

## The experimental investigation of odontogenesis

A. RICHARD TEN CATE\*

Faculty of Dentistry, University of Toronto,  
Toronto, Canada

### Introduction

I have been given no easy task. The history of the experimental investigation of odontogenesis properly documented would readily occupy this entire volume, and I doubt whether the reader of this essay is interested in a detailed chronological account of the many experimental investigations that have led to our present state of knowledge. In any event, excellent accounts up to the late eighties exist (Gaunt and Miles, 1967; Slavkin, 1974; Kollar, 1983; Ruch, 1984; Lumsden, 1987) and the other contributors to this volume will, I suspect, complete the record to the present time. Instead, I have elected to analyze the impact of experimental investigation, coupled with continued technical development, on the evolution of our ideas concerning odontogenesis.

To begin with, it is necessary to define what is meant by odontogenesis. The usual connotation of the term is that it describes events relating to the origins and formation of teeth, a definition that has been expanded by Kollar and Lumsden (1979) to include the overlapping events of initiation, morphogenesis and cell differentiation. I wish to expand this definition even further so that it also includes the origins and formation of tooth-supporting tissues, namely cementum, periodontal ligament and alveolar bone for, as will be described later, these tissues are also truly dental.

To analyze how experimental design has influenced the development of ideas about odontogenesis, it is also necessary to understand how ideas are formed and how they may influence thinking. Any idea is a notion conceived by the mind and is dependent upon the conscious and sub-conscious analysis of a myriad of facts accumulated in many different ways including, in the context of this essay, those derived from direct observation of normal developmental events and from experimental investiga-

tion. Chance, imagination, intuition and reason are also involved. Furthermore, it needs to be recognized that a function of an idea is to be useful and interesting, even if not correct, for attempts to determine the truth of an idea form the foundation for experimental investigation. And while it is true that on occasion an experimental investigation may reveal or confirm a fundamental truth by testing and proving a hypothesis, more often than not such investigation harvests more facts which may assume significance only when reason is applied to formulate further hypotheses which, in turn, demand further experimental investigation. Thus, the cascade of inquiry and discovery is continued.

It also needs to be recognized that underpinning most experimental investigations is the continued development of technologies of increasing sophistication, that now permit the dissection of biological events at the molecular level. But it is important to understand, and make distinction between, experimental design and the techniques used to undertake an experiment. Good experimental design reflects the recognition of the problem, the need to ask crucial questions and possess knowledge of both the potential and the limitations of techniques available to answer the questions posed. As far as odontogenesis is concerned, once the associated technical underpinning is stripped away, experimental design has been fairly circumscribed and essentially involves ablation, transplantation and substitution (recombination) of tissues either *in vivo* or *in vitro*.

In assessing how experimental investigation has influenced our understanding of odontogenesis, and to indicate how such investigations form an evolving, continuous and expanding inquiry, I wish to focus on three aspects of odontogenesis, namely the initiation of the tooth, its morphogenesis, and the pattern of cell differentiation as related to the development of the tissues associated with tooth support. Events related to the differentiation of the

\*Address for reprints: Faculty of Dentistry, University of Toronto, 124 Edward Street, Toronto Canada M5G 1G6.



hard tissue forming cells, the odontoblast and the ameloblast, are covered by other contributors to this volume.

### Initiation of the tooth

Experimental investigation of odontogenesis began some sixty years ago. Before that time most of our ideas concerning the development and structure of teeth came from facts derived from direct and detailed observation of histological sections coupled with increasing improvements in light microscopy and tissue preparation. We do owe a great deal to classical observations made by such pioneers as Tomes (father and son), von Ebner, Retzius, Hertwig and so on, whose names will always be linked with descriptive dental histology. But it was the experimental investigation of some of the broader problems in the field of general embryology that first focused attention on a specific role for neural crest tissue in odontogenesis, and initiated the experimental analysis of tooth formation.

#### The role of neural crest

##### *Amphibian studies*

It was the early work of Adams (1924), Stone (1926) and Ch.P. Raven (1931), which indicated that when neural crest is ablated in amphibian embryos, first arch cartilage fails to develop. But it was Sellman (1946) using a combination of extirpation, replantation and transplantation of neural crest *in vivo* in amblystoma larvae, who precisely localized that portion of the neural crest associated with first arch and dental development, and hinted that the capacity for proper first arch development depended upon an interaction of neural crest cells with stomodeal epithelium as they migrated into the first arch to become ectomesenchyme. Confirmation of Sellman's findings quickly followed using two different experimental approaches, namely *in vitro* and chimeral studies.

Wilde (1955) combined urodele neural crest in combination with stomodeal epithelium *in vitro* to produce fully formed tooth germs. In retrospect, this proved to be an experimental design of importance for it was later used by Lumsden (1984) to demonstrate for the first time that mammalian neural crest was also involved in odontogenesis. Wagner (1949, 1955) produced chimerous larvae and teeth by transplanting *in vivo* various combinations of frog and newt tissue. In the frog mandible teeth are simply keratinous appendages with no phylogenetic relationship to teeth proper (which consist of dentine and enamel) whereas newts develop true teeth. When frog neural crest was transplanted and substituted into the newt, teeth developed which consisted of newt dental organ and frog papilla, a result that was taken to indicate the potential of frog neural crest-derived cells to form not only dental papilla but also to initiate an epithelial response with the formation of a dental organ. The possibility that newt epithelium influences frog mesenchyme was not considered. In the reverse experiment, where newt neural crest was substituted in the frog, no teeth formed, a result which was interpreted as showing a lack of competence on the part of frog epithelium to respond. Again, the possibility that frog epithelium lacks a capacity to influence ectomesenchyme was ignored. A counterpoint to these experiments was performed by Henzen (1957) who substituted oral epithelium of newt into frog and achieved the development of true teeth in the frog, again strongly suggesting a lead role for epithelium in odontogenesis, but interpreted in exactly the

opposite way with a lead role for ectomesenchyme claimed. Thus Gaunt and Miles (1967) summarized,

"Most of the foregoing observations raise questions as to the source of the primary impetus in tooth development. They appear to indicate that in amphibians the ectomesoderm (ectomesenchyme) of the head region is the primary organizer that induces changes in the mouth ectoderm leading to the differentiation of groups of cells into epidermal dental caps."

This was a somewhat surprising conclusion as an interaction between epithelium and mesenchyme in dental development had already been identified, with epithelium as prime in well-designed transplantation experiments involving autografts in the dog where it was shown for the first time that the dental (enamel) organ was responsible for the differentiation of odontoblasts (Huggins *et al.*, 1934). One can only surmise that the conclusions drawn from these experiments were steered by a wish to determine a role for neural crest derived cells in odontogenesis.

In sum, by the early sixties, an appreciation had developed from the result of elegant experimental studies, involving ablation, transplantation and substitution in amphibia (because of the accessibility of the developing larvae), that neural crest-derived tissue was involved in tooth initiation.

##### *Avian studies*

The logical question that followed was whether the events occurring in amphibian development represented a basic tenet of facial and dental development in all species. Attention transferred to the study of neural crest involvement in the facial development of avian embryos, again largely because of the availability of the embryo for study. Johnston (1966) was the first to show that neural crest was involved in the development of first arch structures. He first labeled neural crest cells *in situ* with tritiated thymidine, and then excised and transplanted them into a second embryo. Following further development, and with use of autoradiography, he was able to demonstrate the migration of labeled cells to first arch. Again, following the sequence of amphibian experiments, chimeral experiments quickly confirmed Johnson's observations. Quail cells are distinguished by a distinctive accumulation of heterochromatin in the nucleolus (Le Douarin, 1982) which thus constitutes a natural marker and, in substitution experiments between quail and chick crest, the former cells were easily identified in chick first arch structures.

These experiments unequivocally established that neural crest-derived cells are also involved with facial development in the chick but, as teeth do not develop in avian embryos, a linkage to odontogenesis can be questioned. This is now a hypothetical question as tissue recombination experiments *in vitro* (discussed in detail later) have shown that first arch mammalian ectomesenchyme can unleash a dormant response from avian epithelium to form teeth (Kollar and Fisher, 1980), an observation that seemingly supports a dominant role for ectomesenchyme in odontogenesis.

##### *Mammalian studies*

It is now possible to maintain mammalian embryos in a culture situation, and selectively label neural crest and show migration to first arch following the protocols established in amphibian and avian experiments, but this is a recent development. In historical terms early attempts to maintain mammalian embryos in an external environment were only partially successful. Thus Johnston and Hazleton (1972) achieved some success by exteriorization of



mammalian embryos, labeling neural crest cells with tritiated thymidine and following their fate for a sufficient time to reveal the presence of such cells in first arch. Embryos handled in this way did not, however, survive for a sufficient time to demonstrate the fate of the labeled cells.

Circumstantial evidence, derived from a series of experiments designed to prevent or inhibit the migration of neural crest in mammalian embryos, and observation of the developmental outcome, supported the general thesis. Thus, treatment of mouse embryos with teratogens which interfere with cell division (Bhadinaronk *et al.*, 1974) before crest cell migration results in mesenchymal deficiencies in the facial processes. A Teacher-Collins-like malformation (Morriss-Kay, 1972; Poswillo, 1975) results when rats are treated with excess vitamin A (*in vitro* excess vitamin A has the effect of inhibiting the translocation of mesenchymal cells) before crest migration.

The issue was finally settled by Lumsden (1984) who, borrowing the experimental design used by Wilde (1955), recombined mouse neural crest cells directly with mandibular epithelium in the anterior chamber of the eye and achieved tooth formation. In sum, not until 1984 was proof definite that mammalian neural crest was involved in dentinogenesis, although this was generally assumed to be the case throughout the seventies and dictated experimental design to a significant degree.

#### **Epithelium or mesenchyme as instructor?**

The ability to recombine tissues from different sites and observe further development has had a tremendous impact upon the whole field of developmental biology, including odontogenesis. The history of the development of this technology, while of interest, is not the subject of this essay other than to indicate that initially it involved *in vitro* studies, followed by the use of *in vivo* locations such as the chick chorio-allantoic membrane, or the anterior chamber of the eye, both of which provide in essence a biologically defined culture medium.

While this technology has been most helpful, results have been, at times, contradictory in terms of determining the roles of epithelium and ectomesenchyme in odontogenesis. The emphasis placed on neural crest-derived tissue as prime in odontogenesis stemmed from the experimental studies in amphibia already described, and seemed to be confirmed in a whole series of experiments conducted with mammalian tissue. Recombination of first arch ectomesenchyme with embryonic plantar epithelium changes the developmental direction of the epithelium so that it forms dental organ (Kollar and Baird, 1970). Conversely, the reciprocal recombination of foot mesenchyme with dental organ switches the latter's developmental pathway to form keratinized surface epithelium. Recombination of molar papilla with incisor dental organ results in molar development, and conversely, recombination of incisor papilla with molar dental organ results in incisor development (Kollar and Baird, 1969). The recombination of mammalian (mouse) ectomesenchyme with avian (chick) epithelium (Kollar and Fisher, 1980) results in tooth formation. All these findings suggest the dominance of ectomesenchyme in odontogenesis. Only one study (Miller, 1969) contradicted these conclusions. Miller successfully separated epithelial and mesenchymal tissues and recombined them on the chick chorio-allantoic membrane in various combinations. Thus, incisor ectomesenchyme was combined with molar ectoderm, and vice-versa, as well normal recombinations. Miller found that the morphogenetic

field for the molars was determined by E10 and the incisors by E10.5, and that from then on ectoderm determined tooth type.

Nevertheless, the idea that ectomesenchyme was prime in odontogenesis raised a series of further questions. For example, when is this instructive capacity assumed: is it present within neural crest cells before they migrate, is it assumed as they migrate, possibly requiring endodermal contact as hinted by Sellman (1946), or is it assumed after migration is completed when the cells are resident in the first arch? If the first proposition is correct, the specificity of neural crest needs to be determined. A further question relates to the determination of the migratory pathway to assure that neural crest cells reach the first arch.

The answer to the last question has been resolved using heterotrophic transplantation experiments (Noden, 1975) which showed that migratory pattern for neural crest is not intrinsic to the migrating cells but is determined by resident factors in the tissues through which the cells pass. Latex beads injected into crest migratory routes mimic the normal patterns of dispersal (Bronner-Fraser, 1982) and there is strong evidence that this pathway is determined by the local expression of cell surface and extracellular molecules such as tenascin and syndecan (Mina *et al.*, 1990).

The answers to the remaining questions came from well-designed and ingenious experiments conducted by Lumsden (1984) which have already been referred to. Thus, premigratory cranial and caudal neural crest cells were dissected out from mice and combined in the anterior chamber of the eye with either mandibular or limb-bud epithelium in various combinations. In sum, it was shown that only when neural crest cells were combined with mandibular epithelium did teeth develop, indicating that mammalian neural crest only expresses its odontogenic potential when associated with a regionally appropriate epithelium. As already indicated, these findings also provided the first direct evidence that mammalian neural crest, be it cranial or caudal, participates in tooth formation. They also indicated that neither the process of cell migration itself, nor itinerant contact with pharyngeal endoderm, is a requirement. A slight problem here is that Lumsden was unable to show that mandibular epithelium could induce odontogenic development from a foreign (limb) mesenchyme, but this begs the question whether limb mesenchyme is in fact ectomesenchyme.

Thus, a set of questions posed to confirm ideas related to the dominance of ectomesenchyme provided experimental results to support epithelial dominance, as suggested by Miller (1969), and this represents a good example of how useful and interesting ideas, as indicated at the outset, can be of significance even though not necessarily correct.

Subsequent and additional evidence that epithelium is crucial for the initiation of odontogenesis comes from continued descriptive and experimental studies. In mice a diastema exists between incisor and molar teeth and absence of dental lamina is claimed (Mina and Kollar, 1987) but disputed (Petrokova, 1983). More significant, and more exciting, is the identification and experimental demonstration of the mediating signal within epithelium that initiates tooth development. Over the past few years there have been tremendous advances in technologies (immunohistochemistry, *in situ* hybridization, transgenic animals) which have enabled the identification and localization in tissue sections at the molecular level various growth factors, transcription factors, structural molecules of the extracellular matrix and cell surface, and gene expression. In terms of odontogenesis, the emphasis has until recently been on the identification of the expression and localization of these molecules, and inference of their possible roles by



relating them spatially and temporally to descriptive developmental events. This is a situation reminiscent of the development of histochemical techniques in the early 1950s, when the potential of these techniques was only harnessed to an experimental base following an intense descriptive phase. On the basis of mapping studies of BMP-4, a member of the TGF $\beta$  superfamily, it has been shown that this soluble growth factor is initially expressed in the epithelium, followed by expression in the ectomesenchyme suggesting that it may act as a mediator. That this is the case has been confirmed experimentally (Vainio *et al.*, 1994) by the local application in culture of BMP-4 to presumptive dental ectomesenchyme and demonstrated a response from the ectomesenchyme similar to that produced following recombination of first arch epithelium and ectomesenchyme.

But this now raises the question as to what causes the epithelium to express BMP-4, and a note of caution must be expressed here. In the long run it really is not too important to determine which tissue is prime. It may well be with our appreciation of the Hox genes and their expression that a further shift may occur as developmental events are pushed further back in ontogeny. What is abundantly clear is that a reciprocal relationship of delicate proportions exists during odontogenesis and that temporal parameters are of considerable importance.

#### Temporal parameters

Recognition of temporal parameters enables reconciliation of seemingly accurate but conflicting experimental results.

Kollar (1972) has stated: "Perhaps even more interesting is the possibility of exchanging tissue fragments of differing developmental stages (Rawles, 1963). Experimentally altering the ages of reacting tissues emphasizes and capitalizes on the basic, but often overlooked, fact that embryos have temporal as well as spatial parameters. Tissue capabilities or the effects of inductive processes at one time result in what we recognize as normally expected development. But at some earlier (or later) time the experimental interplay of tissue capabilities and inductive intensities have changed and are out of phase. These novel confrontations may express something new, unusual or instructive. Embryo's age and the aging process may play a decisive role in selecting or limiting developmental expression and determining form".

This point is well exemplified by some critical experiments undertaken by Mina and Kollar (1987) who dissected out the first and second branchial arches of mouse embryos of 9-13 days of gestational age (E9-E13), separated the epithelial and ectomesenchymal components and then recombined them heterotypically and grafted them into the anterior chamber of the eye. The outcome was that when mandibular arch epithelium was combined with second arch mesenchyme, teeth formed only in E9 through E12 day material, with the highest incidence occurring at E11 days. No teeth formed in E13 day material. On the other hand, when mandibular arch ectomesenchyme was recombined with second arch epithelium, tooth formation resulted only in E12 and E13 day grafts. These results indicate that first arch epithelium has odontogenic potential up to E12 days of gestation, and is able to elicit a reaction from ectomesenchyme of the second arch. Thereafter, this odontogenic potential is lost from the epithelium but interestingly is now assumed by the ectomesenchyme. This finding not only indicates an epithelial role in odontogenesis, but emphasizes the importance of chronology and likely explains some of the conflicting results and interpretations from other heterotypic recombination experiments. It is thus becoming clear from the sum

of all the experimental work that in terms of odontogenesis the initial patterning for tooth development resides in the epithelium, and that thereafter the induced ectomesenchyme assumes a dominant role in terms of morphogenesis.

#### Tooth morphogenesis

The same techniques of recombination have been used to address the issue of tooth morphogenesis. It is clear from the now almost classical studies of Kollar and Baird (1969) that the ectomesenchymal dental papilla dictates the shape a tooth assumes once initiation has taken place. How this is achieved is not known exactly, but control of cell division within the internal dental epithelium seems to be a factor.

Far less is known about the determination of heterodonty in mammalian dentitions. Two hypothetical models exist, namely the field model of Butler (1967) and the clone model of Osborn (1973). In the field model it is proposed that tooth shape is determined by two independent variables: a series of tooth-forming locations (in the epithelium?) distributed along the jaw and a continuously graded internal environment (in the mesenchyme) that constitutes a morphogenetic field. Thus, a tooth bud forming at a given location develops according to its position in the field. It was further supposed that the field was divided into three regions, corresponding to incisors, canine and molar. On the other hand, the clone model proposes that each tooth class is derived from a clone of mesenchymal cells programmed (by the epithelium?) to produce teeth of a given pattern.

As Butler (1978) has stated: "Only by more experimental investigation of the embryo can we solve the intriguing problem of the ontogenetic basis of heterodonty. In particular, we need to know more about the processes that precede tooth initiation in the mouse between the 8th and 11th days. The differentiation of the migrating mesenchymal cells needs further investigation and the role of the prospective dental epithelium needs clarification. Does the epithelium, for example, influence the differentiation of the mesenchyme? What determines the locations at which tooth germs are initiated?"

We know in part the answer to the first question — yes, the epithelium does initiate a response from the ectomesenchyme to initiate tooth formation. Whether the epithelium also imparts to the ectomesenchyme the responsibility for determining tooth shape and locations, are questions that have been answered in a series of experiments by Lumsden and his co-workers (Lumsden, 1979, 1984; Lumsden and Buchanan, 1986) involving recombination and intraocular grafting.

Before providing the results of these experiments, it is necessary to mention the work of Glasstone, a pioneer of *in vitro* studies of tooth development, and Main (1966). In one set of experiments Glasstone (1967) bisected 20-day rabbit tooth germs buccolingually at a time before cusp outlines developed, and found that each half continued development to form an entire tooth—albeit somewhat smaller than the original. She also demonstrated that this ability was lost when the experiment was repeated, using 22-day old tooth germs. These observations indicate that, for a time at least, the tissues of the early tooth germ have the capacity for self regulatory development. This observation was confirmed and extended by Slavkin and Bavetta (1968), who showed that this capacity exists as soon as the dental lamina appears. Also germane to the discussion that follows is the work of Main (1966) who showed that when tooth germs were cultured on gelatin sponges for an extended period, regression occurred. All that remained of a once



organized tooth germ were a few ectodermal cells, the remnants of the dental organ, and a few ectomesenchymal cells, the remnants of the dental papilla, scattered amongst the interstices of the sponge. Significantly, when these few cells were harvested and implanted subcutaneously, they reformed their anatomical relationships and produced a tooth with both dentine and cementum tissues present. At that time these findings were used to indicate the retention of a potential to form teeth in disassociated cells, so that not only has the early tooth germ the capacity for self-regulatory development, but seemingly it can retain this for extended periods of time.

In his first series of experiments Lumsden (1979) showed that isolated presumptive first molar tissue of E12 day old and later embryos gave rise to all three molar teeth in their normal sequence with normal shapes and relative sizes. As the second and third molar primordium were totally absent from the explanted tissue, this result, taken with Glasstone's (1967) demonstration of self-regulatory development and Main's demonstration of retention, provides support for the clone theory. At that time, when the dominance of epithelium in the initiation of tooth development was not established, Lumsden referred to the work of Miller (1969) and speculated that a region of epithelium might specify beneath it a zone of ectomesenchymal stem cells that would subsequently grow to lay out the pattern of the molar dentition and, in the mouse at least, that there would be two such areas of epithelium in each jaw quadrant, one specific to an incisor clone and the other specific for three molars.

The tooth forming potentials along the mesiodistal (antero-posterior) axis of the E9-10 mandibular arch (Lumsden, 1984; Lumsden and Buchanan, 1986) were established by sub-dividing the arches into regions or sectors. Whereas both incisor and molar teeth with near normal crown shapes developed in intraocular homografts of complete mandibular arches explanted at E9 and E10, arches that had been divided in the midline gave rise only to molars. The ventral midline region of the arch produced incisors at E10 but not at E9. These results enabled Lumsden to deduce the following. First of all, the development of both molars and incisors in whole mandibular arch grafts indicates that sufficient ectomesenchyme for complete odontogenesis has entered the mandibular arch by E9. Second, the induction of incisor development only in E10 midline grafts, indicates an insufficiency in the amount of ectomesenchyme available at E9. Third, the failure to produce incisors in hemisected mandibular arches can be attributed to the destruction of the epithelial isthmus during the preparation of the grafts. In this instance, the ectomesenchyme is unable to pursue its normal developmental fate in the absence of an initiating signal from the appropriately specified area of epithelium.

Taken together, this means that at E9 during normal development, ectomesenchyme has yet to complete its migration into proximity with the ectoderm in the presumptive incisor region although it has already attained proximity with the ectoderm in the molar region and indicates that ectomesenchyme is equipotential.

Thus, the further experimental work called for by Butler (1978) increasingly supplies support for the clone theory in the determination of heterodonty in mammals.

### Cell differentiation — the tissues of tooth support

In terms of cell differentiation, and in particular the differentiation of odontoblasts (and ameloblasts), a voluminous literature based on experimental investigation exists and is dealt with by other

contributors to this volume. Overlooked in most reviews dealing with odontogenesis is any account of the initiation and development of the tooth supporting tissues, largely because of a sense that these tissues are not truly dental. This is erroneous, for experimental investigation has now clearly established that tooth supporting tissues also differentiate from the neural crest derived cells.

It is worthwhile describing these investigations in some detail for several reasons. First, they represent another example of a cascade of scientific enquiry involving experimental investigation built around chance, hypothesis, imagination, intuition and reason; second, there is personal involvement; third, the issue is not dealt with elsewhere in this volume and fourth, the findings have considerable clinical significance.

Twenty-five years ago I was asked to write a chapter on the development of the periodontium. At that time information on this subject was limited, imprecise and based almost entirely on descriptive studies. The definition of the term "dental follicle" from which the tooth supporting tissues supposedly derived, was imprecise but generally was considered to describe that tissue between the tooth germ and the forming bone of the jaw and was subdivided into either two compartments (bone and tooth) or three (bone, tooth and intermediate). Nor was the contribution of these varying zones to the development of tooth support understood. As to the origin of the follicle, Scott (1953) indicated that it was derived from the dental papilla although the evidence for this was not presented. On the basis of further careful descriptive analysis of histological sections and some histochemical observations, I argued (Ten Cate, 1969) that the term dental follicle should be reserved for the inner or investing layer (the tooth related compartment) as there seemed to be sufficient evidence to indicate that this layer gave origin to both the cementum and periodontal ligament. It was pointed out that this compartment was continuous with the dental papilla, but no comment was made as to its possible origin from the papilla as claimed by Scott (1953). Rather, it was assumed that just as dental organ initiates the formation of the dental papilla, it also initiated the formation of the investing layer. The only experimental evidence available at that time (Hoffman, 1960) contradicted the conclusions I was trying to draw from a descriptive analysis. Hoffman successfully transplanted developing molar teeth, removed from their follicles, from newborn hamsters into a subcutaneous site in adult animals. Development continued in this ectopic location with the formation of cementum, periodontal ligament and "alveolar" bone, and it was argued therefore that the dental organ and dental papilla had the ability to differentiate the tissues of tooth support from any connective tissue, and that the formation of tooth supporting tissue was the "effort of a morphogenetic field to complete itself". However, Hoffman properly noted that "...additional evidence should be obtained to establish conclusively that no transplanted cells were the precursor to the periodontal tissues formed. Without doubt, a certain few cells adhered to the outer enamel epithelium and were transplanted. It seems improbable that these cells could have been responsible for the extensive formation of the periodontium routinely seen around transplanted teeth after 28 days in the host subcutaneous tissues."

To my mind this caution was more than appropriate, for my previous experience in the dissection of tooth germs suggested that the presence of follicle was key to maintaining the integrity of the tooth germ and at that time I commented that, "...it is possible that in removing the tooth germs from their dental sacs Hoffman obtained the same results as removal of human tooth germs, and



that the 'certain few cells' represented the ectomesenchymal cells of the investing layer."

To determine the correctness of the matter, Hoffman's experimental approach was modified (Ten Cate *et al.*, 1972). Murine tooth germs were first dissected out and flash labeled with tritiated thymidine in culture medium. Control sections assured that the follicular cells had captured the label. The tooth germs were then transplanted to a subcutaneous location where development continued with the formation of cementum, periodontal ligament and alveolar bone. The demonstration of labeled cementoblasts and ligament fibroblasts clearly established their derivation from donor material, namely the tooth germ. The origin of bone was not established with certainty as labeling was minimal and could be attributed to background, although the demonstration of lymphocytes on the surface of this bone did suggest the initiation of a rejection response (Ten Cate and Mills, 1972). To support the idea that bone was derived from follicle I reasoned that in Main's (1966) experiment supporting tissues, including bone, should have formed in relation to his ectopic teeth (his published photograph only exhibited pulp, dentine and cementum). Access to Main's material confirmed that indeed bone formed in relation to the teeth.

The issue of whether bone derives from follicle was seemingly nicely settled by altering the experimental design so as to grow teeth in an intraocular location rather than subcutaneously (Yoshikawa and Koller, 1981). A problem with the subcutaneous location is that it is known that a number of foreign agents can induce bone in this location by marshalling osteoblasts from presumably undifferentiated mesenchymal cells. In the anterior chamber of the eye, such potential osteogenic precursor cells are absent. When various recombinations of dental follicle, dental papilla and dental organ were transplanted intraocularly, teeth formed with associated supporting tissues, including bone. Significantly, with this experimental design Yoshikawa and Kollar (1981) were able to show that a recombination of dental organ and dental papilla alone produced all the diverse, fully differentiated structures of tooth support, including bone, implying that follicle derives from papilla.

Palmer and Lumsden (1987) extended the experimental design employed by Yoshika and Kollar (1981) to investigate temporal influence, and obtained conflicting results in that when various recombinations of dental organ and papilla from E16 and newborn, which specifically excluded the investing layer were made, only cementum and periodontal ligament formed. Bone formation only occurred when follicle was included in the recombinations, raising the question once more whether, when dissecting out the tooth germ, contamination of the follicle from the perifollicular mesenchyme is a factor. It is difficult to explain this finding of Palmer and Lumsden (1987) with respect to the absence of bone when follicle is also absent, especially when it is remembered that in Main's experiment bone formed from a recombination of disassociated dental organ and dental papilla cells.

Both studies, however, demonstrated that the dental papilla has the ability to regenerate dental follicle and implies that, in normal development, this structure also develops from dental papilla. That this is the case has been demonstrated using an entirely different approach. Osborn (1984) attempted to analyze the evolution of the gomphosis in mammals based on the different types of attachment found in vertebrate dentitions. Central to his argument was the proposition that from the original ectomesenchyme various clades (differentiation compartments) occur, with one being the primitive attachment ectomesenchyme which has to migrate out from the dental pulp to form attachment tissue. In explaining the evolution

of the gomphosis, Osborn (1984) concluded that during normal development the dental papilla would have to provide cells that migrate out and are primarily responsible for the development of the attachment tissues. This hypothesis was tested experimentally by Osborn and Price (1988) using tritiated thymidine labeling and measuring changes in the labeling index with time in defined areas of the papilla and follicle in the developing third molar of the mouse. They were able to show that indeed during normal development cells do move from the dental papilla to the follicle, and concluded that the papilla, rather than the investing layer, is the source of tooth attachment tissue.

Thus, a series of experiments designed to determine the origin of the supporting tissue of the tooth have clearly demonstrated that these tissues are indeed dental, but have raised further questions as to the precise origin of alveolar bone and the significance of the early dental follicle. Is the follicle formed entirely as a result of cell migration from the dental papilla, or is it originally the investing layer formed to define the tooth germ which secondarily becomes reinforced with cells possessing the potential to form the tissues of tooth support? Seeking the answers to these questions ensures that the flow of enquiry will continue, but what is certain is that the development of tooth support must be considered as an essential part of odontogenesis.

## References

- ADAMS, A.E. (1924). An experimental study of the development of the mouth in the amphibian embryo. *J. Exp. Zool.* 40: 311-379.
- BHAKDINARONK, A., ETO, K. and JOHNSTON, M.C. (1974). X-irradiation and the frequency of cleft lip in A/Jax mice. *J. Dent. Res.* 53: 189.
- BRONNER-FRASER, M. (1982). Distribution of latex beads and retinal pigment epithelial cells along the ventral neural crest pathway. *Dev. Biol.* 91: 50-63.
- BUTLER P.M. (1978). The ontogeny of mammalian heterodonty. *J. Biol. Buccale* 6: 217-227.
- BUTLER, P.M. (1967). Dental merism and tooth development. *J. Dent. Res.* 46: 845-850.
- GAUNT, W.A. and MILES, A.E.W. (1967) Fundamental aspects of tooth morphogenesis. In *Structural and Chemical Organization of Teeth* (Ed. A.E.W. Miles). Academic Press, New York, pp. 151-197.
- GLASSTONE, S. (1967). Morphodifferentiation of teeth in embryonic mandibular segments in tissue culture. *J. Dent. Res.* 46: 611-614.
- HENZEN, W. (1957). Transplantationen zur entwicklungsphysiologischen Analyse der lavalen Mundorgane bei Bombinator und Triton. *W. Roux Arch. Entw.Mech. Org.* 149: 387-442.
- HOFFMAN, R.L. (1960). Formation of periodontal tissues around subcutaneously transplanted hamster molars. *J. Dent. Res.* 39: 781-798.
- HUGGINS, C.B., MCCARROLL, H.R. and DAHLBERG, A. (1934). Transplantation of tooth germ elements and the experimental heterotopic formation of dentin and enamel. *J. Exp. Med.* 60: 199-208.
- JOHNSTON, M.C. (1966). A radioautographic study of the migration and fate of cranial neural crest cells in the chick embryo. *Anat. Rec.* 156: 143-156.
- JOHNSTON, M.C. and HAZELTON, R.D. (1972). Embryonic origins of facial structures related to oral sensory and motor function. In *Third Symposium on Oral Sensation and Perception. The Mouth of the Infant*. Thomas, Springfield, Ill, pp. 76-97.
- KOLLAR, E.J. (1972). Histogenetic aspects of dermal-epidermal interactions. in *Developmental Aspects of Oral Biology* (Eds. H.C. Slavkin and L.A. Baretta). Academic Press, London, pp. 125-149.
- KOLLAR, E.J. (1983). Epithelial-mesenchymal interactions in the mammalian integument: tooth development as a model for instructive interaction. In *Epithelial-mesenchymal Interactions in Development* (Eds. R.H. Sawyer and J.F. Fallon). Praeger, New York, pp. 27-74.
- KOLLAR, E.J. and BAIRD, G.R. (1969). The influence of the dental papilla on the development of tooth shape in embryonic mouse tooth germs. *J. Embryol. Exp. Morphol.* 21: 131-148.



- KOLLAR, E.J. and BAIRD, G.R. (1970). Tissue interactions in embryonic mouse tooth germs. I. Reorganization of the dental epithelium during tooth-germ reconstruction. *J. Embryol. Exp. Morphol.* 24: 159-171.
- KOLLAR, E.J. and BAIRD, G.R. (1970). Tissue interactions in embryonic mouse tooth germs. II. The inductive role of the dental papilla. *J. Embryol. Exp. Morphol.* 24: 173-186.
- KOLLAR, E.J. and FISHER, C. (1980). Tooth induction in chick epithelium: expression of quiescent genes for enamel synthesis. *Science*, 207: 993-995.
- KOLLAR, E.J. and LUMSDEN, A.G.S. (1979). Tooth morphogenesis: the role of the innervation during induction and pattern formation. *J. Biol. Buccale* 7: 49-60.
- LE DOUARIN, N. (1982). *The Neural Crest*. Cambridge Univ. Press, Cambridge.
- LUMSDEN, A.G.S. (1979). Pattern formation in the molar dentition of the mouse. *J. Biol. Buccale* 7: 77-103.
- LUMSDEN, A.G.S. (1984). Tooth morphogenesis: contributions of the cranial neural crest in mammals. In *Tooth Morphogenesis and Differentiation* (Eds. A. Belcourt and J.V. Ruch). *Colloque INSERM* 125. INSERM, Paris, pp. 19-27.
- LUMSDEN, A.G.S. (1987). The neural crest contribution to tooth development in the mammalian embryo. In *Developmental and Evolutionary Aspects of Neural Crest* (Ed. P.F. Maderson). Wiley, New York, pp. 3-43.
- LUMSDEN, A.G.S. and BUCHANAN, J.A.G. (1986). An experimental study of timing and topography of early tooth development in the mouse embryo with an analysis of the role of innervation. *Arch. Oral Biol.* 31: 301-311.
- MAIN, J.H.P. (1966). Retention of potential to differentiate in long-term cultures of tooth germs. *Science* 152: 778.
- MILLER, W.A. (1969). Inductive changes in early tooth development: I. A study of mouse tooth development on the chick chorioallantois. *J. Dent. Res.* 48 (Suppl.): 719-725.
- MINA, M. and KOLLAR, M. (1987). The induction of odontogenesis in non-dental mesenchyme combined with early murine mandibular arch epithelium. *Arch. Oral Biol.* 32: 123-127.
- MINA, M., KOLLAR, E.J., BISHOP, J.A. and ROHRBRACH, D.H. (1990). Interactions between the neural crest and extracellular matrix proteins in craniofacial skeletogenesis. *Crit. Rev. Oral Biol. Med.* 1: 79-87.
- MORRISS-KAY, G. (1972). Morphogenesis of the malformations induced in rat embryos by maternal hypervitaminosis A. *J. Anat.* 113: 241-250.
- NODEN, D.M. (1975). An analysis of the migratory behaviour of avian cephalic neural crest cells. *Dev. Biol.* 42: 106-130.
- OSBORN, J.W. (1973). The evolution of dentitions. *Am. Sci.* 61: 548-559.
- OSBORN, J.W., (1978). Morphogenetic gradients: fields versus clones. In *Development, Function and Evolution of Teeth* (Eds. P.M. Butler and K.A. Joysey). Academic Press, London, pp. 171-201.
- OSBORN, J.W. (1984). From reptile to mammal: evolutionary considerations of the dentition with emphasis on tooth attachment. *Symp. Zool. Soc. Lond.* 52: 549-572.
- OSBORN, J.W. and PRICE, D.G. (1988). An autoradiographic study of periodontal development in the mouse. *J. Dent. Res.* 67: 455-461.
- PALMER, R.M. and LUMSDEN, A.G.S. (1987). Development of periodontal ligament and alveolar bone in homografted recombinations of enamel organs and papillary, pulpal and follicular mesenchyme in the mouse. *Arch. Oral Biol.* 32: 281-289.
- PETROKOVA, R. (1983). Dental lamina develops even within the mouse diastema. *J. Craniofac. Genet. Dev. Biol.* 5: 89-104.
- POSWILLO, D. (1975). The pathogenesis of the Treacher-Collins syndrome (mandibulofacial dysostosis). *Br. J. Oral Surg.* 13: 1-26.
- RAVEN, Ch.P. (1931). Zur entwicklung der ganglienleiste. I. Die kinematik der ganglienleistenentwicklung bei den urodelen. *W. Roux Arch. Entw.Mech. Org.* 125: 210-292.
- RAWLES, M.W. (1963). Tissue interactions in scale and feather development as studied in dermal-epidermal recombinations. *J. Embryol. Exp. Morphol.* 11: 765-789.
- RUCH J.V. (1984). Tooth morphogenesis and differentiation. In *Dentin and Dentinogenesis* (Ed. A. Linde). CRC Press, Boca Raton, pp. 47-79.
- SCOTT, J.H. (1953). How teeth erupt. *Dent. Pract.* 3: 345-349.
- SELLMAN S. (1946). Some experiments on the determination of the larval teeth. In *Ambystoma mexicanum*. *Odont. Tidskr.* 54: 1-128.
- SLAVKIN H.C. (1974) Embryonic Tooth Formation. A tool for developmental biology. *Oral Sciences Reviews*, Vol. 4. Munksgaard, Copenhagen.
- STONE, L.S. (1926). Further experiments on the extirpation and transplantation of mesectoderm in *Ambystoma punctatum*. *J. Exp. Zool.* 44: 95-131.
- TEN CATE, A.R. (1969). Development of the periodontium. In *Biology of the Periodontium* (Eds. A.H. Melcher and W.H. Bowen). Academic Press, London, pp. 53-89.
- TEN CATE, A.R. and MILLS, C. (1972). The development of the periodontium: the origin of alveolar bone. *Anat. Rec.* 173: 69-79.
- TEN CATE, A.R., MILLS, C. and SOLOMON, G. (1971). The development of the periodontium. A transplantation and autoradiographic study. *Anat. Rec.* 170: 365-380.
- VAINIO, S., KARAVANOVA, I., JOWETT, A. and THESLEFF, I. (1994). Identification of BMP-4 as a signal mediating secondary induction between epithelial and mesenchymal tissues during early tooth development. *Cell* 75: 45-58.
- WAGNER, G. (1949). Die bedeutung der neuralleiste fur die kopfgestaltung der amphibienlarven, untersuchungen an chimaeren von Triton und Bombinator. *Rev. Suisse Zool.* 56: 519-620.
- WAGNER, G. (1955). Chimaerische Aahnanlagen aus Triton-schmelzorgan und Bombinator-papille. Mit Beobachtungen über die Entwicklung von Kiemenzahnchen und Mundsinnesknospen in den Triton-larven. *J. Embyol. Exp. Morphol.* 3: 160-188.
- WILDE, C.E. (1955). The urodele neuroepithelium. The differentiation *in vitro* of the cranial neural crest. *J. Exp. Zool.* 130: 573-591.
- YOSHIKAWA, D.K. and KOLLAR, E.J. (1981). Recombination experiments on the odontogenic roles of mouse dental papilla and dental sac tissues in ocular grafts. *Arch. Oral Biol.* 26: 303-307.