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The development and fate of the dental lamina of the mandibular first molar tooth in the rat

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INTRODUCTION

Odontogenesis involves a series of interactions between orally derived epithelium and the ectomesenchyme of the jaw arches. Ontogenetically, however, there are often marked differences in the fate of part of this epithelium, even among species of the same class. In mammals, for example, the dental lamina may break up early in the morphogenesis phase of tooth development, as in man (Moscow & Bloom, 1983), whereas in the developing molar teeth of rats, the lamina apparently persists (Warwick-James & Wellings, 1943; Magnusson, 1968). Dental laminae may therefore provide useful models for studying aspects of epithelial cell behaviour and their interactions with mesenchyme, especially the factors connected with either break-up and/or persistence of the epithelium. The latter are not only matters of general developmental interest, but also of clinical interest, because in man, epithelial residues of the lamina may later form cysts (Moscow & Bloom, 1983), and in the case of odontogenic epithelium, ameloblastomas of the jaws (Manley, 1954; Pindborg, 1970). However, no sequential detailed account of lamina development appears to have been undertaken in any species. In view of this, the lamina of the first mandibular molar of the rat was examined to provide histological baselines for further studies of its epithelium and related mesenchyme.

MATERIALS AND METHODS

The laminar of first mandibular molar teeth of 39 rats, age range 11 d intra-uterine (i.u.) to 16 d postnatal (p.n.), were used. Fetuses were recovered at 2-day intervals from 11 to 21 d i.u. after laparotomy of pregnant dams, anaesthetised and then overdosed with ether vapour; postnatally, pups were killed by cervical dislocation at 1, 3, 5, 10 and 16 d. In all cases, the animals were immediately decapitated and their heads fixed either in 10% formol saline, for processing for light microscopy, or in half-strength Karnovsky's fixative at 4 °C for 24 h for processing for transmission electron microscopy (TEM). Processing for light microscopy included demineralisaton in 5% trichloracetic acid, dehydration in graded ethanols preparatory to routine clearing and embedding in paraffin wax. Serial sections 10 μm thick were cut in the transverse (coronal) plane, stained either with haematoxylin and eosin, Weight and van Geison or Mallory's trichrome connective tissue stain. Processing for TEM included

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demineralisation in 4·17% EDTA, postfixation in 1·0% osmium tetroxide in 0·1 M sodium cacodylate buffer for 2 h followed by routine dehydration and embedding in Epon 812 resin. Semithin and ultrathin sections were cut using glass knives mounted on a Reichert OMU2 ultramicrotome. The former sections were stained with toluidine blue and examined by light microscopy whereas the latter were stained with uranyl acetate and Reynold's lead citrate for examination using a JEOL 100 CX II TEM operated at 80 kV.

RESULTS

Prenatal stages

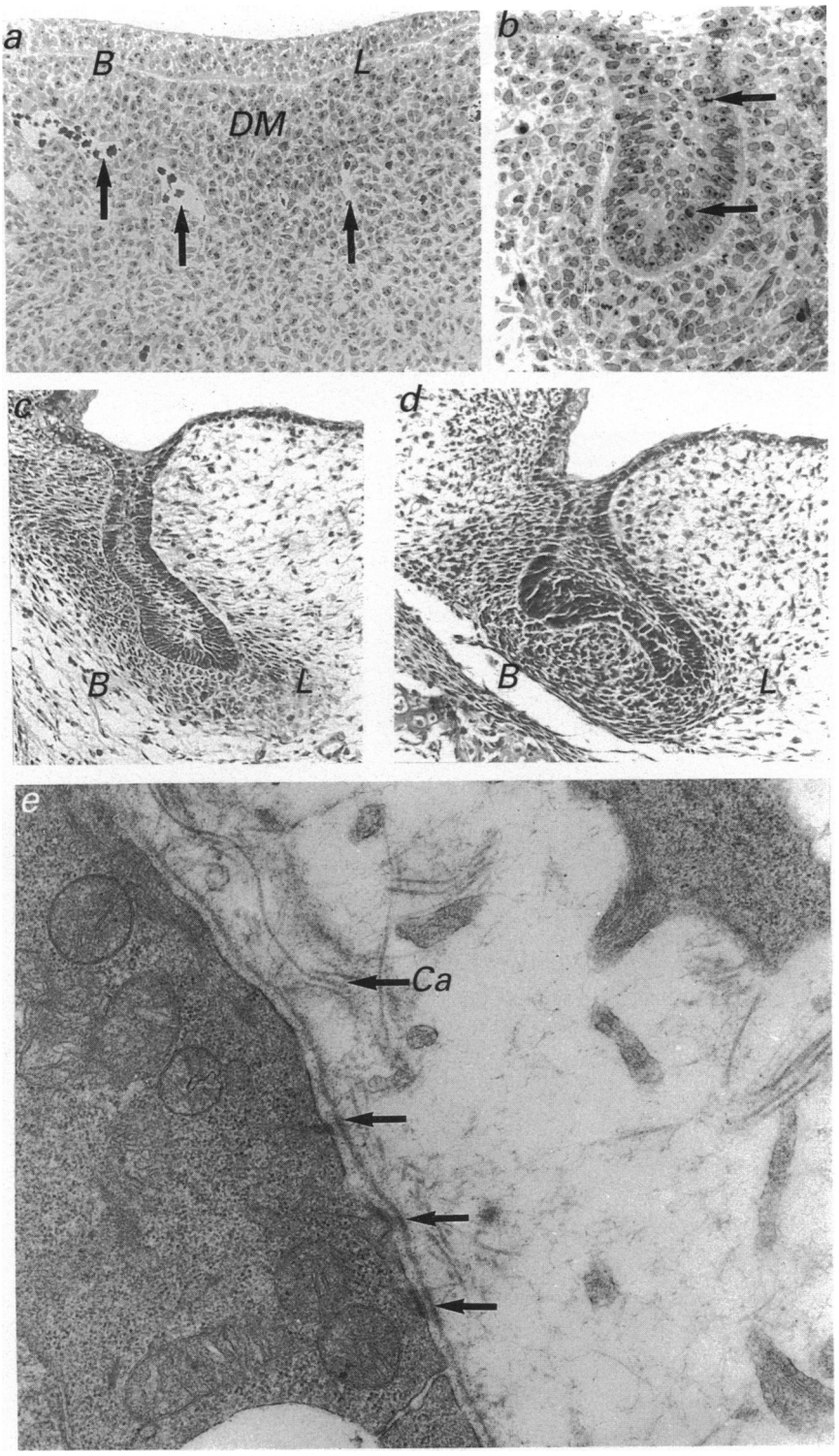
Earliest indications of the site of invagination of the lamina were detectable at 13 days prenatally (Fig. 1*a*) as a shallow indentation of the surface, the epithelium on the buccal aspect of which was thicker than that on the lingual. Directly beneath this site was a mesenchymal condensation, distinguished from the more loosely packed jaw mesenchyme mainly by cell density but often also by intervening capillaries. In the early proliferative stage of laminar invagination, which lasted until approximately 15–16 d i.u., when the bud of the enamel organ was first apparent, the lamina consisted of basal layers buccally and lingually and a central core of less densely staining cells; however, any mitotic figures that were observed were confined to the suprabasal layers (Fig. 1*b*).

At 15 and 17 d i.u., the distinction between the buccal and lingual epithelium on either side of the laminar invagination was more marked than earlier; in addition, the mesenchymal cell density buccal to the lamina was greater than on the lingual side (Fig. 1*c*, *d*). The cells of the basal epithelium of the lamina showed few organelles other than free ribosomes, a few mitochondria and occasional strands of rough endoplasmic reticulum (RER). Their basal lamina was generally smooth if slightly undulating in places (Fig. 1*e*).

By 19 d the enamel organ was well developed, showing the characteristic layers of the 'bell-stage' configuration and at this stage, several changes were detectable in relation to the lamina. The more obvious were keratinisation of the overlying epithelium (in conjunction with the oral epithelium generally) and accumulations of glycogen, mainly in the cells of the oral half of the lamina as well as in the related oral epithelium (Fig. 2*a*, *b*). The latter feature became more evident by 21 d and persisted approximately until the onset of tooth eruption.

Additionally, small bays were detectable in the basal layers of the lamina, principally in the dentally related half (Fig. 2*c–e*). Laminar cells bordering the bays often contained glycogen as well as lipid-like bodies (Fig. 2*d*) but little RER and there was no evidence of basal epithelial cell death to suggest a means of bay formation. In places, the lamina densa of the basal lamina in the bays appeared to be absent or deficient and there were few related hemidesmosomes (Fig. 2*e*).

Fig. 1. (*a*) Light micrograph (LM) of a semithin section of the site of invagination of the lamina. Buccally (*B*) the epithelium is thicker than lingually (*L*). In this section, capillaries (arrows) demarcate the dental mesenchyme (*DM*) from the jaw mesenchyme. 13 d i.u. Toluidine blue, ×200. (*b*) LM of the early lamina at 15 d i.u. The basal layers are more densely stained than the central zone but mitotic figures (arrows) are confined to the suprabasal layers. Toluidine blue, ×307. (*c*) LM of the lamina at 16 d i.u., and (*d*) at 17 d i.u., which shows early enamel organ development; in both sections, buccal and oral epithelium is thicker than lingual and buccal mesenchyme denser than lingual (*B*, buccal; *L*, lingual). Toluidine blue, ×242, and ×233 respectively. (*e*) TEM of basal cells in the lamina showing development of the basal lamina (arrows) and collagenous anchoring fibrils (*Ca*) ×30200.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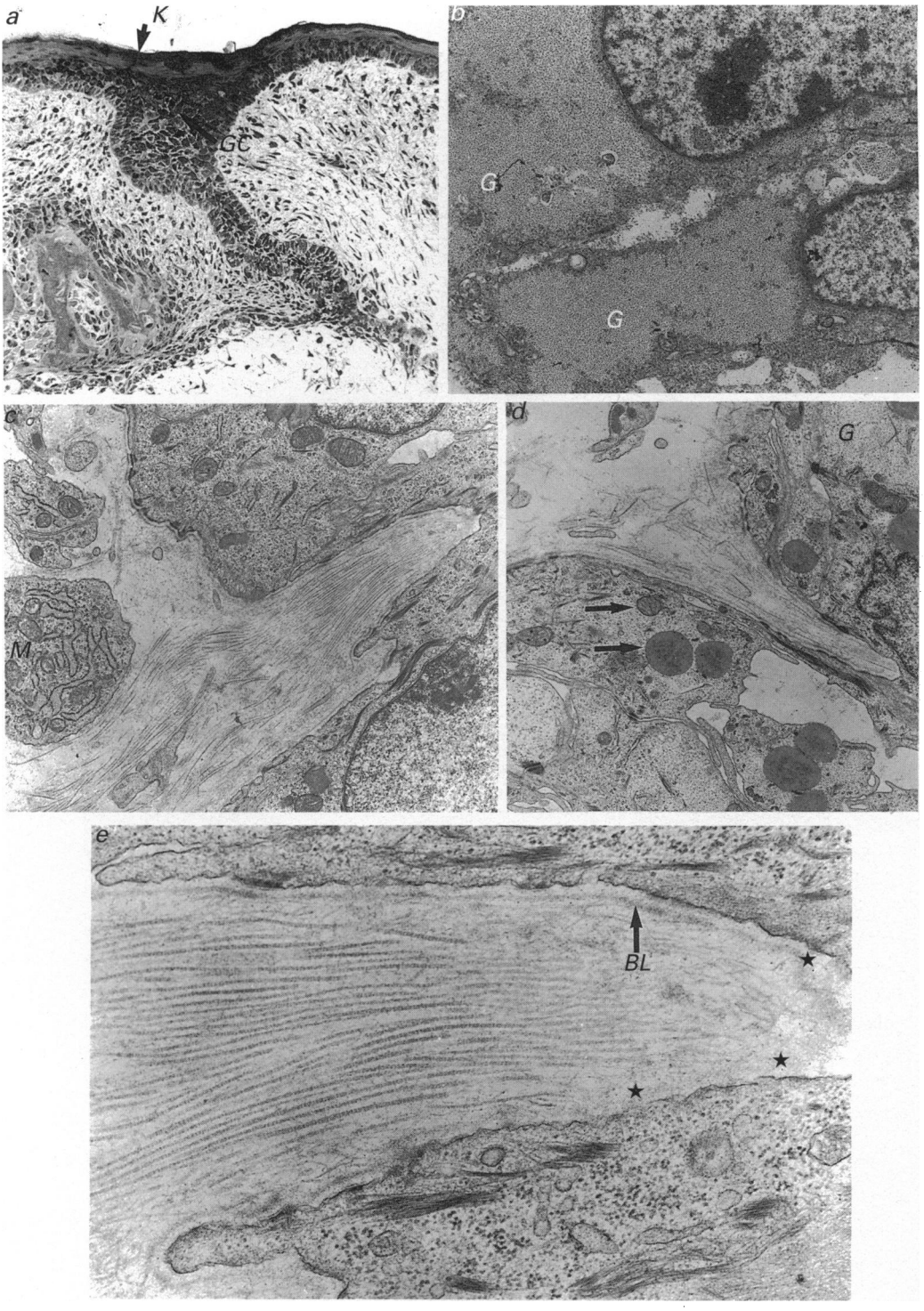
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Each of these bays was occupied by collagen, oriented mostly in the longitudinal axis of the bay, and detectably more dense than the collagen related to the basal lamina elsewhere; in addition, the background extracellular matrix appeared to be more plentiful (Fig. 2*e*). Adjacent mesenchymal cells were rich in dilated RER but at this stage (Fig. 2*c*) the cells themselves did not intrude into the bays.

Postnatal stages

In newborn animals, the general features of the lamina were similar to those seen at full term, except that in many sections, the oral half of the lamina appeared to be thicker and more glycogen-rich than previously. However, on the lingual aspect, where the lamina became continuous with the external enamel epithelium, a secondary tooth bud was apparent on the lingual aspect (Fig. 3*a*); and elsewhere, the external enamel epithelium showed break-up with continuity of external vascular connective tissue into the stellate reticulum (Fig. 3*b*). The secondary tooth bud was still apparent at 5 d, but by this time it too showed collagen-containing bays in its basal layers as well as accumulations of collagen in its deeper intercellular spaces (Fig. 3*c*). The epithelium of the bud, however, did not show any of the glycogen accumulations or lipid associated with bay formation in the lamina itself. At 10 d the bud was no longer detectable.

At 5 and 10 d, the lamina was generally thicker than earlier and at the latter age, in particular, epithelial 'pearls' were often visible in its core (Fig. 4*a*). Although otherwise the lamina appeared to be intact, ultrastructurally, collagen and mesenchyme-like cells occupied intercellular spaces of the dentally related half and these features were more apparent with increase in age (Fig. 4*a*, *c*). Where the inserted mesenchymal cells showed lysosome-like inclusions (Fig. 4*b*) there was little evidence of basal lamina formation by adjacent epithelium but where lysosomes were absent, the epithelium often showed a complete basal lamina (Fig. 4*c*). At the lamina surface, moreover, some basal epithelial cells showed areas of 'watery' cytoplasm and deficiencies in their basal lamina, adjacent to mesenchymal cells that contained lysosomal bodies (Fig. 4*d*).

At 16 d, shortly before the eruption of the tooth into the oral cavity, the lamina orally consisted of a short compact trunk, but its dentally related part was widely fenestrated above the erupting crown and glycogen was no longer apparent in any of its cells; and the fenestrations were occupied by vascular connective tissue, continuous with that of the stellate reticulum (Fig. 5*a*). Cells in the fenestrations contained large accumulations of lysosomes (Fig. 5*c*) and there was evidence of cell death in the related laminar epithelium (Fig. 5*b*). In addition, single lysosome-rich cells were detectable within the laminar epithelium, and much of the related intercellular spaces was occupied by an amorphous substance similar to that contained in these cells (Fig. 5*d*).

Fig. 2. (*a*) LM of the lamina at 19 d i.u. showing keratinisation of the oral epithelium (*K*), and the appearance of glycogen within cells of the oral half of the lamina (darker stained cells, *GC*). Haematoxylin and eosin, ×224. (*b*) TEM showing glycogen accumulations (*G*) in 2 of the basal cells of the lamina. ×7500. (*c–e*) Electron micrographs showing collagen containing bays in the lamina at 21 d i.u. (*c*) No cells or cell processes occupy the bays but adjacent mesenchyme cells (*M*) show dilated endoplasmic reticulum. ×10800. (*d*) Adjacent laminar cells contain glycogen (*G*) and lipid (arrows). ×10000. (*e*) Detail of the bay showing the discontinuities (starred) in the basal lamina (*BL*). ×40000.

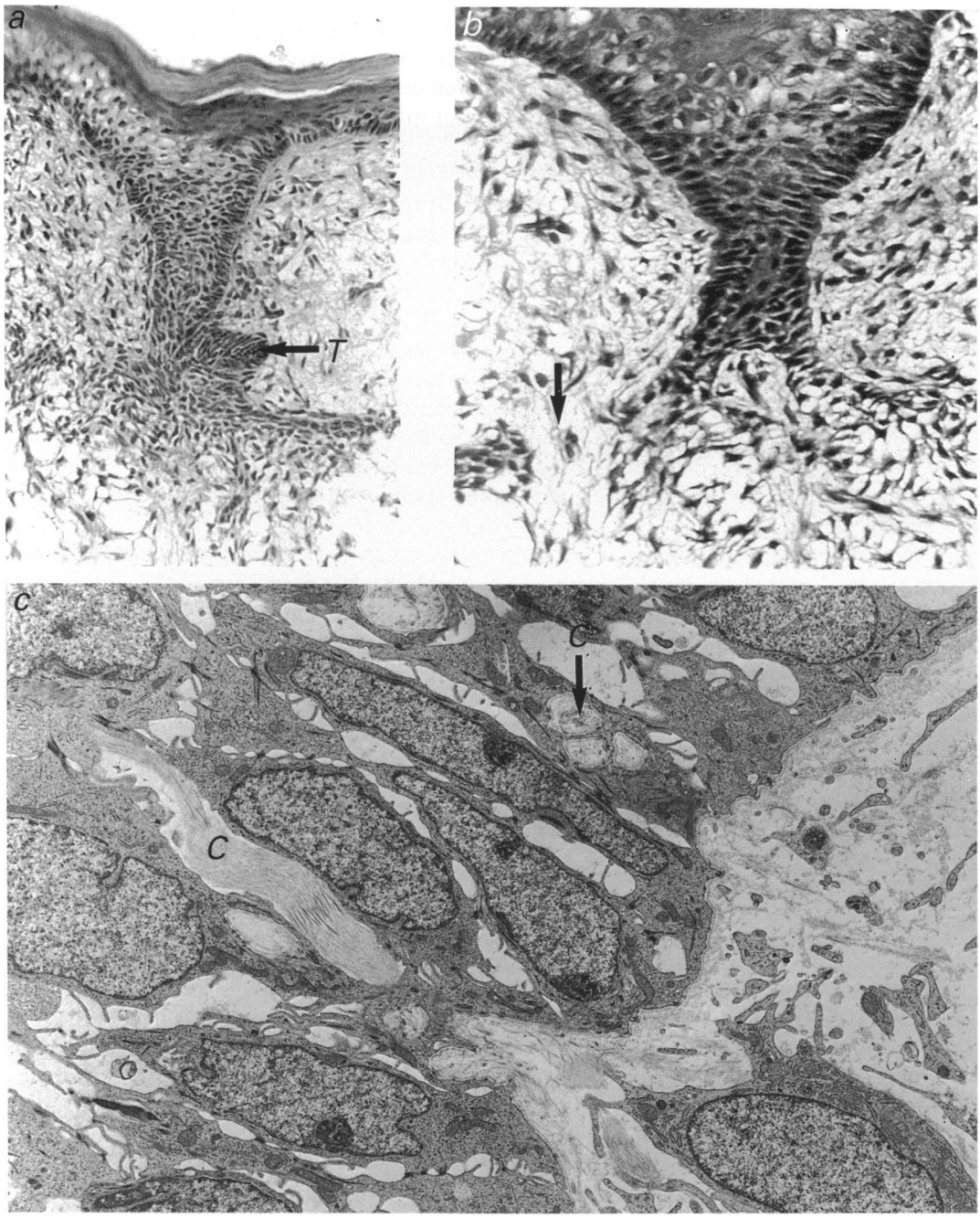

Fig. 3. (*a*) LM of the lamina of newborn rat showing a secondary tooth bud (*T*). Haematoxylin and eosin, ×277. (*b*) Continuity of vascular connective tissue into the stellate reticulum through discontinuous external dental epithelium (arrow). Haematoxylin and eosin, ×455. (*c*) TEM of basal layers of the secondary tooth bud at 5 d showing collagen (*c*) in the intercellular spaces. ×4725.

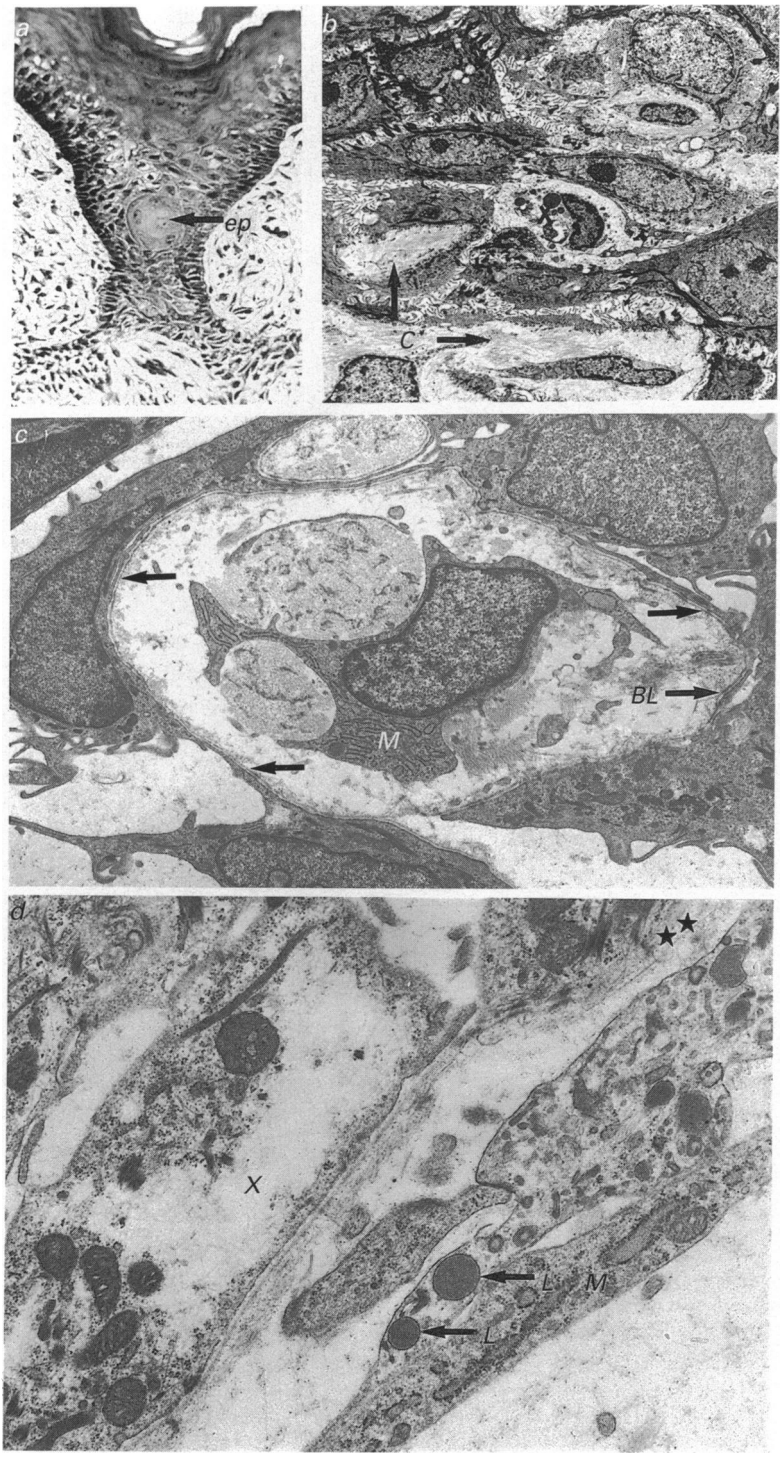

Fig. 4. (*a*) LM at 10 d p.n. showing an epithelial pearl (*ep*) in the centre of the lamina. Haematoxylin and eosin, ×430. (*b*) TEM of the central part of the lamina at the same stage showing collagen (*C*) in the intercellular spaces. Lysosomal bodies can be seen in occasional cells (*X*). ×2100. (*c*) Mesenchyme-like cells (*M*) and collagen surrounded by epithelial cells in the central part of the lamina. Note the continuous basal lamina (*BL*, arrows). ×7500. (*d*) TEM of the lamina at 10 d p.n. showing a lysosome (*L*)-rich mesenchyme cell (*M*) adjacent to basal epithelial cells which show areas of 'watery' cytoplasm (*X*) and a discontinuous basal lamina (starred). ×17500.

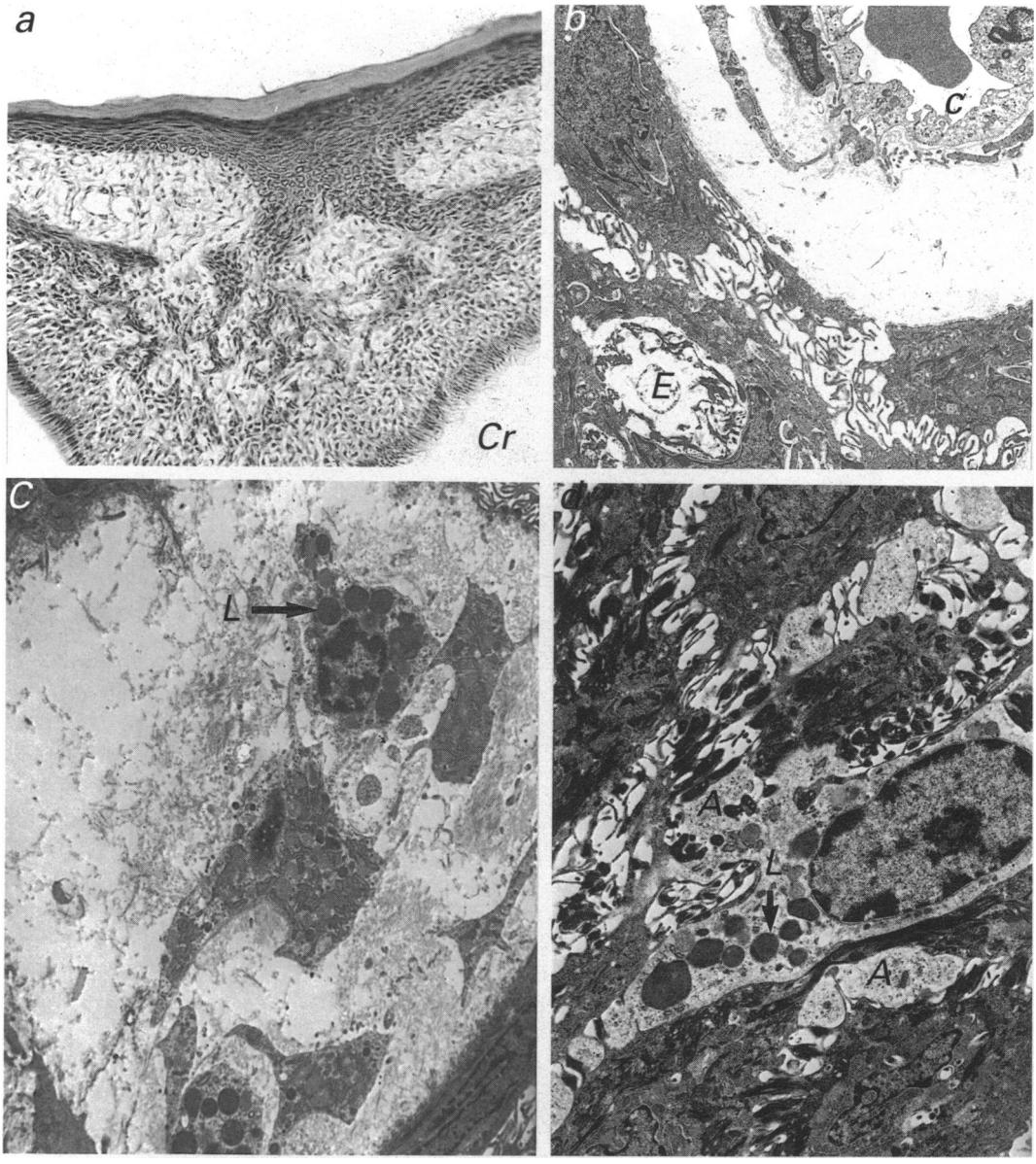

Fig. 5. (*a*) LM of the lamina at 16 d p.n. Note the compact lamina compared with earlier stages and the penetration of connective tissue from below. The proximity of the erupting crown (*Cr*) to the oral epithelium is apparent. Haematoxylin and eosin, ×105. (*b*) TEM of the lamina at 16 d showing spaces in the lamina occupied by capillaries (*C*), and evidence of epithelial cell death (*E*). ×4050. (*c*) Lysosome-rich cells (*L*) are apparent in the fenestrations of the lamina. ×4050. (*d*) Accumulation of amorphous intercellular material (*A*) is evident within the laminar epithelium close to a lysosome-rich mesenchymal cell (*L*). ×6000.

DISCUSSION

While from a light microscopic view the results support previous conclusions that the molar lamina of the rat persists throughout tooth development (Warwick-James & Wellings, 1943; Magnusson, 1968), ultrastructural evidence indicates that epithelial integrity is progressively disrupted by mesenchymal elements, probably beginning as early as 19 d i.u. with collagen and extracellular matrix (ECM) products occupying bays in the basal layers of the lamina. It is noteworthy that these bays appeared first at the late 'bell' stage of the enamel organ, which corresponds to the stage of onset of disruption of the lamina in man (Moscow & Bloom, 1983).

The evidence further suggests that the contents of the bays were produced by the RER-rich mesenchymal cells adjacent to the lamina rather than by the epithelium, and that the secondary tooth bud apart, bay formation was accompanied by altered metabolism of adjacent epithelial cells as well as patchy loss of the basal lamina. However, it was not possible to decide whether these bays arose 'actively' by ECM products invading the basal epithelium, or 'passively', either by epithelial enfolding of deposits of collagen or by localised alterations in epithelial cells which permitted (or even attracted) mesenchymal intrusion. Nevertheless, there is a wealth of literature indicating that epithelial–mesenchymal interactions are mediated via basement membrane components, probably dictated by mesenchymal ECM macromolecules (Kratochwil, 1969; Spooner & Wessels, 1970; Lawson, 1972; Kleinman *et al.* 1982; Kleinman, McGarvey, Hassell & Martin, 1983; Kleinman *et al.* 1984; Bernfield *et al.* 1984; Ferguson & Honig, 1984); growth factors (Ferguson, 1988); neurotransmitters (Zimmerman & Wee, 1984) and possibly also cell to cell contacts (Ferguson, 1988; Sharpe and Ferguson, 1988). It is also known that in tooth development, many of these ECM macromolecules change as development progresses (Thesleff, Stenman, Vaheri & Timpl, 1979; Thesleff & Hurmerinta, 1981; Thesleff, Mackie, Vainio & Chiquet-Ehrismann, 1987). On these bases, plus the finding that later evidence of epithelial cell death was detected in relation to mesenchyme cells containing lysosomes, it seems reasonable to implicate the mesenchyme in the initiation as well as the continuance of laminar disruption. No evidence was found to support the view that the process of bay formation involved either programmed cell death or epithelial cell transformation, such as have been reported in the midline epithelial seam of the secondary palate (Ferguson, 1988; Sharpe & Ferguson, 1988).

Further disruption of the lamina was quite patchy in the pre-eruptive phase (i.e. before day 10 p.n.) and appeared to involve mainly the dentally-related half with collagen and then mesenchymal cells intruding gradually into the more deeply placed intercellular spaces. This was accompanied in some instances by formation of a basal lamina by related epithelial cells, sometimes to the extent of encircling the mesenchymal intrusion (e.g. Fig. 4*c*). We postulate that there may be a phasic progression in the disruptive process, in the manner of physiological resorption generally. Thus, in active phases, little or no evidence of basal lamina formation may be detectable, while in quiescent phases, epithelial cells may regroup and reform attachments to each other as well as a basal lamina. If this is indeed the case, the thickness of the lamina in the rat may be one reason why the lamina remains relatively intact throughout tooth development. The pearl-like inclusions seen occasionally in the lamina at later stages suggest that groups of epithelial cells may even form a complete surround of basal lamina during quiescent stages—something that may echo events in the formation of epithelial residues (microcysts) in laminae that normally disrupt more completely (Moscow & Bloom, 1983).

At the initiation and proliferation states, the site of laminar development was marked by differences in the thickness of buccal and lingual epithelium. Although the factors involved in tooth initiation were not the prime concern of this work, it seems reasonable to suggest that these differences may be a manifestation of a buccolingual determinant of the siting of the lamina. Hitherto, work has concentrated on mechanisms of tooth initiation and the question of tooth position in the anteroposterior axis (Koller & Baird, 1970*a*, *b*; review by Osborn, 1978; Lumsden & Buchanan, 1986; Mina & Kollar, 1987) but it is clear that the invagination site must have the second axis before its position can be established. Further evidence of buccolingual influences during early odontogenesis may also be reflected in the observed differences in buccal and lingual densities of the mesenchyme related to the lamina. It is known, moreover, that ECM products implicated in epithelial–mesenchymal interactions such as tenascin are differentially expressed in the same regions (Thesleff *et al.* 1987). As odontogenesis, like many other developing systems, involves such interactions (Kollar & Baird, 1970*a*, *b*; Thesleff & Hurmerinta, 1981; Lumsden, 1988) it would be surprising if these differences were not indicative of some role in the process of lamina development, including perhaps its guidance towards the developing blastemata of the jaw bones.

The appearance of glycogen in the lamina has been reported previously (Wislocki & Sognnaes, 1950; Magnusson, 1968). Similar glycogen accumulations appear in the external dental epithelium of the enamel organs of the rat (Decker, 1963) and man (Fischlschweiger, Provenza & Sisca, 1967) at the late bell stage of tooth development, as well as in parts of the oral epithelium of rats. However, they are not universally present in epithelium associated with keratinisation (Porter & Lefkowitz, 1965), so the almost synchronous development of these 2 features must be regarded as a coincidence. None of the previous workers has given satisfactory explanations for the transient appearance of this glycogen. Fischlschweiger *et al.* (1967) proposed, somewhat self-evidently, that it reflected altered cell metabolism; and that its diminution was inversely related to the accumulation of lipid, but no such correlation was suggested in the present work. We suggest that these alterations are more likely to reflect epithelial changes in response to localised mesenchymal signalling (Ferguson, 1988). Moreover, although no lipid or glycogen inclusions were detected in the epithelium of the secondary tooth bud preceding deposition of collagen first into bays and later into the deeper intercellular spaces, it seems reasonable to suggest that this deposition may be the primary cause of abortion of the secondary bud development.

Once the process of eruption commences, laminar breakdown appears to accelerate, particularly in the late stages, with lysosome-rich mesenchymal cells intruding into intercellular spaces. The evidence is consistent with the view that here the mesenchymal cells may first of all break down epithelial cell adhesions and then precipitate some epithelial cell death. The spaces thus created appear to become filled with connective tissue elements, extending mostly from the underlying stellate reticulum. Not surprisingly, this apparent acceleration has echoes of the disruption of connective tissue above the crowns of erupting teeth in which the lamina disappears (Ten Cate, 1971), so it seems reasonable to conclude that as in that instance, accelerated breakdown of the lamina is occasioned by tooth eruption.

SUMMARY

The lamina of the first mandibular molar teeth of rats, age range 13 d intrauterine (i.u.) to 16 d postnatal (p.n.), was examined by light and transmission electron micro-

scopy to establish histological baselines of its development and fate. All material was obtained from animals anaesthetised with ether, killed by cervical dislocation and prepared by routine methods for both types of examination. Contrary to earlier reports that the lamina remains intact throughout development, mesenchymal elements disrupt the lamina. These were seen first at 19 d i.u., as collagen-filled bays in the basal epithelial layers, associated with partial loss of related basal lamina. In the early stages, collagen deposition was limited and it was not obviously preceded by epithelial cell death or transformation, even though many bay-related cells showed lipid and glycogen accumulations. Later disruption of the lamina showed more mesenchymal cells as well as collagen in deeper spaces. After the onset of tooth eruption, mesenchymal cells external to and within the lamina contained lysosomal bodies and these plus evidence of related epithelial cell death and capillaries in the laminar spaces became more and more apparent. Similar collagen deposits were observed in a successional tooth primordium, which appeared at term but eventually aborted between days 5 and 10 p.n. Thus disruption of the lamina by connective tissue began earlier than has been reported previously and progressed as the tooth erupted towards the oral cavity. The evidence suggests that this disruption is initiated and sustained by mesenchymal cell activity rather than by programmed cell death or transformation of the epithelium.

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