix itself either associated loosely with the extracellular proteins or free in the tissue fluid. Some consideration of this will be given in the following section.

Matrix

The extracellular matrix, which is presumed to afford mechanical support as well as to provide structural information to the developing mineral phase, also undergoes profound alteration during this stage.

The extracellular organic matrix comprises a range of proteins many of which are unique to enamel. These proteins can be divided into two broad groups: 1) the amelogenins and their processing products which form the bulk of the matrix (i.e. about 90%) and 2) the non-amelogenins which comprise some 10% or so of the matrix (Termine *et al.*, 1980; Belcourt *et al.*, 1982). This latter group contains the proline rich non-amelogenins (Fukae and Tanabe, 1987), tuft related proteins (Weatherell *et al.*, 1968; Robinson *et al.*, 1975), tuftelin (Deutsch *et al.*, 1991) and certain serum proteins notably albumin (Limeback *et al.*, 1989; Strawich and Glimcher, 1990). The relative proportions of these groups and their processing products change during each developmental stage (Fig. 2). This is presumably associated with development of function for each protein group. There are in addition a number of non-structural entities such as proteolytic and phosphorolytic enzymes which effect the extracellular matrix processing mentioned above. Detailed consideration of these enzymes, however, is beyond the scope of this chapter.

The initial secretion of matrix which occurs on the surface of the pre-formed dentine is enriched with non-amelogenin components (Robinson *et al.*, 1977; Seyer and Glimcher, 1977). The precise identities of the proteins present at this stage is unclear but the so-called tuft proteins have been reported to be present on the dentine surface in mature tissue (Weatherell *et al.*, 1968; Robinson *et al.*, 1975). Recent work has also demonstrated a relationship, albeit immunologically, between certain proline rich non-amelogenins (Mr 13-17 kDa) and tuft proteins (Amizuka *et al.*, 1992). Immunological evidence has also placed material related to tuftelin at this early stage (Deutsch *et al.*, 1991). Tuftelin is apparently an acidic protein unique to enamel. It has been identified from a cDNA clone and antibodies to synthesized peptides were used to establish a possible location (Deutsch *et al.*, 1991). Whatever tuftelin is, it may,

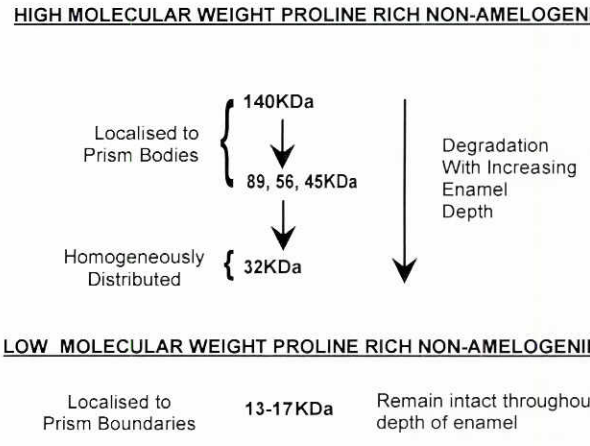

Fig. 4. Extracellular processing of proline rich non-amelogenin matrix proteins with ultrastructural locations. *Compiled from Uchida et al., 1991a,b.*

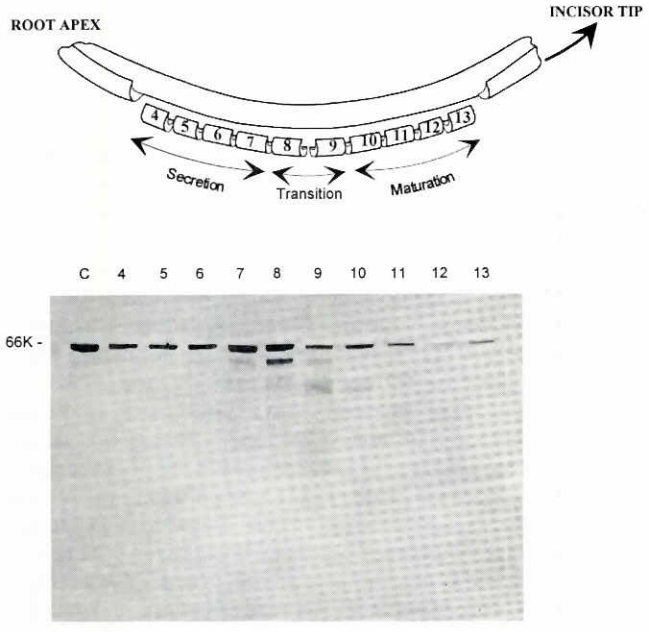

Fig. 5. Distribution of albumin at each developmental stage of rat incisor enamel as indicated by Western blotting using polyclonal antibodies to rat serum albumin. *Albumin is present as the intact molecule (molecular weight 66 kDa) throughout secretion. During transition and maturation albumin breakdown products become evident followed by almost complete removal of albumin from the later maturation stage. C, control rat serum.*

together with tuft proteins and possibly proline rich non-amelogenins, be concerned with initial mineral deposits on the dentine surface. However, the precise identity of a specific nucleating species has not been obtained. Current feeling is that nucleation of the mineral phase in enamel may in fact be in some way related to the dentine interface involving either the extracellular dentine matrix (Fearnhead, 1979) or the dentine mineral itself (Arsenault and Robinson, 1989).

Following this initial phase, secretion proceeds by the acquisition of primarily amelogenin until it and its accumulating processing products comprise some 90% of the total matrix protein (Termine *et al.*, 1980; Belcourt *et al.*, 1982). It is during the early part of this process that prismatic structure begins to develop. Immunohistological data suggest that at least some of the proline rich non-amelogenins (the 13-17 kDa group) tend to be confined to the prism boundary/interprismatic regions of the tissue (Uchida *et al.*, 1991b). In contrast, high molecular weight proline rich non-amelogenins (56-140 kDa) appear to be restricted to the prism bodies in the more recently secreted surface layers of enamel (Uchida *et al.*, 1991a,b). Interestingly, from the point of view of control of crystal growth, some elements of this group appear to be, at least in part, mineral bound.

It is tempting to conclude that an interplay between these two protein groups, i.e. amelogenins and proline rich non-amelogenins, is responsible for crystal morphology and orientation, leading to the development of prismatic/interprismatic architecture.

In this respect it is pertinent to consider aspects of the chemistry of enamel extracellular matrix processing in relation to mineral development. The dominant features of the bulk extracellular matrix are major degradation products of the nascent amelogenin (Eggert *et al.*, 1973; Termine *et al.*, 1980; Fincham *et al.*, 1982a,b; Brookes *et al.*, 1995). These are respectively molecules of 23, 20 and 25 kDa (SDS values). In the older, deeper tissue, the 20 kDa molecule dominates the matrix while both 25 kDa parent and the 23 kDa molecules can be regarded as more transient, being present in quantity only in the recently secreted surface layer of enamel. The older, deeper layers also contain a range of lower molecular weight components which appear to be derived from the parent 25 kDa amelogenin as shown in Fig. 3. Degradation generates distinct cumulative products throughout the secretory stage, although some protein turnover is also apparent. During the late secretory stage, loss of matrix protein becomes more pronounced and the water content of the tissue begins to increase as lost matrix is replaced by tissue fluid (Robinson *et al.*, 1988).

A consideration of these chemical changes strongly indicates that degradation and removal of amelogenin is a necessary prerequisite for crystal growth. This has raised two questions: 1) Is matrix removal merely effected to produce space into which crystals can grow? or 2) Is the matrix acting in the capacity of a crystal growth inhibitor which must be removed to permit crystal growth? Elucidation of the precise relationship between matrix and mineral is hampered by the fact that much of the matrix appears to be in the solid phase.

In vitro investigations, using matrix components rendered soluble by demineralizing the tissue, showed that the nascent 25 kDa amelogenin, present in the newly secreted surface layer of enamel, selectively bound to synthetic mineral and inhibited further mineral deposition. In contrast, degradation products of the nascent 25 kDa amelogenin (e.g. 20 kDa amelogenin) had a much reduced affinity for the mineral and did not greatly inhibit further mineral deposition (Aoba *et al.*, 1987). This data should be regarded with some caution, however, since experiments were conducted at pH 6.0 rather than pH 7.26 (the value reported by Aoba and Moreno (1987) to be the pH of the secretory stage enamel tissue fluid). The sensitivity of these experiments to pH is evident since the difference in binding affinity existing between the 25 kDa nascent amelogenin and its 20 kDa degradation product at pH 6.0 disappeared when the experiment was conducted at pH 7.8. In addition, it is not clear whether the matrix proteins used in the experiments still held their native conformations following extraction from the tissue. The specific conformation of the matrix proteins could have significant effects on their mineral binding properties.

It is possible that *in vivo* degradation of the 25 kDa nascent amelogenin occurring soon after its secretion allows for the increased crystal growth that has occurred in older, deeper layers of the tissue. It should also be pointed out that crystals in the deeper layers of tissue are older and their increased size may simply be due to their longer exposure to the mineralizing environment of the enamel matrix rather than the removal of the inhibitory nascent 25 kDa amelogenin.

The chemistry of amelogenin/mineral binding still remains to be clarified *in vivo*. No specific mineral binding groups have been identified. Although previous investigations have focused on the role of the amelogenins in matrix/mineral interactions, more recent studies have raised questions concerning the role of proline rich non-amelogenins.

High molecular weight proline rich non-amelogenins (140 kDa and below) have been shown to undergo a similar controlled degradation process to the amelogenins resulting in the generation of various processing products followed by a more comprehensive

degradation to much smaller peptides (Uchida *et al.*, 1991a,b) (Fig. 4). These high molecular weight proline rich non-amelogenins and a related 32 kDa breakdown product appear to exhibit a high affinity for mineral (Tanabe *et al.*, 1990) and their controlled degradation may be significant in terms of controlling crystal growth. In contrast, the 13-17 kDa proline rich non-amelogenins, which seem to be restricted to prism boundaries, appear to be stable, at least throughout enamel secretion.

Of perhaps equal importance with respect to crystal growth is the possible role of serum proteins in enamel, notably serum albumin. Albumin has been shown to be present in secretory stage enamel (Limeback *et al.*, 1989; Strawich and Glimcher, 1990) with maximum concentrations occurring between the late secretory/early maturation stages (Robinson *et al.*, 1994). The presence of albumin is potentially important since it has been shown to both bind to apatite and inhibit apatite crystal growth (Garnett and Dieppe, 1990; Robinson *et al.*, 1992). The precise status of albumin in secretory stage enamel *in vivo* is unclear. It may be bound to the mineral (as suggested by a requirement for demineralization for its extraction) or it may merely be associated with the extracellular matrix.

There is evidence to suggest that albumin degradation does occur prior to the maturation stage resulting in a loss of albumin from the maturing tissue (Fig. 5). This may be significant in terms of the inhibitory properties of albumin described above. Albumin may serve to "damp down" crystal growth during secretion or (because of its calcium binding ability) to act as a calcium buffer in the tissue. It is conceivable that no chemical binding occurs between matrix components and mineral. The close proximity of solid state amelogenin, non-amelogenin and/or serum proteins to the crystal surfaces might simply provide a physical restraint on crystal growth. In this respect the physical ultrastructure of the matrix would be expected to guide and direct the development of crystal morphology, exerting a moderating influence on growth.

Transitional stage

As the ameloblasts approach the surface limit of the enamel, secretion of matrix slows and ultimately stops. Withdrawal of matrix which has begun during secretion becomes more evident and the water which replaces it begins to increase dramatically (Robinson *et al.*, 1982, 1988). This generates extensive porosity within the tissue which has a considerable bearing on its behavior.

Mineral phase

During the transitional stage, growth of crystals has already occurred to some extent in the deeper layers of the enamel. However, the average mineral content of the tissue has not significantly altered, and even the oldest inner layers of enamel are still far from the mature state (Robinson *et al.*, 1981b, 1982; Robinson and Kirkham, 1984). Towards the end of this stage, however, there is evidence of an increase in mineral content overall which is indicative of an increase in crystal growth. The crystals presumably grow to replace fluid which had in turn replaced the now rapidly degrading enamel matrix.

The chemical nature of the mineral phase also appears to alter at this stage. Magnesium and fluoride in particular have been shown to be selectively acquired by the enamel during the transitional stage (Hiller *et al.*, 1975; Robinson *et al.*, 1981c, 1984; Aoba and Moreno, 1990) (see Fig. 1). This may be due in part to the changes in ameloblast/enamel organ cell biology occurring at this stage (Reith, 1963; Smith and Warshawsky, 1975; Robinson *et al.*, 1981a) but in addition, the hugely hydrated and porous nature of the tissue must inevitably have an influence on the access of magnesium and fluoride ions to the mineral phase (Robinson *et al.*, 1988). Since magnesium and fluoride have opposite effects on the growth of hydroxyapatite, the net effect of this accumulation is difficult to discern (Robinson, 1984). It is also possible that some of this fluoride is not mineral bound and may reside in the consider-

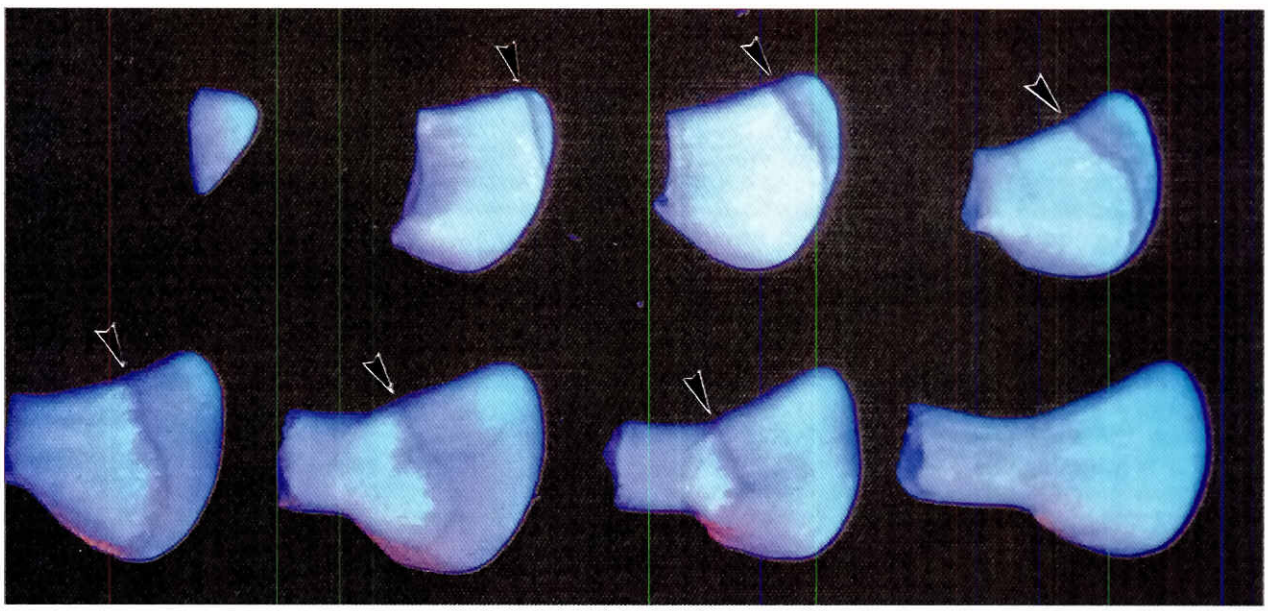

Fig. 6. Developing foetal bovine incisors from earliest crown formation to eruption viewed in ultra-violet light (Robinson *et al.*, 1978). *The dark line (arrowed) representing the beginning of maturation can be seen advancing from the incisal edge towards the cervical margin following the growth of the tooth crown.*

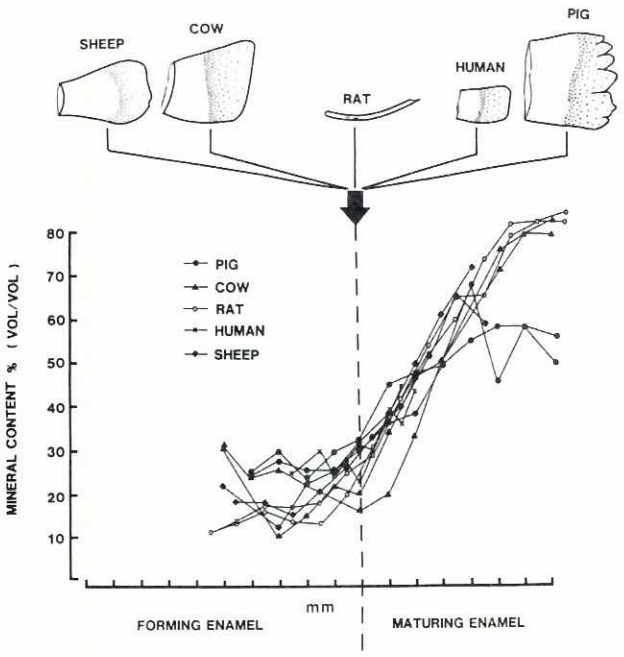

Fig. 7. Mineral content per volume of enamel at each developing stage from several species. *The pattern of mineral uptake is similar regardless of species or tooth type (see Robinson and Kirkham 1984). The broken line represents the beginning of maturation.*

able amount of fluid found at this stage (Weatherell *et al.*, 1977). This information has had some considerable bearing on fluoride research when attempting to decide the stage at which the developing tooth can take up the most effective amounts of fluoride to provide a beneficial effect with regard to caries prevention (Wagner *et al.*, 1993).

Matrix

At the transitional stage the matrix exhibits considerable evidence of degradation in that there is a very obvious increase in the proportion of low molecular weight components (Fig. 1) (Glimcher *et al.*, 1977; Robinson *et al.*, 1982). Much of this material relates to degradation products of the amelogenins, although as described above, proline rich non-amelogenin degradation may also generate low molecular weight fragments. Also present during transition are enzymes which are capable not only of generating discrete breakdown products but also of degrading matrix components completely. Considerable matrix loss is also apparent since much of it has been clearly replaced by tissue fluid.

The parent amelogenin molecule is no longer in evidence in transition stage enamel and amelogenin fragments at about 5-10 kDa predominate. These fragments, presumably including TRAP (tyrosine rich amelogenin peptide), are found together with any remaining 20 kDa amelogenin component (Fincham *et al.*, 1982a; Robinson *et al.*, 1982). The role of these proteins is still significant since it is thought that even this residual matrix must be removed before full crystal growth can occur (Robinson *et al.*, 1989). Albumin which reaches a peak in overall concentration at this stage also shows considerable evidence of degradation (Robinson *et al.*, 1994) (see Fig. 5). Since this molecule has shown an ability to bind to apatite and to inhibit crystal growth its role as a modulator of the development of the mineral must be seriously considered.

Maturation stage

The maturation stage of enamel development follows on directly from transition. The large amount of water acquired by replacement of the matrix dries out very quickly if the tooth is removed from the jaw producing a sharp boundary between the transitional stage and the white opaque tissue of maturing enamel (Robinson and Kirkham, 1985). This has been used as an internal marker to identify stages of development (see Fig. 6). By the maturation stage changes in ameloblast morphology begun during transition are completed. For example, Tomes processes disappear, the internal structure of the ameloblasts is totally rearranged and in many species cell length reduces by about 50% (Reith, 1963; Smith and Warshawsky, 1975; Robinson *et al.*, 1981a).

Mineral phase

The overall mineral content of the enamel begins to rise steeply at the beginning of maturation (Fig. 7) (Robinson *et al.*, 1982; Robinson and Kirkham, 1984a). This is presumably due to a massive increase in uptake of mineral ions (Robinson *et al.*, 1974). This rise continues until values characteristic of mature enamel are achieved i.e. 80-90% mineral by volume. Final mineral levels vary somewhat from species to species. For example, human deciduous enamel is often as high as 95% mineral by volume (Kirkham, unpublished results) while human permanent enamel is usually on average 85% mineral by volume. In the domestic pig, enamel often does not reach more than 60% mineral by volume (Kirkham *et al.*, 1988).

It is also important to point out that enamel does not mineralize homogeneously. In the thicker enamels, inner tissue is often less well mineralized than outer tissue. The reasons are not clear but may be related to the local capacity of the matrix to facilitate crystal growth.

The chemistry of enamel mineral during maturation also varies. The selective uptake of fluoride and magnesium reaches a maximum at the beginning of maturation, thereafter decreasing towards the mature tissue (Hiller *et al.*, 1975; Weatherell *et al.*, 1975, 1977; Robinson *et al.*, 1984) as shown in Fig. 1. Some of this decline may be due to increasing acquisition of mineral low in both magnesium and fluoride. Measurements made on a volume basis, however, suggest that there is a real decrease in both magnesium and fluoride as maturation proceeds (Robinson *et al.*, 1984). This has raised some speculation as to the location of both ions in the tissue. Fluoride as a substituent of the apatite lattice is unlikely to be lost since it has a stabilizing effect on enamel mineral. It must be assumed that a considerable portion of this fluoride is either free in solution or loosely bound in some way to the disappearing organic matrix. This may be very significant, however, since ameloblasts adjacent to such tissue might be exposed to relatively high levels of fluoride locally with the possibility of effects on enamel development.

Matrix

The matrix composition of the maturation phase which gradually becomes mature enamel reflects the final stages of matrix degradation and withdrawal. Early maturation contains a gradually decreasing concentration of the dominant amelogenin processing products, notably the 20 kDa molecule together with some smaller fragments of small molecular size including amino acids. Whether these are derived from amelogenin or non-amelogenin is not totally clear. The final composition at the end of the maturation process

seems to reflect that of tuft protein together with very small fragments presumably originating from both amelogenin and non-amelogenin processing (Glimcher *et al.*, 1964).

Acknowledgments

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