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Morphogenesis of human tooth primordia: the importance of 3D computer-assisted reconstruction

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ABSTRACT Three-dimensional reconstruction of embryological structures from histological serial sections is necessary when researching questions concerning formal development and mutual influences due to neighboring relationships. Graphical reconstructions can be performed by personal computers. In the present paper the need for 3D-reconstructions is explained, and examples showing the development of human dental primordia and their surrounding structures of specimens ranging between 18 and 64 mm CRL are presented. The contour-line plots that are provided by the software HISTOL are graphically reworked to anatomical drawings. Spatial impediment as one of the factors responsible for development of tooth form is discussed on the basis of the 3D-reconstructions.

KEY WORDS: *human tooth primordia, morphogenesis, computer-aided reconstruction, 3D-reconstruction, spatial condition*

Introduction

The need for 3-dimensional findings

Highly polemical arguments were used by Adloff (1909, 1913a,b, 1914) and by Ahrens (1913b) during their controversy concerning the so-called prelacteal lamina. This supposedly was an additional lamina that should have indicated a dentition prior to the first dentition. Adloff vehemently maintained that the additional invaginations of the epithelium found by himself represented the existence of a prelacteal lamina in man as well as in other species. This was a time when evolutionary theories greatly influenced embryological studies, and a tiny folded or invaginated structure in a histological section of any embryonic specimen was enough to trigger a speculative and highly imaginative train of thought, in the course of which these formations became rudimentary remnants of old organs or initial signs of future organ formations (Goll, 1972).

Solid 3-dimensional models that Ahrens (1913a,b) constructed from serial sections, and computer-aided graphical reconstructions that we made (Radlanski and Jäger, 1991) from early dentogenous epithelial invaginations revealed clearly that additional foldings of this kind arise from the vestibular lamina, and not from the dental lamina. It is therefore difficult to see how this gave rise to speculations of precursors of dental formations. Ahrens, at all events, reproached Adloff, who did not even use serial sections, for examining only one section, thereby rendering his interpretations of the problem inadmissible. Single sections cannot give information concerning the spatial arrangement of a structure or an arrangement of several structures and their mutual relationships. Not knowing the angle from which the sectioned structure was cut by the microtome knife is too severe a handicap. Not only disfiguring projection phenomena by a more or less oblique cutting angle (Plackova, 1963) but the clear fact that a single section cannot represent the bodily arrangement of a structure led Born (1883) and His (1887) to the construction of solid wax plate models from serial sections.

Knowledge of the form of a developing organ and the perception of its spatial arrangement with its neighboring structures is a necessary prerequisite if we understand embryological development as a sequence of multifactorial interactions. Despite the fact that direct, programmatic genetic control of organ formation is implausible (Fuhrmann, 1985), biochemical aspects obtained from *in vitro* experiments are highly interesting. But it is not enough to know about, for example, a bone growth factor, if we do not now why a certain bony protrusion is formed at a specific place at a specific time and to a specific extent. At present, we know very little about the changes of form and shape of developing structures, and the enlarged plastic models of embryological developmental stages which are discussed in the literature are only single and fortuitous action shots of a process that is in fact continuous.

Knowledge of spatial arrangement of developing structures led His (1874, 1887) to the conception that organs are given their forms and their structures by mutual influence which they exercise over each other during growth. Genetic activity has been considered to be of a more reactive character during this process (Blechschmidt, 1948); it was found that locally different developmental movement of tissue is a principle in morphogenesis, and that the position and form of an organ are interdependent (Blechschmidt, 1960). In experimental embryology spatial impediment as a morphogenetic factor causing form changes has been proven (Steding, 1967).

Teeth are an interesting embryological tool, because their completed forms are hard tissues with cusps, ridges and fissures, long before they emerge from opposite locations and come into

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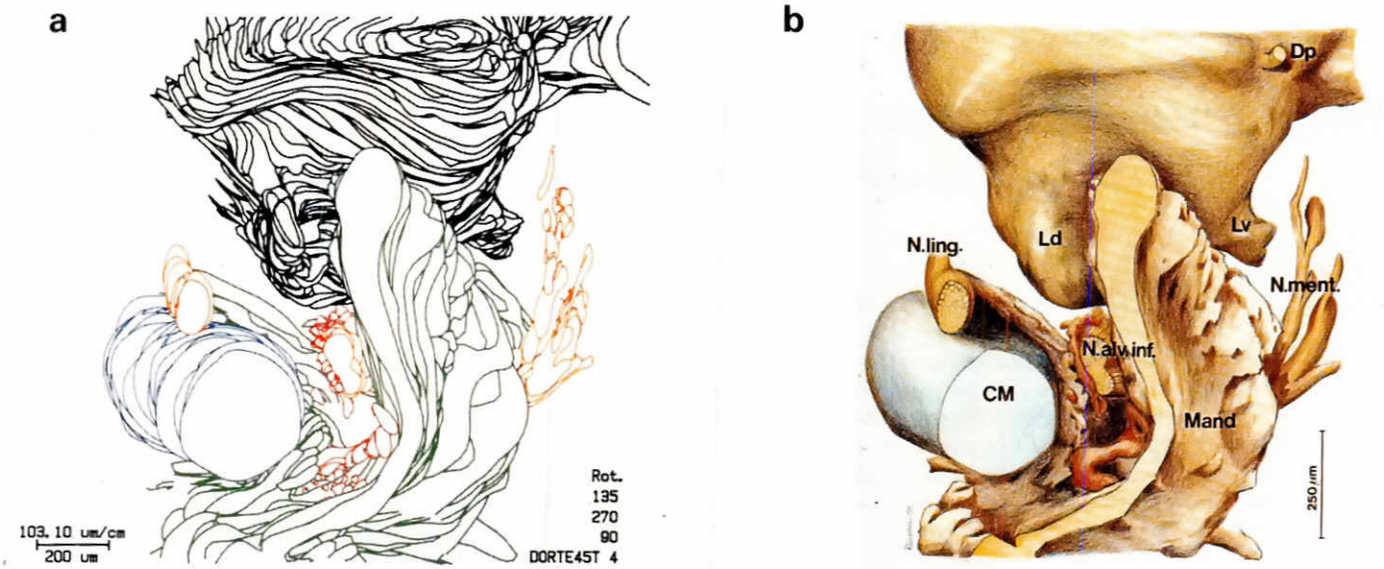

Fig. 1. Morphogenesis of human tooth primordia: the importance of 3-D computer assisted reconstruction. (a) *Graphic reconstruction produced as a contour-line plot with the aid of HISTOL software. Selected structures are plotted with different colors (epithelium black, mandible green, Meckel's cartilage blue, nerves orange, vessels red). The software can omit or reproduce any of the differently labeled structures. The complete reconstruction can be rotated around any axis.* **(b)** *The contour-line plot is difficult to understand, and readers used to normal illustrations generally need the help of an anatomical drawing. The drawing is done by hand and under stereoscopic control of the hidden line plot. Posterior view of the spatial relationship between the epithelial invaginations of the dental lamina (Ld), vestibular lamina (Lv), mandible (Mand), Meckel's cartilage (CM), and the alveolar inferior nerve (N. alv. inf.), the mental (N. ment.) and the lingual nerve (N. ling.). The red structures are the inferior alveolar arteries and veins. The parotid duct (Dp) is cut. Human 37 mm CRL fetus.*

function free of interferences. It would be interesting to discover interdependencies between position and form of the tooth primordia, and if the spatial conditions under which the primordia develop are of importance for the formation of the specific form of each single tooth (Radlanski *et al.*, 1988, 1989; Radlanski and Jäger, 1991; Radlanski, 1993). Since these questions concern arrangement in space, answers can only be found if spatial conditions are made visible. Technical limitations do not allow any direct anatomical preparation, so 3D-reconstruction from serial histological sections is the method of choice (Strasser, 1886; Peter, 1906; Ware and LoPresti, 1975; Gaunt and Gaunt, 1978; Meyer and Domanico, 1988).

The technical approach of 3-dimensional reconstructions from histological serial sections

The classic wax plate reconstruction technique was introduced by Born (1883). Here the contours were directly transferred from the microscope to the wax plate, cut out and piled up as a reconstruction. Thickness of wax plates and magnification of the drawings had to match each other. Wax, however, is not sufficiently dimensionally stable, because it gives way under the pressure of the piled-up wax plates. In addition its stability is dependent on a constant temperature. Reconstructions made from transparent materials like celloidine (Schiefferdecker, 1882), glass (Thomee, 1928), or gelatine (Rolshoven, 1937) are less useful and are only

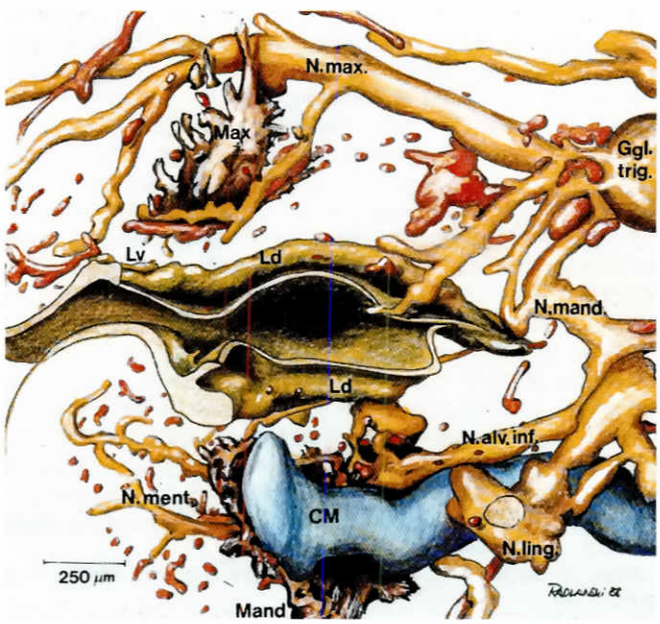

Fig. 2. Human 18 mm CRL embryo. *Reconstruction of the right half of the oral cavity and a selection of its surrounding structures in a medial view. The sagittal section plane is located in the midfacial region. Towards the left margin of the illustration the contours of the upper and the lower lips are sectioned. The extensions of maxillary (Max) and mandibular (Mand) bony formations and the course of Meckel's cartilage (CM) are shown as bodily reconstructions. The vestibular lamina (Lv) and the dental lamina (Ld) can be seen as two separate invaginations from a common epithelial invagination in the maxillary region, whereas the common invagination is not separate in the midfacial mandibular region. Neuronal structures (Ggl. trig., N. max., N. Mand., N. alv. inf., N. ment., N. ling.) are relatively thick at this stage. Blood vessels (red) are rather insular than continuous in specific regions.*

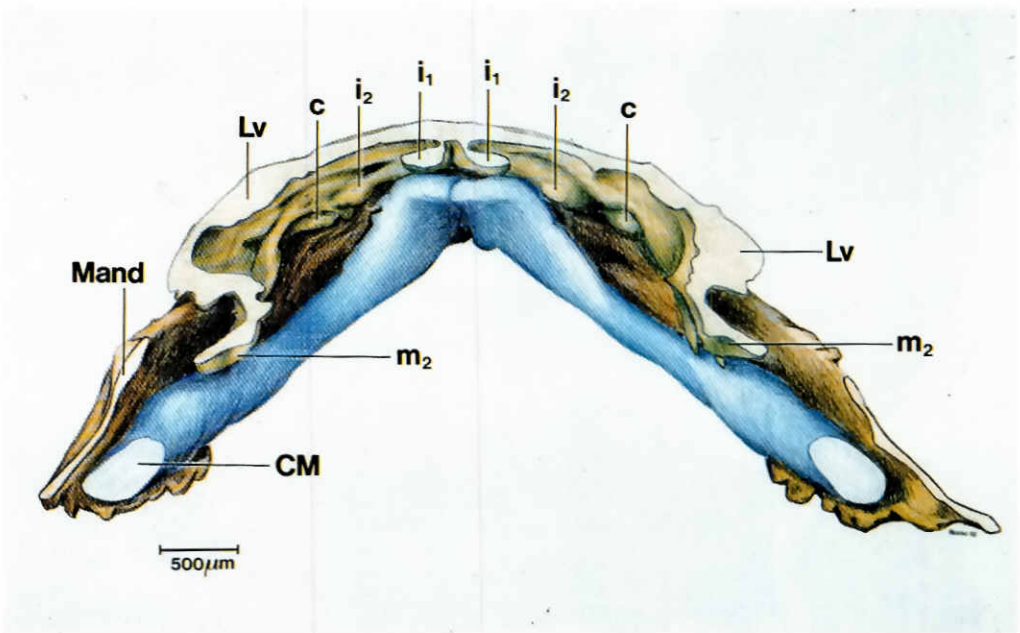

Fig. 3. Human 21 mm CRL embryo. *Cranial aspect of the spatial relationship between the dental lamina, the vestibular lamina (Lv), mandibular bone (Mand), and Meckel's cartilage. The dental primordia i_1, i_2, c, m_1 and m_2 are in their bud stage, but m_1 is not visible because of protruding epithelial foldings. Note the proximity of the anterior ends of Meckel's cartilage and the primordia i_1.*

suitable for smaller reconstructions. An improvement of the wax plate reconstruction was introduced by the casting mold technique, where the wax model serves as a temporary mold, into which a more stable material is poured (Dankmeijer, 1940). Blechschmidt (1954) developed a reconstruction technique using plastic resins, which enabled models of about 1 m in height to be built. Ooe (1956) produced his reconstruction models of tooth development from cardboard. Although today there are materials available that are easier to work with (e.g. styrofoam plates), the construction of a solid model still requires more effort. Here graphic reconstructions are an alternative, if the arrangement of the structures is not too complex (Kastschenko, 1886, 1887; Odhner, 1911; Lison, 1936; Levinthal and Ware, 1972; Gaunt and Gaunt,1978). The contours are drawn in such a way that when all sections are superimposed, the hidden lines are omitted. The result is a representation of the surface as a contour-line plot (Fig. 1a). If different and distorted coordinate systems are used, it is possible to obtain views that vary from the plane of section. Today it is possible to use computers (Marino *et al.*, 1980; Poelmann and Verbont, 1985, 1987) and personal computers (Gras and Killmann, 1983; Street and Mize, 1983; Gras, 1984; Huijsmans *et al.*, 1986; Beursgens *et al.*, 1987; Lozanoff *et al.*, 1988; El Gammal *et al.*, 1989; Hilbelink and Gasser, 1990; Radlanski and Jäger, 1990, 1992a,b) for graphic reconstruction, which accelerates the process.

Findings

As a consequence of the developmental stage of the specimens all findings represent formation of the primary dentition.

In the 18 mm CRL embryo (Fig. 2) the epithelial thickenings and invaginations of dental and vestibular lamina extend into their underlying mesenchyme. The 3D-reconstruction reveals enough space for these formations to grow into the depth. Special attention should be paid to the location where the epithelial laminae are formed: directly above the epithelial thickening there is a furrow in the epithelium covering the oral epithelium.

When the primordia have reached their bud stage in the 21 mm CRL embryo (Fig. 3), the spatial conditions under which they develop also require special attention. The primordia i_1 are very close to the anterior end of Meckel's cartilage and they show a correspondingly flat outline. Not so close to Meckel's cartilage are the primordia i_2, which are not as flat, being more oval or even rounded. The canine primordia are even further from Meckel's cartilage, and they extend laterally into a bony groove. The space for the development of the molar primordia is characterized by the downward curvature of Meckel's cartilage. This reconstruction not only shows the special spatial condition under which each single primordium can develop; it also presents the spatial relation that the dental lamina as a whole has towards its surrounding structures: distal expansion of the dental lamina is prevented by the ascending path of Meckel's cartilage and mandibular bone. The same is true for the maxillary primordial dental arch, because the ascendant mandibular ramus reaches close to the distal end of the dental lamina there.

Due to the rapid enlargement of bony structures the spatial conditions under which the primordia develop in the 37 mm CRL fetus (Fig. 4) have clearly changed in comparison to the situation found in the 18 mm embryo (Fig. 2). The flatness of the primordia i_1 and i_2 and the proximity of Meckel's cartilage are obvious arrangements that one can take from the reconstruction. The mandibular canine primordium extends into a lateral bony groove, and the dental lamina at m_2 is free of bone distally. In the maxillary region there is a noticeable proximity between bone and vestibular and dental epithelial formations, as shown as an example for the canine region in Fig. 5a,b.

When the cap stage is reached in the 47 mm fetus the bony structures covering Meckel's cartilage underneath the primordium i_2 are rather compact (Fig. 6), but they are prone to resorption, together with Meckel's cartilage, when the early bell stage has been reached in the 64 mm fetus (Fig. 7).

In the molar region, there is no such resorption of Meckel's cartilage, but rather a change of shape and size of the bony groove.

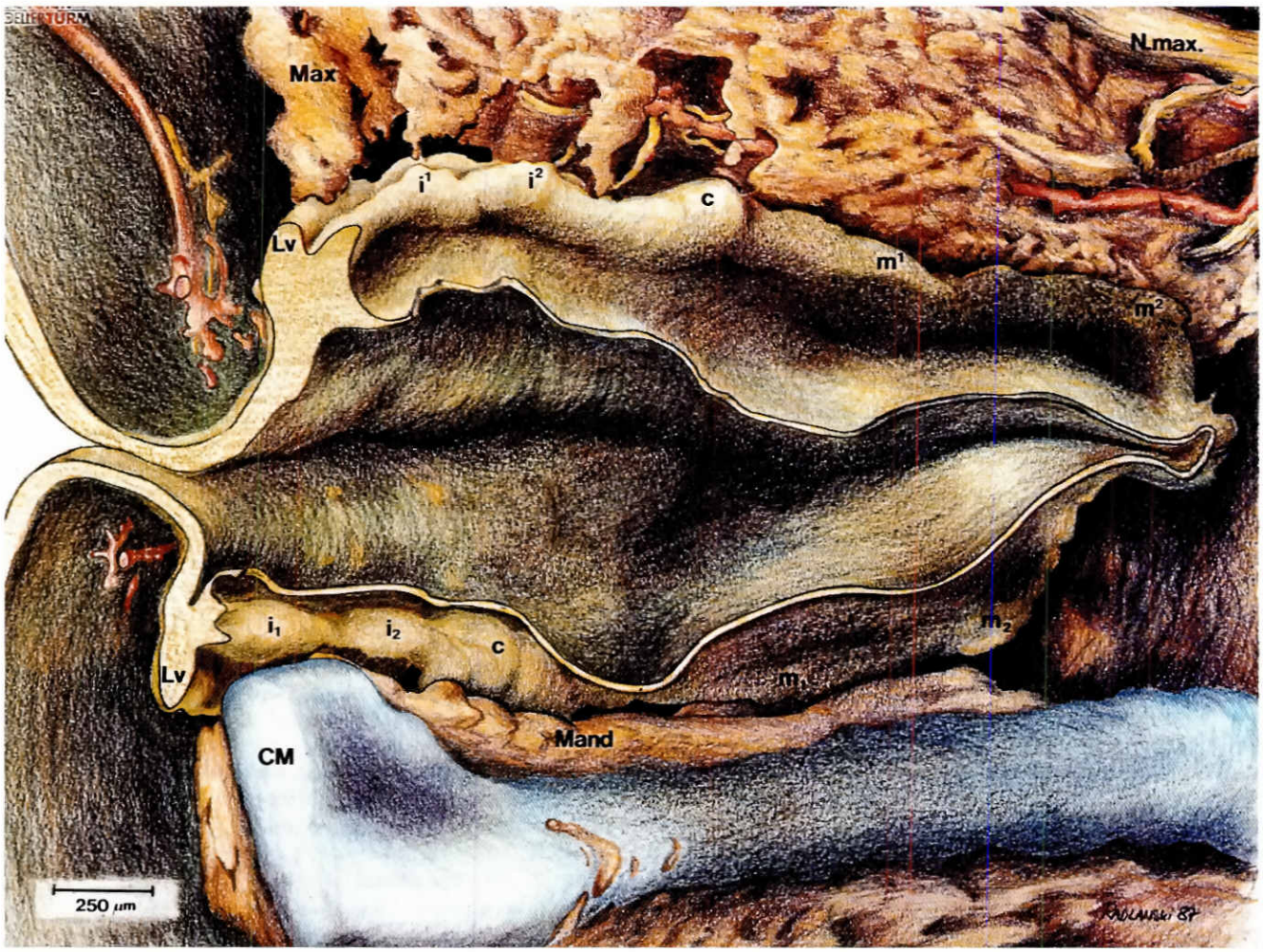

Fig. 4. Human 37 mm CRL fetus. *Reconstruction of the right half of the oral cavity and a selection of its surrounding structures in a medial view. The sagittal section plane is located in the midfacial region. Towards the left margin of the illustration the contours of the upper and the lower lips are sectioned. They merge into the epithelial invaginations of the vestibular lamina (Lv). The dental primordia i1, i², and m² have reached the bud stage, the primordia c¹ and m¹ are in the early cap stage. Extensions of the maxilla (Max) reach down into the furrow between the dental and the vestibular lamina (Lv). In the mandibular region, i_2 and m_2 are in their bud stage, and the primordia i_1, c_1, and m_1 have reached the early cap stage. Meckel's cartilage (CM) protrudes close to bud i_1. Parts of mandibular bone (Mand) arise between the other primordia and Meckel's cartilage.*

The cap m_1 extends into the bony groove that extends underneath it (Fig. 8). This v-shaped groove becomes more acute overall, and much deeper, to give room for the primordium m_1 that has attained its early bell stage in the 64 mm CRL fetus (Fig. 9).

Discussion

It has been pointed out that one needs to know about the 3D-form (unfortunately there is no specific English translation of the German word *Gestalt*, which means more than *form, figure, outline*, or *shape*. In the context of this paper the three-dimensional outer appearance of the embryological formations is meant) of embryological structures and their arrangement in space if one wants to trace possible mutual influences. The best way to gain knowledge about the anatomical relation would be a preparation technique usual in gross anatomy. In some cases this is possible even in micromorphological levels if the scanning electron microscope is applied. This, however, requires a preparation technique where the tissue which has been removed to reveal the structures of interest is inevitably lost.

One way out is the technique of serial histological sectioning and subsequent total or partial 3D-reconstruction. Here, primary "preparation" takes place when the contours are traced under the microscope, and when non-interesting structures are omitted. Furthermore, preparation-like steps can be carried out when structures are selectively omitted by software operations. The advantage of the serial sectioning technique is that although the specimens' continuity is interrupted by several thousand sections, no structure is really lost, because it can be re-established by reconstruction.

There is one major argument against this technique, and this concerns the question of correct alignment of the numerous single sections to a 3-dimensional arrangement. Many attempts have been made to improve the method of subjective judgment in piling up the sections by inserting straight structures for reference which were co-embedded in the paraffin block and included in the section and its reconstruction (Ongaro *et al.*, 1991). Technical difficulties

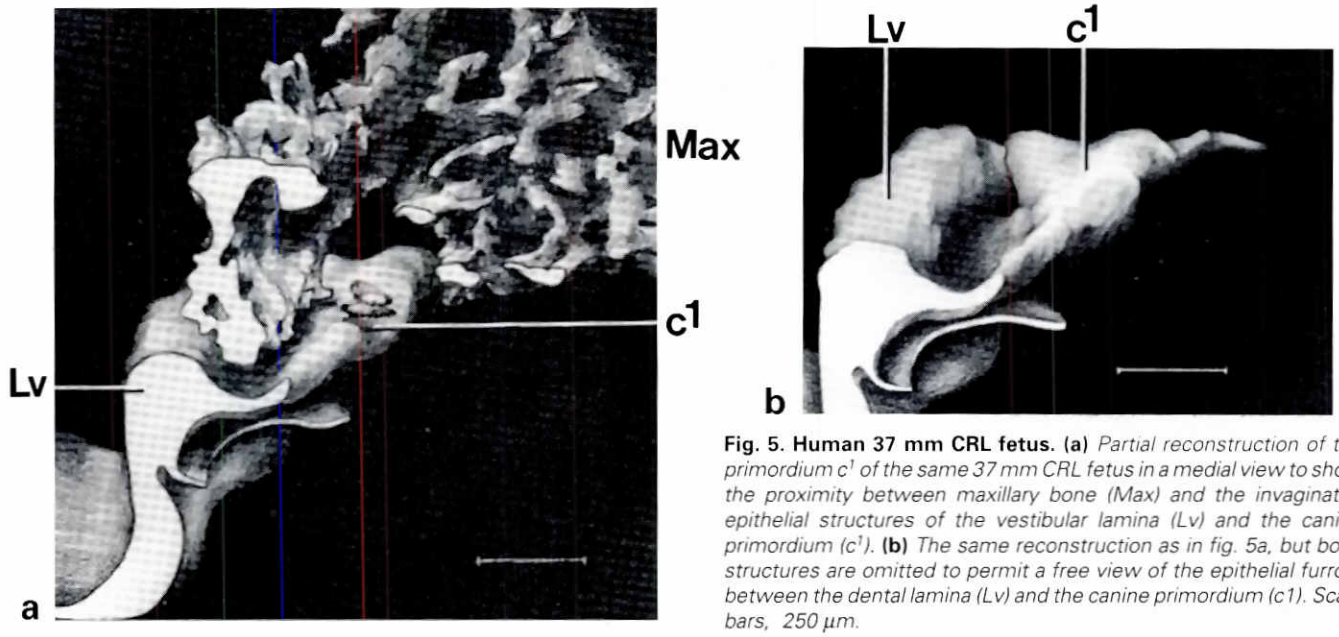

Fig. 5. Human 37 mm CRL fetus. (a) *Partial reconstruction of the primordium c¹ of the same 37 mm CRL fetus in a medial view to show the proximity between maxillary bone (Max) and the invaginated epithelial structures of the vestibular lamina (Lv) and the canine primordium (c¹).* **(b)** *The same reconstruction as in fig. 5a, but bony structures are omitted to permit a free view of the epithelial furrow between the dental lamina (Lv) and the canine primordium (c1). Scale bars, 250 µm.*

such as positional changes during the sectioning procedure or inaccuracy due to size differences prevented these efforts from becoming routine. If large and well known structures such as outer facial contours are integrated in the reconstructions, one can easily check correct alignment, and when, for example, the eyes turn out to be more oval than round, it is clear that there is some distortion in the reconstruction. So reference structures can be found in the specimen itself, and a set of stereoscopic photographs taken prior to histological procedure is useful in most cases.

When only single sections are applied, the outlines of the structures are highly dependent on the plane of section. Projection phenomena may be so severe and erroneous that no secure statement can be made about the real 3D-form of the structure sectioned (Hofstadter, 1979). Besides the inadmissible mixing up of evolutionary and embryological tracks of thought, this was the main factor in the dispute between Adloff (1909, 1913a,b, 1914) and Ahrens (1913a,b). 3D-reconstructions of the vestibular lamina could clarify the situation (Radlanski and Jäger, 1991).

When the spatial arrangement of the dental primordial structures and their surroundings are evaluated from the 3D-reconstructions, certain interdependencies and principles of development can be interpreted.

The epithelial covering of the oral cavity thickens and invaginates for reasons of space. The location is specified by the furrow that forms due to extension and rolling in of the lips against the intraoral epithelium (Blechschmidt,1960). Once the dental and vestibular lamina have invaginated the underlying mesenchyme seems not to exercise much resistance, and the reconstructions reveal sufficient space before bony structures are reached.

The early form of the tooth buds resemble characteristic patterns of the later teeth. It must be stated here that, for example, the incisor primordia are *not* flat because the later incisor crowns must have such an outline. Quite the reverse, in fact: the final teeth obtain their specific form because the primordia have formed them that way. So if an answer is to be found concerning the determination of dental form, we will have to look for it in the conditions that lead to the form of the primordia.

The proximity of dental epithelium and neighboring structures is obvious in the reconstructions, and more research is necessary to clarify what kind of influence is exercised beyond spatial impediment and pressure. The overall arrangement of the structures (dental lamina, Meckel's cartilage, and bone) suggest that the formation of buds is a consequence of the mutual spatial impediment of these structures (Radlanski *et al.*, 1988, 1989; Radlanski and Kubein-Meesenburg, 1990). Under experimental conditions, spatial impediment of growing epithelium leads to its thickening and subsequently to invagination into the underlying mesenchyme which is then condensed (Steding, 1967). In epithelial protrusions, epithelial cells are generally wedge-shaped (Blechschmidt, 1948, 1960), and were recognized in the bud stage of dental primordia (Radlanski *et al.*, 1989; Radlanski, 1993).

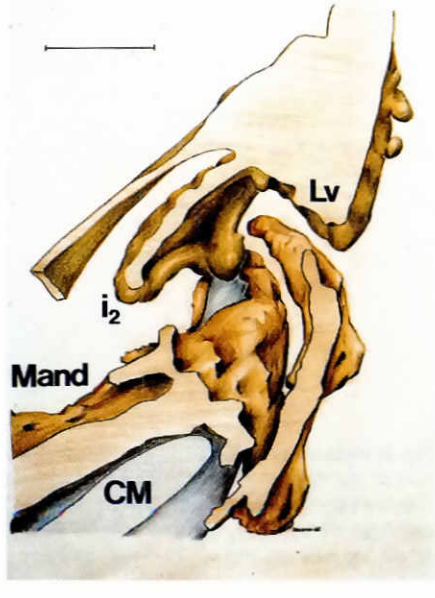

Fig. 6. Human 47 mm CRL fetus. *Reconstruction of the right primordium i_2 and its spatial relations to the vestibular lamina (Lv), mandibular bone (Mand) and Meckel's cartilage (CM). The small protrusions at the vestibular aspect of the vestibular lamina are early primordia of regional minor salivary glands. Lateral view. Scale bar, 250 µm.*

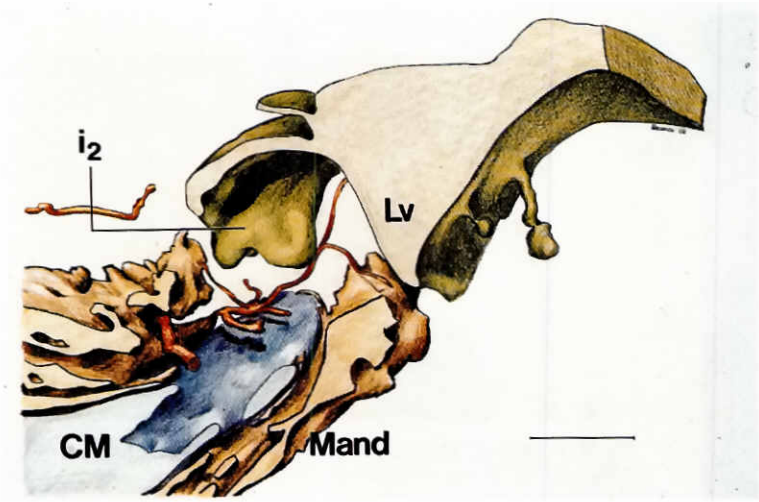

Fig. 7. Human 64 mm CRL fetus. *Reconstruction of the right primordium i_2 and its spatial relations to the vestibular lamina (Lv), mandibular bone (Mand), Meckel's cartilage (CM) and some surrounding blood vessels (red). The protrusions at the vestibular aspect of the vestibular lamina are primordia of regional minor salivary glands. Lateral view. Scale bar, 250 µm.*

The space for the expanding dental primordia is either created by active resorption of the underlying structures, as can be seen in the frontal mandibular region (Figs. 6 and 7) or, in the molar region, the groove of the mandible gets deeper as the primordium enlarges (Figs. 8 and 9). Here the vertical development of mandible leaves space for the molars to develop. This process is mediated by osteoclastic activity of the bony regions underneath the primordia, but the control of this process is still unknown. Grüneberg's findings (1937) show that in mice, which suffer from a bone resorption defect, teeth confined to crypts which do not enlarge sufficiently are conspicuously small and crimpled in form.

We cannot fully interpret the histochemical findings that deal with communication activities of the cells until we have sufficient knowledge of the spatial arrangement of developing structures. Only then we will know about the distances that the mediator substances have to bridge.

The technique and performance of computer aided 3D-reconstruction is rapidly improving, and computer capability increases from generation to generation. Contour-line plots are being replaced by surface-rendered reconstructions with modern software that can almost compete with anatomical drawings by hand. In addition, animation is available that makes the reconstruction spin around and thus enables instant views from any direction (Radlanski *et al.*, 1995).

However, as yet computers are unable to carry out automatic scanning of histological sections to record the contours to be traced. Here a differential diagnosis of the various structures of the

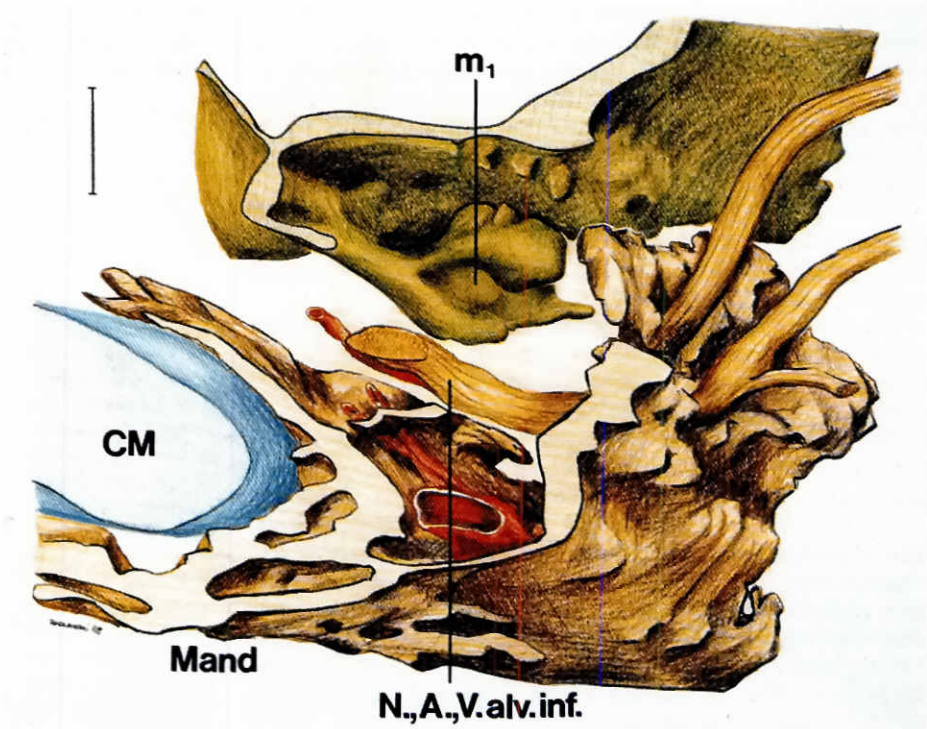

Fig. 8. Human 47 mm CRL fetus. *Reconstruction of the right primordium m_1 and its spatial relations to mandibular bone (Mand), Meckel's cartilage (CM) and nerves and vessels (N., A., V. alv. inf.). Lateral view. Scale bar, 250 µm.*

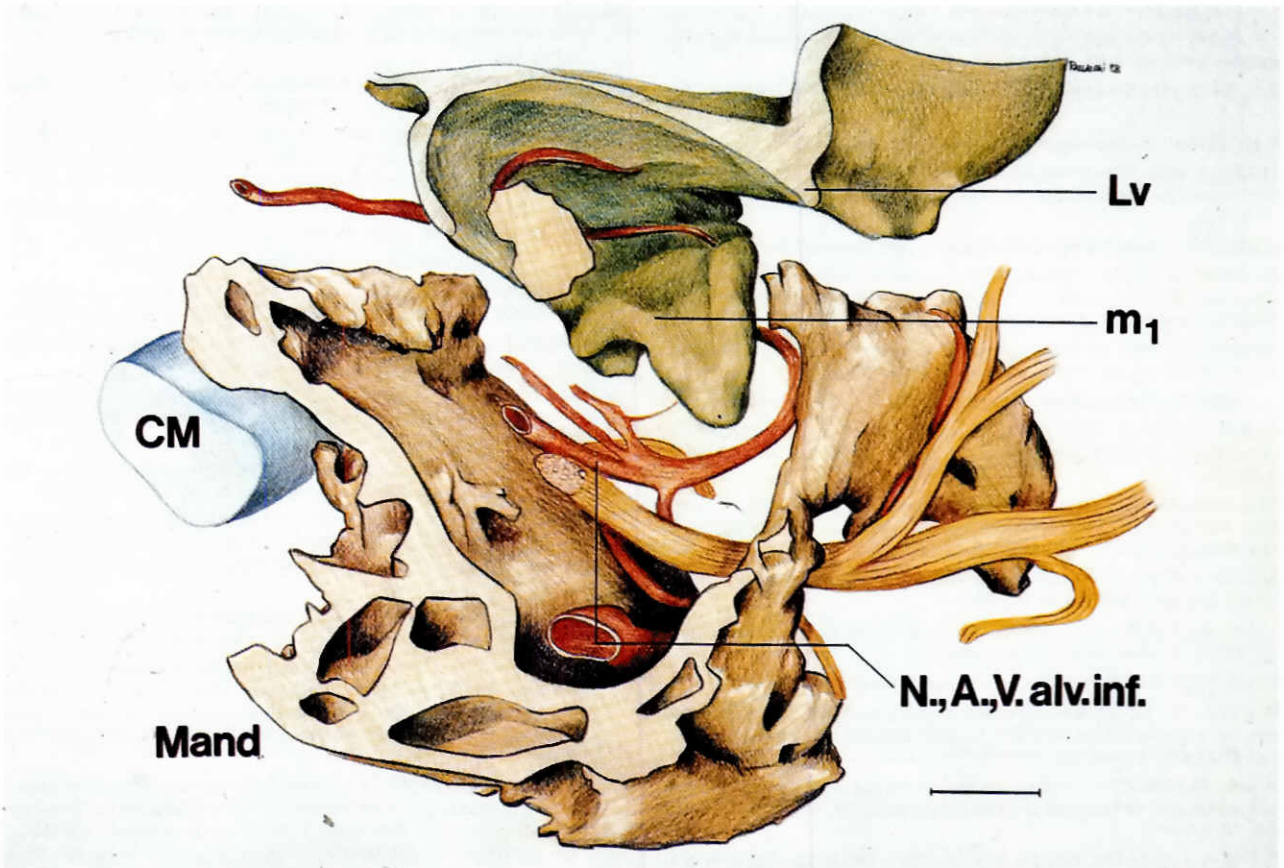

Fig. 9. Human 64 mm CRL fetus. *Reconstruction of the right primordium m1 and its spatial relations to the vestibular lamina (Lv), mandibular bone (Mand), Meckel's cartilage (CM) and nerves and vessels (N., A., V. alv. inf.). Lateral view. Scale bar, 250 µm.*

histological material still requires a trained human eye because parameters such as brightness, cell density, or cellular arrangement are not sufficient to identify borders of tissue structures.

Materials and Methods

The 3D-reconstructions used for description in this paper are a selection taken from a more extensive study (Radlanski, 1993).

Specimens

The specimens selected for this paper are human embryos and fetuses, with sizes of 18, 21, 37, 47, and 64 mm CRL (Crown-rump-length). All specimens were prepared as serial histological sections. Prior to histologic processing, the heads of all specimens were documented by means of stereoscopic photography. This served as an aid for the alignment of the single sections to form the reconstructions. The specimens were fixed in Bouin's solution, transferred into alcohol, and decalcified prior to histological sectioning. Paraffin embedding was carried out according to standard histological procedures, and all specimens were cut as 10 µm thick serial sections and stained by hematoxylin and eosin.

Technique

For the computer-aided graphical reconstruction in each section of interest the contours of epithelium, bone, cartilage, vessels and nerves were determined histologically under the light microscope. The microscope was equipped with a drawing mirror that allowed enlargement (x30 to 130) and tracing of the contours of each section on transparent paper (Gaunt and Gaunt, 1978). The drawings were piled on a light box, and the correct alignment of the single sections was checked using the outer contours of the face, which had been photographed prior to sectioning. In addition, special known structures like the eyes or Meckel's cartilage were used to check the correctness of alignment. For the computer-aided 3-dimensional reconstruction, HISTOL software (© H. König, Tübingen; Distributor: LIST, Darmstadt, Germany) was employed. The contours of the structures, which had been traced on transparent paper, were entered into an IBM-compatible personal computer via a graphic tablet (Digicad plus, KONTRON). Hardcopies were obtained by means of a six-color plotter (KPL 710, TAXAN).

The HISTOL software allows reconstruction of the three-dimensional form and its reproduction in the "hidden-line-mode" as contour-line maps, as they are known in manual graphic reconstruction techniques. The reconstruction can be rotated on the screen and viewed from several directions. For our purposes, mostly stereoscopic plots, viewed under a ZEISS stereoscope, were used. Different structures can be labeled separately and can be plotted in different colors (Fig. 1a). In addition, different structures can be temporarily omitted and quickly restored, to permit viewing of structures hidden by other structures lying closer to the viewer. Further, variable magnifications of details can be obtained. Because it is not easy for most people to understand the contour-line plots at first glance, I graphically reworked the plots (Fig. 1b).

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