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Original Article

Degradation of the dental basement membrane during mouse tooth development *in vitro*

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ABSTRACT During tooth development, the basement membrane is degraded at the late bell stage, but the developmental significance of this event is not known. Organ culture offers a method where developmental processes can be manipulated in controlled conditions. We cultured bell-stage tooth germs either in a chemically defined or a serum-containing medium and analyzed the degradation of the basement membrane by different methods. Type IV collagen was present throughout the dental basement membranes at the epithelial-mesenchymal interface at the onset of culture. After 10 days of culture, irrespective of the medium used, type IV collagen and laminin had disappeared from the cuspal areas but were present at the cervical loop. As was the case *in vivo*, the expression of 72 kDa type IV collagenase gene was intense in the differentiating preodontoblasts and in the odontoblasts during secretion of the first predentin matrix near the cuspal tips. Ultrastructural observations showed that the basal lamina had been removed in all cultured tooth organs. Also, the breakdown of the basement membrane occurred irrespective of the presence of mineral in the dentin matrix. Our observations suggest in contrast to earlier observations, that there are no major differences in basic events leading to dentino- and amelogenesis, when tooth organs are cultured in the presence or absence of serum.

KEY WORDS: *odontogenesis, tooth organ cultures, basement membrane, cell differentiation*

Introduction

Although the disappearance of the dental basement membrane has been documented by different methods in numerous studies during the last decades, the developmental significance of this event has remained obscure (Kollar and Baird, 1970; Thesleff and Hurmerinta, 1981; Ruch *et al.*, 1983). The basement membrane between the inner enamel epithelium and the dental papilla mesenchyme has been shown to trigger odontoblast terminal differentiation (Thesleff *et al.*, 1977, 1978; Thesleff, 1978; Ruch *et al.*, 1982, 1983; Ruch, 1987). Ultrastructural and immunohistochemical studies in different mammals have shown that the basement membrane is degraded during terminal differentiation of ameloblasts and that it disappears completely before initial enamel deposition begins (Reith, 1967; Silva and Kailis, 1972; Kallenbach and Piesco, 1978; Matthiessen and Römert, 1980; Hurmerinta and Thesleff, 1981; Skobe *et al.*, 1981; Katchburian and Burgess, 1983; Sawada *et al.*, 1987, 1990).

In lower vertebrates, such as frogs (*Xenopus* sp. and *Rana* sp.), the differentiation of odontoblasts and ameloblasts and the timing of the degradation of the basement membrane resemble the events in mammals, although, the cell-cell contacts are much less frequent in frogs than they are in mammals. However, in the shark, the basement membrane remains intact throughout ameloblast differentiation and even increases in thickness during the deposition and calcification of the enamel matrix (Kallenbach and Piesco, 1978).

Predentin secreted by the differentiated odontoblasts has been thought to have an important role in inducing the differentiation of the epithelial cells into ameloblasts (Hurmerinta and Thesleff, 1981; Ruch, 1987). Numerous electron microscopical studies have demonstrated that, simultaneously with the onset of predentin mineralization, the basal lamina becomes discontinuous and disappears (Pannese, 1962; Kallenbach, 1971; Meyer *et al.*, 1977). The disappearance of the basal lamina allows the formation of cell-cell contacts between the preameloblasts and the odontoblasts, which has been proposed to mediate the inductive signal for ameloblast differentiation (Kallenbach, 1971; Silva and Kailis, 1972; Slavkin and Bringas, 1976; Kallenbach and Piesco, 1978).

The degradation of the basement membrane has been suggested to be a result of ameloblast phagocytosis (Sawada *et al.*, 1987, 1990), but also lysosomal enzymes derived from ameloblasts as well as proteolytic enzymes originating from odontoblasts could

Abbreviations used in this paper: PBS, phosphate buffered saline; FCS, fetal calf serum; MMP, matrix metalloproteinase.

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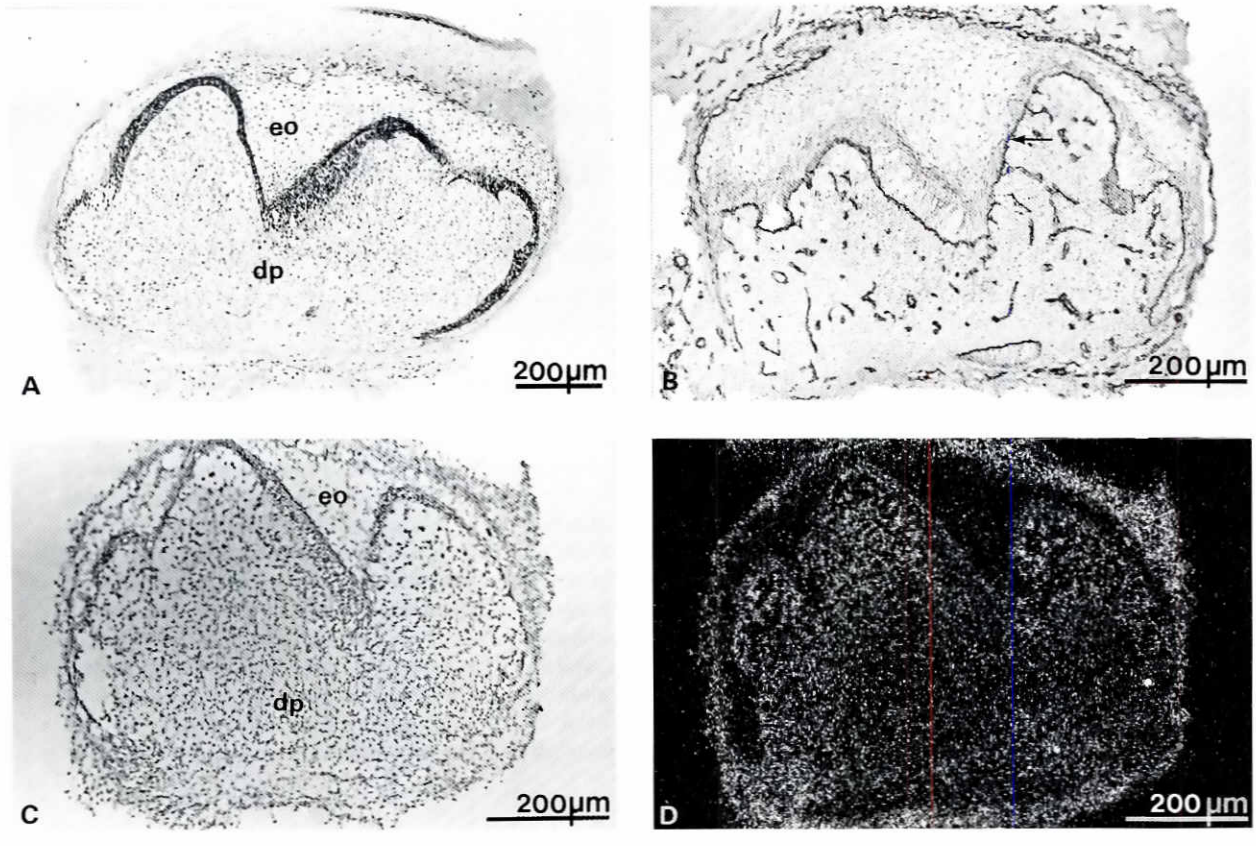

Fig. 1. At the onset of culture the first mandibular molar from 18-day-old mouse embryo is at the bell stage of development. *Cusp pattern is evident but secretion of dentin matrix has not started* **(A)**. *Comparison between in situ hybridization of 72 kDa type IV collagenase* **(C,D)** *and immunostaining of type IV collagen* **(B)** *in the basement membrane is shown.* **(C,D)** *Expression is elevated in the cuspal tips of the bell stage tooth.* **(B)** *In the immunostained section the dental basement membrane (arrow) is present throughout the epithelio-mesenchymal interface. eo, enamel organ; dp, dental papilla mesenchyme.* **(A)** *1 μm Epon section;* **(B)** *type IV collagen immunostaining;* **(D)** *dark-field image of* **(C)**. *A, x60; B-D, x80.*

be involved (Katchburian and Holt, 1969; Betti and Katchburian, 1982; Katchburian and Burgess, 1983). It was suggested recently that preodontoblasts contribute to the degradation of the dental basement membrane (Sahlberg *et al.*, 1992) by expressing 72 kDa type IV collagenase (gelatinase A/MMP-2). Transcripts of this proteinase were localized by *in situ* hybridization during tooth development in the mesenchyme of embryonic and postnatal mouse molars.

It has been reported that the dental basal lamina remains intact during ameloblast differentiation and during formation of enamel extracellular matrix, when tooth germs are cultured in a serumless chemically defined medium (Slavkin *et al.*, 1983). Fibronectin and laminin, visualized immunohistochemically, were localized throughout the epithelio-mesenchymal interface after ameloblast differentiation had occurred and the basal lamina appeared ultrastructurally intact. However, when tooth organs were cultured in medium supplemented with 10% fetal calf serum the basal lamina was degraded as was the case *in vivo* (Slavkin *et al.*, 1977).

Our aim was to use tooth organ culture for analysis of the role of the basement membrane and its degradation during ameloblast differentiation, enamel secretion and mineralization in tooth development *in vitro*. Embryonic mouse molar tooth germs were cultured either in a chemically defined medium or in a serum-containing medium and the basement membrane area was observed in a transmission electron microscope (TEM). The basement membrane molecules, type IV collagen and laminin, were localized by immunohistochemistry. *In situ* hybridization was used for detection of the transcripts of 72 kDa type IV collagenase and von Kossa staining for detection of mineral deposits in dentin and enamel.

Results

Light-microscopic observations

18-day-old embryonic molar in vivo

At the onset of culture the molar tooth had reached the bell stage of development and histologically a distinct cusp pattern was seen (Fig. 1A). At the central cusp and at one of the lateral cusps odontoblasts had differentiated and in the remaining areas only preodontoblasts were found. In immunostained sections collagen type IV (Fig. 1B) was localized throughout the basement membrane at the epithelio-mesenchymal interface. At this stage the expression of 72 kDa type IV collagenase was restricted to mesenchymal cells and the epithelia were completely negative. Transcripts were increased in the preodontoblasts adjacent to the basement membrane near the cuspal tips (Fig. 1C,D).

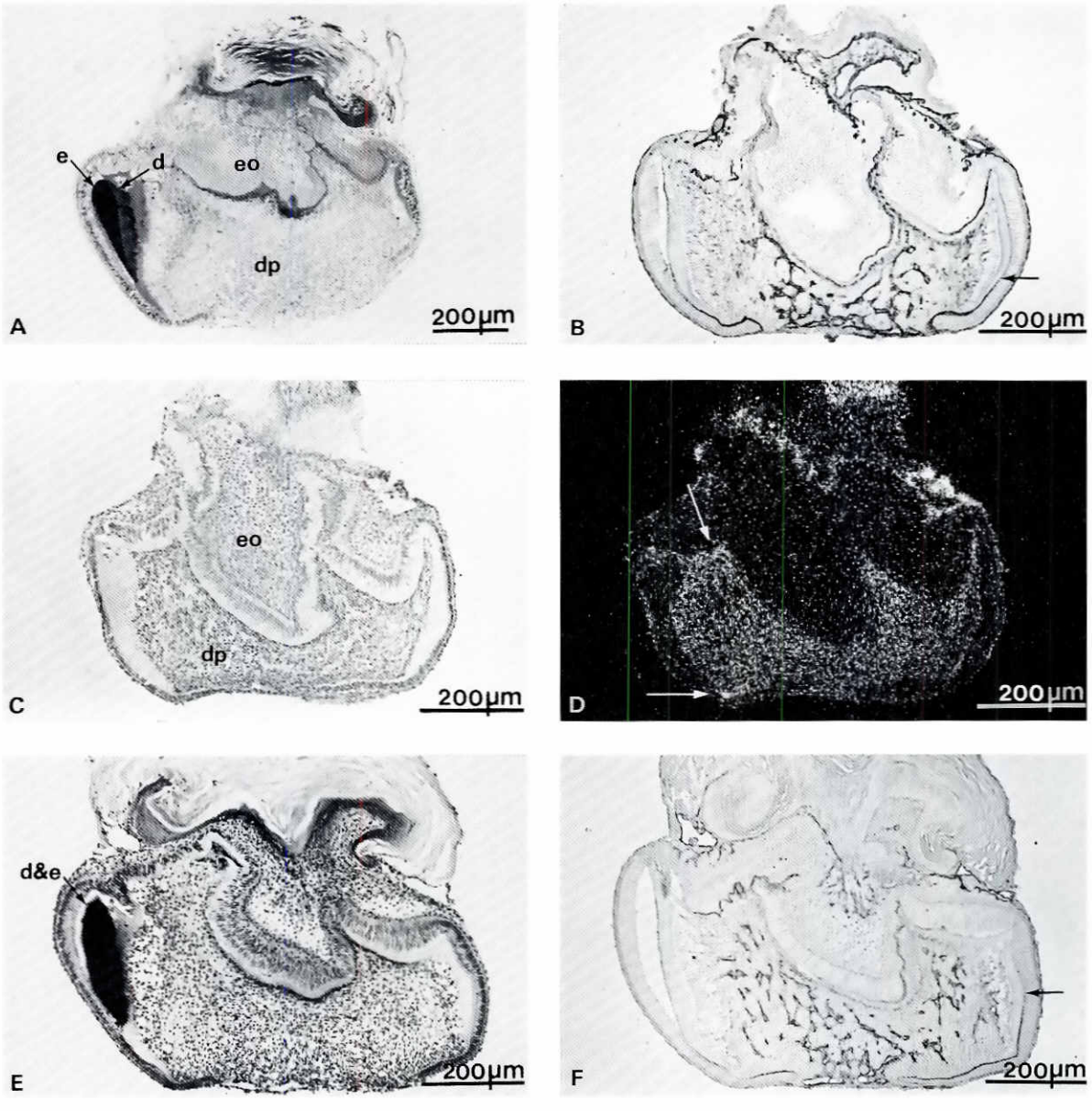

Fig. 2. Molar tooth germs from 18-day-old embryo cultured for 10 days in a serum-containing medium (A). *Comparisons between in situ hybridization of 72 kDa type IV collagenase* **(C,D)**, *immunostaining of type IV collagen* **(B,F)** *and laminin* **(G)** *in the dental basement membrane and von Kossa staining* **(E)** *for calcium precipitates.* **(C,D)** *The expression is evenly distributed in the dental mesenchymal cells except the odontoblasts in a small area at the cervical loop region and near one cusp tip (arrows). Type IV collagen* **(B,F)** *and laminin* **(G)** *in the dental basement membrane have vanished in areas where dentin matrix has been secreted (arrows). The stained structures in the pulp are capillaries.* **(E)** *In one of the lateral cusps an area of mineralized dentin and enamel is shown by von Kossa staining.* **(F)** *The adjacent section is immunostained and shows that type IV collagen in the dental basement membrane has disappeared from areas where dentin matrix has been secreted and from areas where mineralization is verified. dp, dental papilla mesenchyme; eo, enamel organ; d, dentin; e, enamel.* **(A)** *1 μm Epon section;* **(B,F)** *type IV collagen immunostaining;* **(D)** *dark-field image of* **(C)**; **(E)** *von Kossa staining;* **(G)** *laminin immunostaining. A, x60; B-G, x80.*

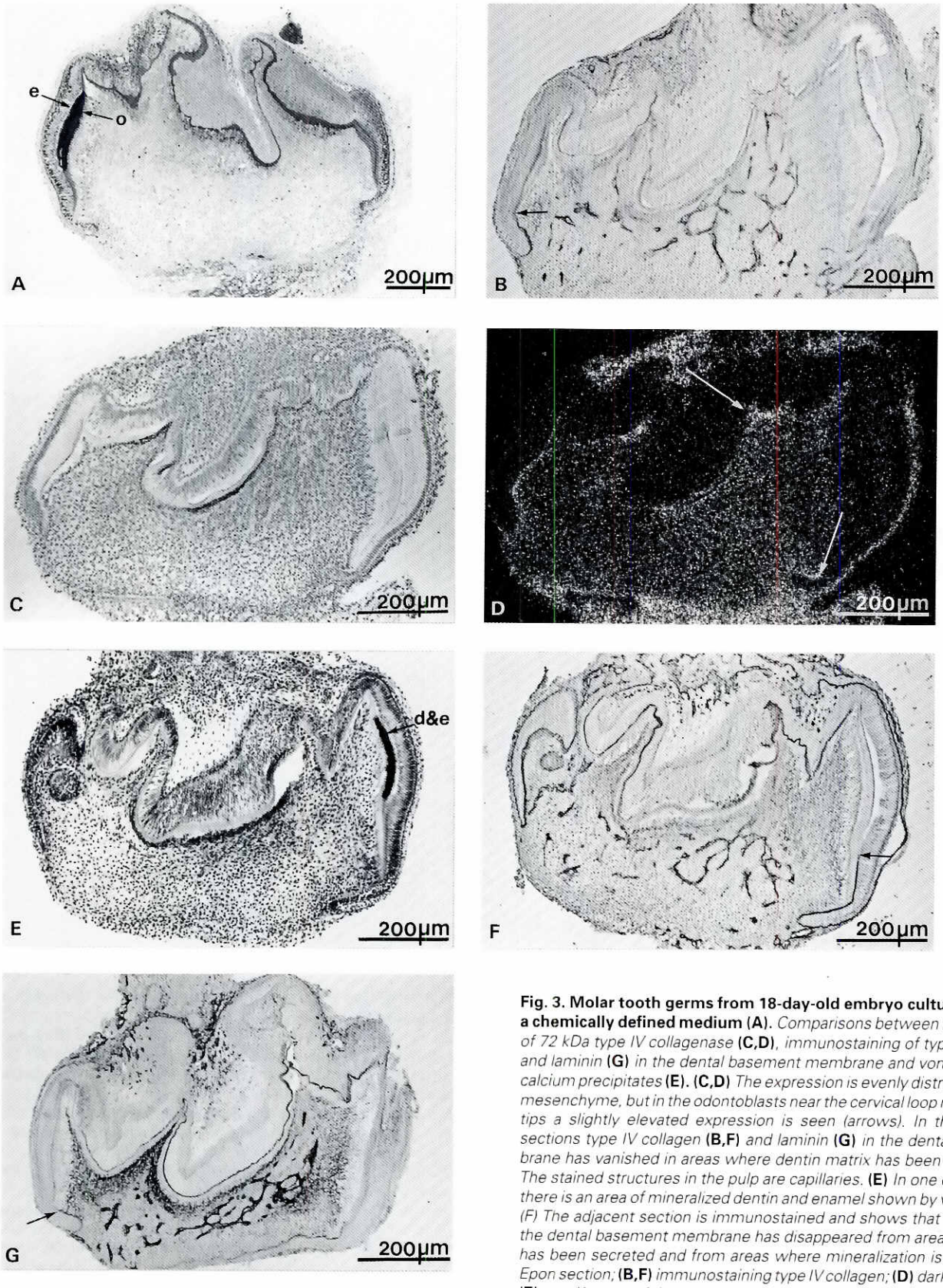

Fig. 3. Molar tooth germs from 18-day-old embryo cultured for 10 days in a chemically defined medium (A). *Comparisons between in situ hybridization of 72 kDa type IV collagenase* **(C,D)**, *immunostaining of type IV collagen* **(B,F)** *and laminin* **(G)** *in the dental basement membrane and von Kossa staining for calcium precipitates* **(E). (C,D)** *The expression is evenly distributed in the dental mesenchyme, but in the odontoblasts near the cervical loop region and the cusp tips a slightly elevated expression is seen (arrows). In the immunostained sections type IV collagen* **(B,F)** *and laminin* **(G)** *in the dental basement membrane has vanished in areas where dentin matrix has been secreted (arrows). The stained structures in the pulp are capillaries.* **(E)** *In one of the lateral cusps there is an area of mineralized dentin and enamel shown by von Kossa staining. (F) The adjacent section is immunostained and shows that type IV collagen in the dental basement membrane has disappeared from areas where predentin has been secreted and from areas where mineralization is verified.* **(A)** *1 µm Epon section;* **(B,F)** *immunostaining type IV collagen;* **(D)** *dark-field image of* **(C)**; **(E)** *von Kossa staining;* **(G)** *laminin immunostaining. A, x60; B-G, x80.*

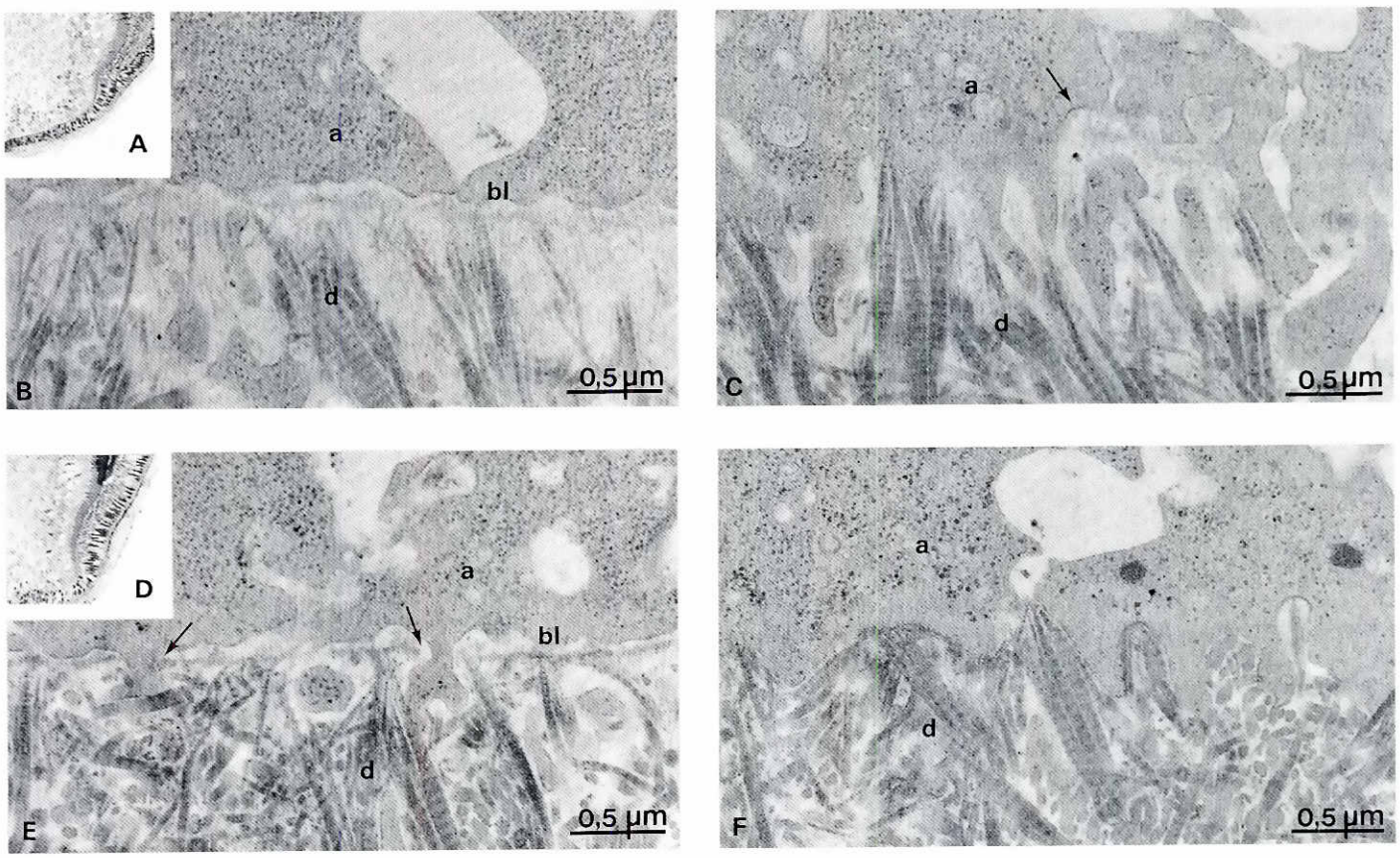

Fig. 4. Histological and ultrastructural observations of the epithelial-mesenchymal interface in first molar tooth germs from 18-day-old mouse embryo *cultured for 10 days in a serum-containing medium* **(A-C)** *or in a chemically defined medium* **(D-F). (A,D)** *Light micrograph of part of the lateral aspect of the cusp including the cervical loop.* **(B)** *The basal lamina separating the epithelial cells from the mesenchymal cells is continuous, whereas* **(C)** *towards the cuspal tip the dentin-ameloblast junction becomes highly irregular, as numerous ameloblast processes are extending into the dentin matrix. Few remnants (arrow) of the basal lamina are persisting.* **(E)** *Small breaks (arrows) in a mostly continuous basal lamina can be observed.* **(F)** *Further towards the cusp tip the basal lamina has completely disappeared. a, ameloblast; d, dentin matrix; bl, basal lamina.* **(A,D)** *1 µm Epon section;* **(B,C,E,F)** *TEM micrographs. A,D, x60; B,C,E,F, x25,000.*

10-day culture in serum-containing medium

Predentin secretion was evident in the cusp tips and along the slopes (Fig. 2A). At the lateral aspects of the cusps dentin was mineralized and adjacent to that, mineralized enamel matrix was observed. Type IV collagen was localized in the dental basement membrane (Fig. 2B) discontinuously at the lateral slopes of the cusps and at the cervical loops. Transcripts of 72 kDa type IV collagenase were uniformly distributed throughout the mesenchyme and expression was slightly elevated in the odontoblasts at the cervical loops (Fig. 2C,D). Control sections, adjacent to those hybridized with the antisense probe, were hybridized with the sense probe. In these sections no specific hybridization was noticed. Where dentin deposition was advanced, or mineralization of dentin and enamel had occurred as detected by von Kossa staining (Fig. 2E), no type IV collagen was detected (Fig. 2F). The distribution patterns of laminin (Fig. 2G) was similar to the distribution of type IV collagen in the dental basement membrane area.

10-day culture in a chemically defined medium

As in the presence of serum, odontoblasts had secreted predentin at the cusp tips and along the slopes. Mineralized dentin and enamel were restricted to the lateral aspects of the cusps (Fig. 3A). In regions where dentin deposition was advanced, type IV collagen was not detected, whereas it was still detectable where dentin matrix deposition had not started or had done so only recently (Fig. 3B). The transcriptions of 72 kDa type IV collagenase were slightly increased in the odontoblasts in the cervical loop region, whereas in the remaining mesenchyme a uniform distribution was seen (Fig. 3C,D). The distribution of laminin (Fig. 3G) was similar to the distribution of type IV collagen. These findings correspond with the results from tooth germs cultured in a serum-containing medium. Also the findings concerning the von Kossa staining (3E) and type IV collagen staining (3F) were similar to the findings in tooth germs cultured in a serum-containing medium.

Ultrastructural observations

In the developing tooth, cell differentiation starts from the cuspal tips and proceeds apically. A developmental gradient is seen so that the most advanced stage remains in the cuspal area (Slavkin, 1974). We examined the epithelial-mesenchymal interface starting at the cervical loop area.

10-day culture in serum-containing medium

At the cervical loop undifferentiated epithelial and mesenchymal cells were seen. More cuspally odontoblasts and ameloblasts had secreted dentin and enamel (Fig. 4A). In the TEM the basal lamina was intact in the cervical loop area. In the dentin matrix striated collagen fibers were predominantly oriented perpendicular to the basal lamina (Fig. 4B). Further towards the cusp tip the epithelial cell processes, extending into the predentin, had left the ameloblast-dentin junction highly irregular with few remnants of the basal lamina observable (Fig. 4C).

10-day culture in a chemically defined medium

In the light microscope the same features were seen as in the previous specimen (Fig. 4D). In the TEM the basal lamina appeared to be continuous in the cervical area and more cuspally small breaks were seen in the basal lamina (Fig. 4E). Further towards the cusp tip the basal lamina had disappeared completely (Fig. 4F).

Discussion

Basement membrane degradation has been suggested to be a prerequisite for ameloblast differentiation by allowing the transmission of the inductive signal between mesenchyme and epithelium, either via cell-matrix or cell-cell contacts. We have speculated that the contacts between the differentiating ameloblasts and the predentin surface are important, as these contacts could involve interactions with matrix molecules of the predentin or with growth factors bound to predentin. The cell-cell contacts would allow transmitting of signals between cells. However, in lower vertebrates, such as frogs, the number of cell-cell contacts is low and their role in controlling differentiation could be a minor one. A causal relationship between basement membrane removal and initiation of biomineralization has also been discussed. We attempted to use the organ culture method described by Slavkin *et al.* (1983) to analyze the causal relationship of basement membrane removal.

In this *in vitro* study (Slavkin *et al.*, 1983) tooth organs were cultured in a chemically defined medium and ameloblast differentiation and enamel matrix secretion was reported to start before removal of the basal lamina. This experimental system would have offered an ideal opportunity to analyze the developmental significance of basement membrane degradation. However, in our hands, no major differences could be detected between teeth cultured in the presence or absence of serum.

Our ultrastructural findings indicate that the basal lamina was removed in cultured tooth organs irrespective of the medium used and also that the basal lamina degradation was initiated even at sites where mineral deposits could not be detected. This is in accordance with Gorter de Vries *et al.* (1986), who observed that the basal lamina vanished also when dentin mineralization did not take place *in vitro*. We therefore conclude that basement membrane degradation occurs independently of mineralization. It is, however, possible that basement membrane removal is a prerequisite for mineralization.

Several components of the dental basement membrane, including collagen type IV, laminin, fibronectin and heparan sulfate proteoglycan, have been localized in developing teeth by means of indirect immunofluorescence (Thesleff *et al.*, 1979, 1981; Laurie *et al.*, 1982; Lesot *et al.*, 1982). The observations in these studies that collagen type IV, laminin, fibronectin and proteoglycan were absent from the basal surface of polarizing epithelial cells in the cuspal areas are in line with ultrastructural findings concerning the timing of basement membrane degradation. The *in vitro* findings in our study on collagen type IV and laminin correspond to the *in vivo* conditions. However, Slavkin *et al.* (1983) reported that in tooth germs cultured in a chemically defined medium, laminin and fibronectin detected by indirect immunofluorescence persisted in the basement membrane adjacent to terminally differentiated ameloblasts.

Type 72 kDa IV collagenase is an extracellular matrix metalloproteinase (MMP). It cleaves native type IV collagen molecules at a single site into 1/4 and 3/4 size fragments (Fessler *et al.*, 1984; Murphy *et al.*, 1989). It is not known for certain that this collagenase degrades basement membranes, but it is widely believed to do so because of associations with metastasizing tumors (Pyke *et al.*, 1992). The expression of this collagenase was studied earlier in mouse molar tooth germs *in vivo* by *in situ* hybridization. The results of this study showed an increased expression in the differentiating preodontoblasts in the cuspal tips of the bell-staged first mandibular molars from 18-day-old mouse embryo (Sahlberg *et al.*, 1992). In the 2-day-old postnatal mouse, where mineralization of dentin had started, the odontoblasts did not show any elevated expression as compared to the rest of the mesenchyme. The expression of 72 kDa type IV collagenase in our cultured teeth correlated with the above mentioned *in vivo* findings (Sahlberg *et al.*, 1992), as increase of transcripts in the preodontoblasts preceded the disappearance of the basement membrane, and the basement membrane was lost in most regions where odontoblasts had ceased to express the enzyme. Hence, the expression of 72 kDa type IV collagenase appears not to depend on the presence of serum in the culture medium.

In conclusion, in our experimental conditions tooth germs cultured in a serum-containing medium and a chemically defined medium showed no major differences in cellular and extracellular matrix changes associated with dentinogenesis and early amelogenesis. In particular, basement membrane degradation appeared to occur in the same developmental sequence under both culture conditions and no differences could be detected in the molecular changes associated with the removal of the basement membrane.

Materials and Methods

Preparation of tissues

The pregnant mice were killed by cervical dislocation and mandibular first molars (n= 121) were removed from 18-day-old (the day of vaginal plug= day 0) CBA/C57BL hybrid mouse embryos. The tooth germs with attached oral mucous membrane were aseptically dissected in phosphate buffered saline (PBS), under a dissecting microscope. The tooth germs were kept at 4°C in PBS until explanted *in vitro*. The first molars from the 18-day-old embryos represented the bell stage of tooth development (Fig. 1A).

Culture procedures

A Trowell-type organ culture was used, in which the explants were grown at the medium-gas interface, supported by a piece of Nuclepore filter (0.6 μm pore size) on a stainless steel grid. Six explants were cultured in each dish supplied with 2 ml of fresh medium every other day. The culture medium consisted of BGJb medium (GIBCO, New York, USA) supplemented with 2.0 mM L-glutamine, 50 μg/ml streptomycin, 10 IU/ml penicillin, 150 μg/ml ascorbic acid (Fluka 11140). 10% fetal calf serum (FCS) was

added to the medium. Organ cultures were incubated at 37°C in a humidified atmosphere consisting of 5% CO_2 in air.

Fixation and preparation of sections

To prepare tissue sections for immunohistochemistry, *in situ* hybridization and von Kossa staining, the cultured tooth germs (n= 68) were fixed in 4% paraformaldehyde in PBS at 4°C over night, dehydrated and embedded in paraffin wax. Sections of 7 µm thickness were placed on silanized glass slides, dried over night at 37°C, and stored in tight boxes at 4°C until used. Adjacent sections were used for *in situ* hybridization and immunohistochemistry and on other adjacent sections immunohistochemistry and the von Kossa staining were applied.

For histological and ultrastructural observations cultured tooth germs (n= 53) were fixed in a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.08 M cacodylate buffer (pH 7.4) followed by 1 h postfixation in 1% osmium tetraoxide in the same buffer. The explants were rinsed in the buffer, dehydrated in graded ethanols and embedded in Epon 812. The blocks were trimmed and cut for light- and transmission electron microscopy (TEM).

Histological and ultrastructural staining

The toluidine blue staining for histological observations was performed on 1 µm Epon sections. For demonstration of calcium phosphate precipitates within the dentin and enamel matrices the von Kossa histochemical staining was applied on 7 µm paraffin sections. These specimens were subsequently counterstained lightly with hematoxylin. Ultrathin Epon sections were mounted on formvar- and carbon-coated copper grids, stained with 4% aqueous uranyl acetate for 4 min and with lead citrate for 2 min and viewed with a Philips 301 electron microscope operated at 60 or 80 Kev.

In situ hybridization

The probes for 72 kDa type IV collagenase have been described elsewhere (Reponen *et al.*, 1992). The plasmids were linearized with *Eco*RI (pSP64, sense) and *Hind*III (pSP65, antisense), and [^{35}S]-uridine S'-triphosphate(= 1000 Ci/nmol, Amersham)-labeled RNA-probes were transcribed using a transcription kit from Promega. The length of the probes was approximately 530 bp. They were precipitated with ethanol, dissolved in hybridization buffer and used at 50,000±2500 cpm/µl.

In situ hybridization was performed as described by Wilkinson and Green (1990). Briefly, the deparaffinized sections were pretreated with proteinase K (Sigma), hybridized with the labeled probes in a humid chamber in 60% formamide over night at 50°C and washed under high-stringency conditions. The dried slides were dipped in autoradiographic emulsion (Kodak NTB2) and exposed for 11 days at 4°C in the presence of silica gel. After development the emulsion was fixed and the sections were stained with hematoxylin and mounted.

Immunohistochemistry

Immunohistochemistry was performed as described by Thesleff *et al.* (1987). In short, the deparaffinized sections were pretreated with pepsin (2 mg/ml 0.01 N HCl) for 15 min at 37°C, incubated with normal goat serum for 30 min at room temperature, washed in PBS and incubated with guinea pig antiserum to mouse collagen type IV (kindly provided by Professor J.M. Foidart, Liege, Belgium) or rabbit antiserum to rat laminin (Telios Pharmaceuticals Inc., San Diego, CA, USA) for 30 min at 37°C. After washes in PBS, biotinylated secondary antibodies against guinea pig IgG (Vector Laboratories, Burlingame, CA, USA) or rabbit IgG made in goat (Vector Laboratories) were applied for 30 min at 37°C and washed. For the color reaction the Vectastain ABC Elite Kit (Vector Laboratories) was used. Control sections were incubated with normal guinea pig serum or normal rabbit serum instead of the primary antibody.

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References

BETTI, F. and KATCHBURIAN, E. (1982). Proteolytic activity of developing dentine of rat tooth germs revealed by the gelatine film substrate technique. *Arch. Oral Biol. 27:* 891-896.

FESSLER, L.I., DUNCAN, K.G., FESSLER, J.H., SALO, T. and TRYGGVASON, K. (1984). Characterization of the procollagen IV cleavage products produced by a specific tumor collagenase. *J. Biol. Chem. 259:* 9783-9789.

GORTER DE VRIES, I., AMELOOT, P.C., COOMANS, D. and WISSE, E. (1986). An ultrastructural study of dentinogenesis and amelogenesis in rat molar tooth germs cultured *in vitro*. *Cell Tissue Res. 246:* 623-634.

HURMERINTA, K. and THESLEFF, I. (1981). Ultrastructure of the epithelial-mesenchymal interface in the mouse tooth germ. *J. Craniofac. Genet. Dev. Biol. 1:* 191-202.

KALLENBACH, E. (1971). Electron microscopy of the differentiating rat incisor ameloblast. *J. Ultrastruct. Res. 35:* 508-531.

KALLENBACH, E. and PIESCO, N.P. (1978). The changing morphology of the epithelium-mesenchyme interface in the differentiation zone of growing teeth of selected vertebrates and its relationship to possible mechanisms of differentiation. *J. Biol. Buccale 6:* 229-240.

KATCHBURIAN, E. and BURGESS, M.C. (1983). Lysosomes and removal of the basal lamina of ameloblasts in early stages of odontogenesis. *Cell Biol. Int. Rep. 7:* 407-415.

KATCHBURIAN, E. and HOLT, S.J. (1969). Role of lysosomes in amelogenesis. *Nature 223:* 1367-1368.

KOLLAR, E.J. and BAIRD, G.R. (1970). Tissue interactions in embryonic mouse tooth germs. *J. Embryol. Exp. Morphol. 24:* 159-171.

LAURIE, G.W., LEBLOND, C.P. and MARTIN, G.R. (1982). Localization of type IV collagen, laminin, heparan sulfate proteoglycan, and fibronectin to the basal lamina of basement membrane. *J. Cell Biol. 95:* 340-344.

LESOT, H., OSMAN, M. and RUCH, J.V. (1982). Immunofluorescent localization of collagens, fibronectin, and laminin during terminal differentiation of odontoblasts. *Dev. Biol. 82:* 371-381.

MATTHIESSEN, M.E. and RÖMERT, P. (1980). Ultrastructure of the human enamel organ. II. Internal enamel epithelium, preameloblasts, and secretory ameloblasts. *Cell Tissue Res. 205:* 371-382.

MEYER, J.M., FARBE, M., STAUBLI, A. and RUCH, J.V. (1977). Relations cellulaires au cours de l'odontogénèse. *J. Biol. Buccale 5:* 107-119.

MURPHY, G., WARD, R., HEMBRY, R.M., REYNOLDS, J.J., KUHN, K. and TRYGGVASON, K. (1989). Characterization of gelatinase from pig polymorphonuclear leucocytes. *Biochem. J. 258:* 463-472.

PANNESE, E. (1962). Observations on the ultrastructure of the enamel organ. III. Internal and external enamel epithelium. *J. Ultrastruct. Res. 6:* 186-204.

PYKE, C., RALFKIÆR, E., HUHTALA, P., HURSKAINEN, T., DANØ, K. and TRYGGVASON, K. (1992). Localization of messenger RNA for M$_r$ 72,000 and 92,000 Type IV collagenase in human skin cancers by *in situ* hybridization. *Cancer Res. 52:* 1336-1341.

REITH, E.J. (1967). The early stages of amelogenesis as observed in molar teeth of young rats. *J. Ultrastruct. Res. 17:* 503-526.

REPONEN, P., SAHLBERG, C., HUHTALA, P., HURSKAINEN, T., THESLEFF, I. and TRYGGVASON, K. (1992). Molecular cloning of murine 72 kDa type IV collagenase and its expression during mouse development. *J. Biol. Chem. 267:* 7856-7862.

RUCH, J.V. (1987). *Determinisms of Odontogenesis.* RBC Cell Biology Reviews, Vol. 14. UBC Press, Leioa.

RUCH, J.V., LESOT, H., KARCHER-DJURICIC, V., MEYER, J.M. and MARK, M. (1983). Epithelial-mesenchymal interactions in tooth germs: mechanisms of differentiation. *J. Biol. Buccale 11:* 173-193.

RUCH, J.V., LESOT, H., KARCHER-DJURICIC, V., MEYER, J.M. and OLIVE, M. (1982). Facts and hypotheses concerning the control of odontoblast differentiation. *Differentiation 21:* 7-12.

SAHLBERG, C., REPONEN, P., TRYGGVASON, K. and THESLEFF, I. (1992). Association between the expression of murine 72 kDa type IV collagenase by odontoblasts and basement membrane degradation during mouse tooth development. *Arch. Oral Biol. 37:* 1021-1030.

SAWADA, T., YAMAMOTO, Y., YANAGISAWA, T., TAKUMA, S., HASEGAWA, H. and WATANABE, K. (1990). Evidence for uptake of basement membrane by differentiating ameloblasts in the rat incisor enamel organ. *J. Dent. Res. 69:* 1508-1511.

SAWADA, T., YANAGISAWA, T. and TAKUMA, S. (1987). Epithelial-mesenchymal junctional area in an early stage of odontogenesis in *Macaca fuscata*. *Adv. Dent. Res. 1:* 141-147.

SILVA, D.G. and KAILIS, D.G. (1972). Ultrastructural studies on the cervical loop and the development of the amelo-dentinal junction in the cat. *Arch. Oral Biol. 17:* 279-289.

SKOBE, Z., STERN, D. and PROSTAK, K. (1981). Ultrastructure of differentiating preameloblasts from tooth germs of the permanent dentition of *Macaca mulatta* and *Macaca arctoides*. *Calcif. Tissue Int. 33:* 603-618.

SLAVKIN, H.C. (1974). Embryonic tooth formation: a tool in developmental biology. *Oral Sci. Rev. 4:* 1-39.

SLAVKIN, H.C. and BRINGAS, P., Jr. (1976). Epithelial-mesenchyme interactions during odontogenesis. IV. Morphological evidence for direct heterotypic cell-cell contacts. *Dev. Biol. 50:* 428-442.

SLAVKIN, H.C., BROWNELL, A.G., BRINGAS, P., JR., MacDOUGALL, M. and BESSEM, C. (1983). Basal lamina persistence during epithelial-mesenchymal interactions in murine tooth development *in vitro*. *J. Craniofac. Genet. Dev. Biol. 3:* 387-407.

SLAVKIN, H.C., TRUMP, G.N., BROWNELL, A.G. and SORGENTE, N. (1977). Epithelial-mesenchymal interactions: mesenchymal specificity. In *Cell and Tissue Interactions* (Eds. J.W. Lash and M.M. Burger). Raven Press, New York, pp. 29-46.

THESLEFF, I. (1978). Role of the basement membrane on odontoblast differentiation. *J. Biol. Buccale 6:* 241-249.

THESLEFF, I. and HURMERINTA, K. (1981). Tissue interactions in tooth development. *Differentiation 18:* 75-88.

THESLEFF, I., BARRACH, H.J., FOIDART, J.M., VAHERI, A., PRATT, R.M. and MARTIN, G.R. (1981). Changes in the distribution of type IV collagen, laminin, proteoglycan, and fibronectin during mouse tooth development. *Dev. Biol. 81:* 182-192.

THESLEFF, I., LEHTONEN, E. and SAXEN, L. (1978). Basement membrane formation in transfilter tooth culture and its relationship to odontoblast differentiation. *Differentiation 10:* 71-79.

THESLEFF, I., LEHTONEN, E., WARTIOVAARA, J. and SAXÉN, L. (1977). Interference of tooth differentiation with interposed filters. *Dev. Biol. 58:* 197-203.

THESLEFF, I., MACKIE, E., VAINIO, S. and CHIQUET-EHRISMANN, R. (1987). Changes in distribution of tenascin during tooth development. *Development 101:* 286-289.

THESLEFF, I., STENMAN, S., VAHERI, A. and TIMPL, R. (1979). Changes in the matrix proteins, fibronectin and collagen, during differentiation of mouse tooth germs. *Dev. Biol. 70:* 116-126.

WILKINSON, D.G. and GREEN, J. (1990). *In situ* hybridization and the three-dimensional reconstruction of serial sections. In *Postimplantation Mammalian Embryos* (Eds. A.J. Copp and D.L. Cockroft). Oxford University Press, Oxford, pp. 155-171.

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