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IMMUNOFLUORESCENT LOCALIZATION AND EXTRACTABILITY OF FIBRONECTIN IN HUMAN DENTAL PULP

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Summary—Fibronectin, visualized in premolar pulps by indirect immunofluorescence, was abundant in the odontoblast layer, around blood vessels and in the core of the pulp. Similarity of alignment of fibronectin with the argyrophilic fibres and von Korff fibres was evident. Fibronectin was extracted from pulps after first removing blood by washing with water, confirmed by eventual negative reaction on α_2-macroglobulin. Extraction of fibronectin from this remaining tissue was most effectively achieved by treatment with collagenase or hyaluronidase, though in all cases some fibronectin remained, indicating that fibronectin in pulp is not exclusively associated with collagen and/or proteoglycans. The fibronectin quantified by electro-immunoassay and expressed as percentage of dry weight was 0.030 per cent in the water extract, 0.094 per cent in the collagenase extract and 0.109 per cent in the hyaluronidase extract. Twice as much fibronectin was extracted from the apical pulp as from the coronal and middle parts, in accord with earlier findings of a higher collagen content in the radicular part. It is suggested that with the loss of collagen type **III** during odontoblast differentiation and its reappearance with advancing vascularization of the dental papilla, the amount of fibronectin is similarly altered.

INTRODUCTION

Previous biochemical studies on the extracellular matrix of human dental pulp revealed the concentration of collagen, its degree of cross-linking, as well as the types and amounts which are present (van Amerongen, Lemmens and Tonino, 1983). Pulp tissue is a loose connective tissue containing, next to collagen as main organic components, proteoglycans and glycoproteins (Seltzer and Bender, 1975). An interesting substance belonging to the glycoproteins is fibronectin, which is an important ingredient of connective tissue matrix and basement membranes (Linder *et al.*, 1975; Stenman and Vaheri, 1978). Two types of fibronectin are distinguished, plasma and cellular fibronectin which are similar in structure and properties. The molecule consists of two similar or identical subunits of molecular weight 220,000, held together by disulphide-bonding (Hynes and Yamada, 1982). Interactions with a wide variety of other macromolecules are ascribed to it. Fibronectin binds to collagens, especially to type **III** collagen (Engvall, Ruoslahti and Miller, 1978) and to glycosaminoglycans and proteoglycans (Perkins, Ji and Hynes, 1979; Yamada *et al.*, 1980). According to Stenman and Vaheri (1978), fibronectin is a component of reticulin, the descriptive name for fibres staining black by silver impregnation. Fibronectin interacts with many cells (Pierschbacher, Hayman and Ruoslahti, 1981) and appears to be involved in a variety of activities. A basic function suggested is that it promotes the adhesion or spreading of cells on materials like collagen and fibrin (Kleinman, Klebe and Martin, 1981; Culp, 1978). Plasma fibronectin may play an important role in the removal of collagenous debris from blood after injury (Saba and Jaffe, 1980). Fibronectin is also implicated in embryonic differentiations and would thus play a role during development of, e.g. the tooth germ (Thesleff *et al.*, 1979). Because of its extracellular location and biological activities, it has been considered to mediate cell–matrix interactions and thus play an important role in the maintenance of normal tissue order (Baum and Wright, 1980). As Thesleff *et al.* (1979) and Lesot, Osman and Ruch (1981) showed that the dental mesenchyme lost fibronectin staining when differentiating into odontoblasts, we wanted to find out if fibronectin was present at all in human pulps and if so, where it could be localized. Until now little is known about the quantities of fibronectin in connective tissues. Bray (1978) and Bray, Mandl and Turino (1981) extracted fibronectin from human lung and placental villi with collagenase and heparin, demonstrating its presence and association with collagen and proteoglycans. Our purpose was to extract and quantify fibronectin from human pulps and to determine its nature of association in the extracellular matrix.

MATERIALS AND METHODS

Purification of fibronectin

Fresh or frozen (−20°C) human plasma was used. Fibronectin was purified as described by Engvall *et al.* (1978) and Dessau *et al.* (1978). In brief, the plasma (50 ml) was passed through a column (2 × 30 cm) of Sepharose 4B (Pharmacia, Uppsala, Sweden) and then applied to a gelatin-CNBr Sepharose 4B column (1 × 10 cm). The column was washed with 0.9 per cent sodium chloride in 0.02 M phosphate buffer, pH 7.4 (PBS) and 1 M urea in 0.05 M tris–HCl, pH 7.5. Fibronectin was eluted with 4 M urea in 0.05 M tris, pH 7.5, dialysed against 0.039 M tris–phosphate buffer, pH 8.6, and subsequently applied to a DEAE–cellulose column (1 × 10 cm). The column was washed successively with 0.039 M tris–phosphate buffer, pH 8.6, 0.127 M tris–phosphate,

pH 6.2, 0.220 M tris–phosphate, pH 5.3 and 0.5 M tris–phosphate, pH 4.2. Fibronectin was eluted at pH 5.3 and dialysed against 0.05 M tris–HCl, pH 7.5 and used for initiation of antisera or stored at 4°C with 0.02 per cent sodium azide.

The purity of the fibronectin was determined by 5 per cent (wt/vol) polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS) (Sykes and Bailey, 1971). After staining with Coomassie Blue, only one band was present, corresponding with a molecular weight of about 440,000 (cf. Dessau *et al.*, 1978). After reduction with 0.01 M dithiothreitol (DTT) and subsequent electrophoresis, only two bands were visible with molecular weights of about 200,000–220,000.

Double diffusion (Ouchterlony, 1958) of several fibronectin concentrations against rabbit antiserum to human albumin showed no detectable precipitation lines.

Preparation of anti-fibronectin serum

Anti-fibronectin was raised in rabbits by subcutaneous injection of 0.4 mg fibronectin in 1 ml PBS and 1 ml Freund's complete adjuvant. After 4 weeks, a booster was given with 0.2 mg fibronectin in 1 ml PBS. The animals were bled two weeks later. On immuno-electrophoresis, rabbit serum showed a high titre of anti-fibronectin. Antiserum was purified further by absorption with fibronectin-free human plasma immobilized on glutaraldehyde gel (Avrameas and Ternynck, 1969). The IgG fraction of the absorbed serum was isolated by affinity chromatography with protein A-Sepharose CL-4B (Pharmacia, Uppsala, Sweden) by the method of Ey, Prowse and Jenkin (1978). This fraction contained 5 mg IgG/ml.

Immunofluorescence microscopy

Dental pulps were obtained from human premolars immediately after extraction for orthodontic reasons from children aged 9–15 yr as described by van Amerongen *et al.* (1983), embedded in 10 per cent gelatin in 0.9 per cent sodium chloride and stored in liquid nitrogen. Sections 6 μm thick were cut in a cryostat (at −20°C) and fixed for 30 min in 2 per cent *p*-formaldehyde in PBS at 4°C.

For immunofluorescence, we used the indirect technique (Stenman and Vaheri, 1978; Chen *et al.*, 1977). Fixed sections were washed three times for 10 min in PBS and incubated for 30 min at 37°C with 20 μl rabbit anti-fibronectin IgG diluted in PBS (1:20, 1:40, 1:80, 1:160). Sections were then washed again with PBS and treated with fluorescein-isothiocyanate conjugated goat anti-rabbit IgG (GAR-FITC, Nordic, Immun. Lab., Tilburg, The Netherlands) diluted in PBS (1:80) for 30 min at 37°C. After three 10-min washes, the sections were mounted under coverslips with a solution containing 100 mM sodium chloride in 50 mM veronal buffer, pH 8.6 and glycerol, 1:1 (vol/vol). In control experiments, the rabbit anti-human fibronectin IgG was substituted either with normal rabbit IgG, with anti-fibronectin pretreated with purified plasma fibronectin, or with PBS. The sections were observed in a Leitz Orthoplan microscope equipped with a fluorescence epi-illuminator Ploempak 2.1. Photographs were taken on Kodak Technical Pan film (100 ASA). For comparison, some premolar sections were prepared with conventional histological techniques and stained following the silver impregnation technique of Gomori (1938).

Extraction of tissue fibronectin

Premolars extracted from children aged from 9 to 15 yr were immediately frozen and stored at −20°C. After defrosting, pulps were removed and minced on ice. To obtain sufficient material, two pulps were pooled, yielding an average wet weight of 50 mg. Minced tissue was washed twice in 5 ml deionized water for 30 min at 4°C. Unless otherwise stated, all extractions were conducted under constant stirring or shaking. Centrifugations took place at 50,000 ***g*** for 30 min at 4°C and the supernatants were dialysed against a large volume of deionized water. After lyophilization, fractions were redissolved in 50 μl electrophoresis buffer, pH 8.6, which contained in 1 l:tricine, 4.3 g; tris, 9.8 g; calcium lactate, 0.106 g and sodium azide, 0.3 g. The effectiveness of the extraction of fibronectin was tested by treating the washed pulp tissue with one of the following solutions: (1) One millilitre of 1 per cent Triton X-100 (scintillation grade) and 4 M urea in electrophoresis buffer pH 8.6, for 16 h at 4°C. (2) Five millilitres of 1 per cent Triton X-100 in sodium phosphate buffer pH 11.0, containing 1.5 mM phenylmethylsulphonyl fluoride (PMSF) and 5 mM benzamidine for 2 h at 4°C (Olden and Yamada, 1977). Centrifugation took place at 100,000 ***g*** for 60 min. (3) Five millilitres of 0.4 per cent SDS and 4 M urea in tris–tricine buffer pH 8.6 (see above), containing 2.4 per cent DTT (a 20-fold excess in relation to dry weight) and 1 mM PMSF for 16 h at 4°C. This method was based on the solubilization of tissue fibronectin by reduction of the disulphide bonds with DTT in the presence of a proteolytic inhibitor. (4) Five millilitres of 2 per cent SDS in 0.05 M tris–HCl, pH 7.5, for 20 h at room temperature. (5) Two millilitres of chloroform–methanol (2:1, vol/vol) for 60 min at 4°C (Folch, Lees and Sloane Stanley, 1957). (For removal and disruption of lipid-like material in the tissue.) (6) One hundred microlitres of 0.05 M sodium phosphate buffer, pH 7.1, containing 2 mM PMSF and 1 mg heparin from porcine intestinal mucosa (169.9 anticoagulant units per mg, Sigma, W. Germany) for 4 h at room temperature (Bray *et al.*, 1981). The samples were centrifuged at 7700 ***g*** for 20 min. (7) Bacterial collagenase (0.3 mg) (395 U/mg, Millipore Corporation, Freehold, NY 07728) of which the non-collagenase proteolytic activity was suppressed (Benya *et al.*, 1973) in 1 ml buffer (0.1 M sodium chloride, 0.05 M tris–HCl, pH 7.6, 10 mM calcium chloride) for 20 h at 37°C. (8) Bovine testicular hyaluronidase (1.25 mg) (348 U/mg, Serva, W. Germany) in 100 μl of 0.1 M sodium acetate buffer, pH 5.2 containing 0.15 M sodium chloride, for 12 h at 37°C and three times with 1.25 mg hyaluronidase in 50 μl of the same buffer at 12 h intervals (37°C). (9) Three 5 s-ultrasonic treatments of the tissue in 0.05 M tris–HCl, pH 8.6, 4 M urea and 20 mM DTT with a sonifier D 12 (Branson Sonic Power Company, Danbury, Conn.), followed by extraction for 2 h at 4°C in the same buffer. Finally, some experiments

Table 1. Extraction of fibronectin from human premolar pulps

Extraction medium	Fibronectin (percentage of dry weight)* mean	SD†	n‡
Water	0.030	0.007	9
Collagenase in buffer, pH 7.6	0.094	0.032	26
Hyaluronidase in buffer, pH 5.2	0.109	0.019	10

*Ratio of wet weight/dry weight in pulps was 8.33 (van Amerongen *et al.*, 1983). †SD = standard deviation. ‡n = number of separate determinations.

were carried out in which some of the above procedures were combined in a random order of selection.

All sediments remaining were washed with deionized water, embedded in 10 per cent gelatin in 0.9 per cent sodium chloride and stored in liquid nitrogen. To judge the effectiveness of the extraction procedures, the stored sediments were sectioned (6 μm) and prepared for immunofluorescence (see above). The effectiveness of the collagenase treatment was checked by a hydroxyproline assay (Guis, Slootweg and Tonino, 1973).

For quantification of fibronectin, all supernatants were analysed following the slightly modified electro-immunoassay technique of Laurell (1972). A solution with 0.9 per cent agarose (Sea Kem, F.M.C. Corporation, Rockland, U.S.A.) in tris–tricine buffer (see above) was mixed with the anti-fibronectin IgG fraction (0.4 per cent, vol/vol). Electrophoresis was carried out for 16 h at 10°C (10 mA/gel, 150 V). Pooled human serum of known fibronectin content was used as a standard. The percentage fibronectin was calculated on the basis of the dry weights of human premolar pulps (van Amerongen *et al.*, 1983). A check for non-specific precipitation was carried out by exchanging the anti-fibronectin IgG for normal rabbit IgG. The amount of blood present in extracts was estimated by a determination of α_2-macroglobulin based on single radial immunodiffusion as described by Mancini, Carbonara and Heremans (1965). To check possible differences in fibronectin content between the coronal and the radicular part, some pulps were divided into three equal sections: a coronal, a middle and an apical part, these being extracted as described under 7 and 8.

RESULTS

The indirect immunofluorescence technique showed that fibronectin was ubiquitously present in the pulp (Figs 1 and 2). Fibronectin was observed between the odontoblasts as well as in the core of the pulp. Its distribution was homogeneous except for a marked fluorescence around the blood vessels (Fig. 2). When compared to pulp sections, stained by the silver impregnation technique (Fig. 3), the similarity of alignment of the predominant argyrophilic fibres and von Korff fibres with fibronectin was evident. The anti-fibronectin used for immunofluorescence was found to be specific, as controls were all negative. When immunofluorescence slides were kept longer than three days at 4°C, a few controls showed slight autofluorescence. The use of different dilutions of anti-fibronectin for incubation procedures had no influence on the results with the exception of the largest dilution (1:160) with which fluorescence was weak and faded away soon.

The extractability of fibronectin as determined by electro-immunoassay is shown in Table 1. Hyaluronidase and collagenase were most effective compared to the other treatments which failed to extract detectable amounts of fibronectin. Only treatment with heparin yielded a minute amount of fibronectin (0.002 per cent) which was at the limit of the detection method. Comparison of the extracted amounts of fibronectin after hyaluronidase or collagenase treatment gave no statistically significant difference. When different extraction procedures were carried out consecutively, only treatment with collagenase, following an extraction with hyaluronidase, always delivered an additional amount of 0.007 per cent fibronectin (SD $\pm$ 0.002). The amounts of fibronectin which could be extracted by treatment with collagenase or hyaluronidase were twice as high in the apical part than in the coronal and middle parts of the pulps. Hydroxyproline assay revealed that 96 per cent of the collagen present was degraded and removed from the tissue by collagenase treatment.

In blood, the concentrations of fibronectin (Hynes and Yamada, 1982) and α_2-macroglobulin (Schultze and Heremans, 1966) are known. The ratio of these concentrations was used for the control of the effectiveness of the removal of blood from the samples by the preceding water-washings. These washings removed an amount of fibronectin (Table 1) which corresponded precisely with the amount expected on the basis of the observed content of α_2-macroglobulin in the washings. After the washing with water, none of the other extracts contained α_2-macroglobulin, indicating that all fibronectin isolated after water-washings was extracted from the tissue and not from the plasma.

Immunofluorescence studies on sediments remaining after extraction showed that some fibronectin was still present, although the tissues were completely degraded and in disorder, especially after collagenase treatment. Controls were negative.

DISCUSSION

Pulps from young human premolars contained fibronectin both in the core of the pulp and in the odontoblast layer. Immunofluorescence showed a strong resemblance in the alignment of fibronectin

and argyrophilic and von Korff fibres, suggesting that these fibres do contain or are accompanied by fibronectin, in agreement with the findings of Bornstein and Ash (1977), Stenman and Vaheri (1978) and Unsworth *et al.* (1982), all of whom suggest that the co-distribution of fibronectin and collagen is due to an affinity of one for the other. We previously found that the collagen content of the radicular part of the pulp was substantially higher than that of the coronal part (van Amerongen *et al.*, 1983). In view of the affinity of fibronectin for collagen it is therefore interesting that our present findings also reveal that the apical part contains a substantially higher amount of fibronectin than other parts of the pulp.

Our findings are unexpected in view of the work of Thesleff *et al.* (1979) and Lesot *et al.* (1981). They studied the changes in fibronectin and collagen during differentiation of mouse tooth germ and found that the dental mesenchyme lost fibronectin staining when differentiating into odontoblasts. Based on this finding, it is unexpected that in human teeth, which are still active in dentine formation, fibronectin occurs in the odontoblast layer. For comparison, we investigated incisors of mature Wistar rats (300 g) in a similar way as the human teeth and found that fibronectin had the same distribution. Similar results were obtained by Linde *et al.* (1982). These findings can only be explained when it is supposed that, after the disappearance of fibronectin during the period of differentiation of the mesenchyme into odontoblasts, fibronectin re-appears later. As suggested by Ruoslahti, Engvall and Hayman (1981), such changes in fibronectin may be secondary to the cellular differentiation but the changes in attachment and motility of cells that must accompany the appearance and disappearance of fibronectin may provide important signals directing differentiation. The interest is increased by the finding, by Thesleff *et al.* (1979), that procollagen type **III** is lost from the dental mesenchyme during odontoblast differentiation but re-appears with advancing vascularization of the dental papilla, and that it is present in the dental basement membrane during the bud and cap stages and then disappears from the cusp area as odontoblast differentiation occurs. These findings of Thesleff *et al.*, combined with the fact that type **III** collagen has strong affinity for fibronectin (Engvall *et al.*, 1978), suggest that the re-appearance of type **III** collagen after an initial disappearance is accompanied by the re-appearance of fibronectin. Thus the conclusion of Shuttleworth, Berry and Wilson (1982) that it is the decrease in fibronectin and not type **III** collagen which histologically results in decreased reticular fibres should be regarded with caution. The studies of Thesleff *et al.* (1979) and Shuttleworth *et al.* (1982) were not carried out on teeth as mature as those we studied.

Except from the water-soluble fraction, fibronectin was difficult to extract from dental pulp tissue. Most methods, including those described for the isolation of fibronectin from cell cultures (e.g. Olden and Yamada, 1977) were ineffective for the isolation of fibronectin from pulp. In the few successful instances of extraction of fibronectin from other tissues the treatment is with collagenase (Bray, 1978) or heparin (Bray *et al.*, 1981). These workers suggested that the application of heparin provided a simple and effective method for the extraction of tissue-fibronectin and for routine quantitative studies but we found that extraction of fibronectin from human dental pulp with heparin yielded only insignificant amounts of fibronectin. The treatment of the tissue with collagenase was the first method by which substantial amounts of fibronectin could be isolated from pulp (Table 1). Only one other method was equally effective, namely treatment of the tissue with hyaluronidase. When, after extraction, the residues were examined by immunofluorescence for the presence of residual fibronectin, the sections were consistently positive. No mention of such a residual fibronectin appears to have been made previously. Thus it must be concluded that even the most exhaustive extraction procedures were still not entirely effective. On the basis of immunofluorescence, it is not possible to make an estimation of the amount of fibronectin that remains captured in the residue. We can only speculate on the possible cause of some fibronectin remaining after extraction. That fibronectin may remain captured by cells or cell fragments in the residue seems unlikely because treatment of the sample with cell-disrupting media like SDS, Triton or chloroform and methanol yielded no detectable fibronectin. That fibronectin is withheld within a matrix of proteoglycans and/or collagens can also be excluded as most, if not all, of these would be degraded by the enzyme pretreatments. It seems therefore most likely that fibronectin is strongly associated with some other glycoproteins in the residue that are still intact.

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REFERENCES

Amerongen J. P. van, Lemmens I. G. and Tonino G. J. M. (1983) The concentration, extractability and characterization of collagen in human dental pulp. *Archs oral Biol.* **28**, 339–345.

Avrameas S. and Ternynck T. (1969) The cross-linking of proteins with glutaraldehyde and its use for the preparation of immunoadsorbents. *Immunochemistry* **6**, 53–66.

Baum B. J. and Wright W. E. (1980) Demonstration of fibronectin as a major extracellular protein of human gingival fibroblasts. *J. dent. Res.* **59**, 631–637.

Benya P., Berger K., Golditch M. and Schneir M. (1973) Recommendations concerning the use of collagenase for isolating collagen-associated molecules from tissues. *Analyt. Biochem.* **53**, 313–316.

Bornstein P. and Ash J. F. (1977) Cell surface-associated structural proteins in connective tissue cells. *Proc. natn. Acad. Sci. U.S.A.* **74**, 2480–2484.

Bray B. A. (1978) Cold-insoluble globulin (fibronectin) in connective tissues of adult human lung and in trophoblast basement membrane. *J. clin. Invest.* **62**, 745–752.

Bray B. A., Mandl I. and Turino G. M. (1981) Heparin facilitates the extraction of tissue fibronectin. *Science* **214**, 793–795.

Chen L. B., Maitland N., Gallimore P. H. and McDougall J. K. (1977) Detection of the large external transformation—sensitive protein on some epithelial cells. *Expl Cell Res.* **106**, 39–46.

Culp J. A. (1978) Biochemical determinants of cell adhesion. *Curr. Top. membr. transp.* **11**, 327–396.

Dessau W., Jilek F., Adelmann B. C. and Hörmann H. (1978) Similarity of antigelatin factor and cold insoluble globulin. *Biochim. biophys. Acta* **533,** 227–237.

Engvall E., Ruoslahti E. and Miller E. J. (1978) Affinity of fibronectin to collagens of different genetic types and to fibrinogen. *J. exp. med.* **147,** 1584–1595.

Ey P. L., Prowse S. J. and Jenkin C. R. (1978) Isolation of pure IgG_1, IgG_{2a} and IgG_{2b} immunoglobulins from mouse serum using protein A-sepharose. *Immunochemistry* **15,** 429–436.

Folch J., Lees M. and Sloane Stanley G. H. (1957) A simple method for the isolation and purification of total lipids from animal tissues. *J. biol. Chem.* **226,** 497–509.

Gomori G. (1938) Silver impregnation of reticulin in paraffin sections. *Am. J. Path.* **13,** 933–1001.

Guis M. B., Slootweg R. N. and Tonino G. J. M. (1973) A biochemical study of collagen in the periodontal ligament from erupting and non-erupting bovine incisors. *Archs oral Biol.* **18,** 253–263.

Hynes O. and Yamada K. M. (1982) Fibronectins: Multifunctional modular glycoproteins. *J. Cell Biol.* **95,** 369–377.

Kleinman H. K., Klebe R. J. and Martin G. R. (1981) Role of collagenous matrices in the adhesion of growth of cells. *J. Cell Biol.* **88,** 473–485.

Laurell C. B. (1972) Electroimmuno assay. *Scand. J. clin. Lab. Invest.* **29,** Suppl. **124,** 21–37.

Lesot H., Osman M. and Ruch J. V. (1981) Immunofluorescent localization of collagens, fibronectin and laminin during terminal differentiation of odontoblasts. *Devl Biol.* **82,** 371–381.

Linde A., Johansson S., Jonsson R. and Jontell M. (1982) Localization of fibronectin during dentinogenesis in rat incisor. *Archs oral biol.* **27,** 1069–1073.

Lindner E., Vaheri A., Ruoslahti E. and Wartiovaara J. (1975) Distribution of fibroblast surface antigen in the developing chick embryo. *J. exp. med.* **142,** 41–49.

Mancini G., Carbonara A. O. and Heremans J. F. (1965) Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry* **2,** 235–254.

Olden K. and Yamada K. M. (1977) Mechanism of the decrease in the major cell surface protein of chick embryo fibroblasts after transformation. *Cell* **11,** 957–969.

Ouchterlony O. (1958) Diffusion-in-gel methods for immunological analysis. In: *Progress in Allergy* (Edited by Kallos D.) Vol. V, pp. 1–78. S. Karger, Basel.

Perkins M. E., Ji T. H. and Hynes R. O. (1979) Cross-linking of fibronectin to sulfated proteoglycans at the cell surface. *Cell* **16,** 941–953.

Pierschbacher M. D., Hayman E. G. and Ruoslahti E. (1981) Location of the cell-attachment site in fibronectin with monoclonal antibodies and proteolytic fragments of the molecule. *Cell* **26,** 259–267.

Ruoslahti E., Engvall E. and Hayman E. G. (1981) Fibronectin: current concepts of its structure and function. *Collagen Res.* **1,** 95–128.

Saba T. M. and Jaffe E. (1980) Plasma fibronectin (opsonic glycoprotein): its synthesis by endothelial cells and role in cardiopulmonary integrity after trauma as related to reticuloendothelial function. *Am. J. med.* **68,** 577–594.

Seltzer S. and Bender I. B. (1975) *The Dental Pulp*, 2nd edn, pp. 63–98. Lippincott, Philadelphia, Pa.

Schultze H. E. and Heremans J. F. (1966) Survey of the plasma proteins. In: *Molecular Biology of Human Proteins*, Vol. I, pp. 173–235. Elsevier, Amsterdam.

Shuttleworth C. A., Berry L. and Wilson N. H. F. (1982) Biosynthesis of glycoproteins by rabbit dental pulp fibroblasts in culture. *Archs oral Biol.* **27,** 645–650.

Stenman S. and Vaheri A. (1978) Distribution of a major connective tissue protein, fibronectin, in normal human tissues. *J. exp. med.* **147,** 1054–1064.

Sykes B. C. and Bailey A. J. (1971) Molecular weight heterogenity of the α-chain subunits of collagen. *Biochem. biophys. Res. Commun.* **43,** 340–345.

Thesleff I., Stenman S., Vaheri A. and Timpl R. (1979) Changes in the matrix proteins, fibronectin and collagen during differentiation of mouse tooth germs. *Devl Biol.* **70,** 116–126.

Unsworth D. J., Scott D. L., Almond T. J., Beard H. K., Holborow E. J. and Walton K. W. (1982) Studies on reticulin. I: Serological and immunohistological investigation of the occurence of collagen type III, fibronectin and the non-collagenous glycoprotein of Prass and Glynn in reticulin. *Br. J. exp. Path.* **63,** 154–166.

Yamada K. M., Kennedy D. W., Kimata K. and Pratt R. M. (1980) Characterization of fibronectin interactions with glycosaminoglycans and identification of active proteolytic fragments. *J. biol. Chem.* **255,** 6055–6063.

Plate 1 overleaf.

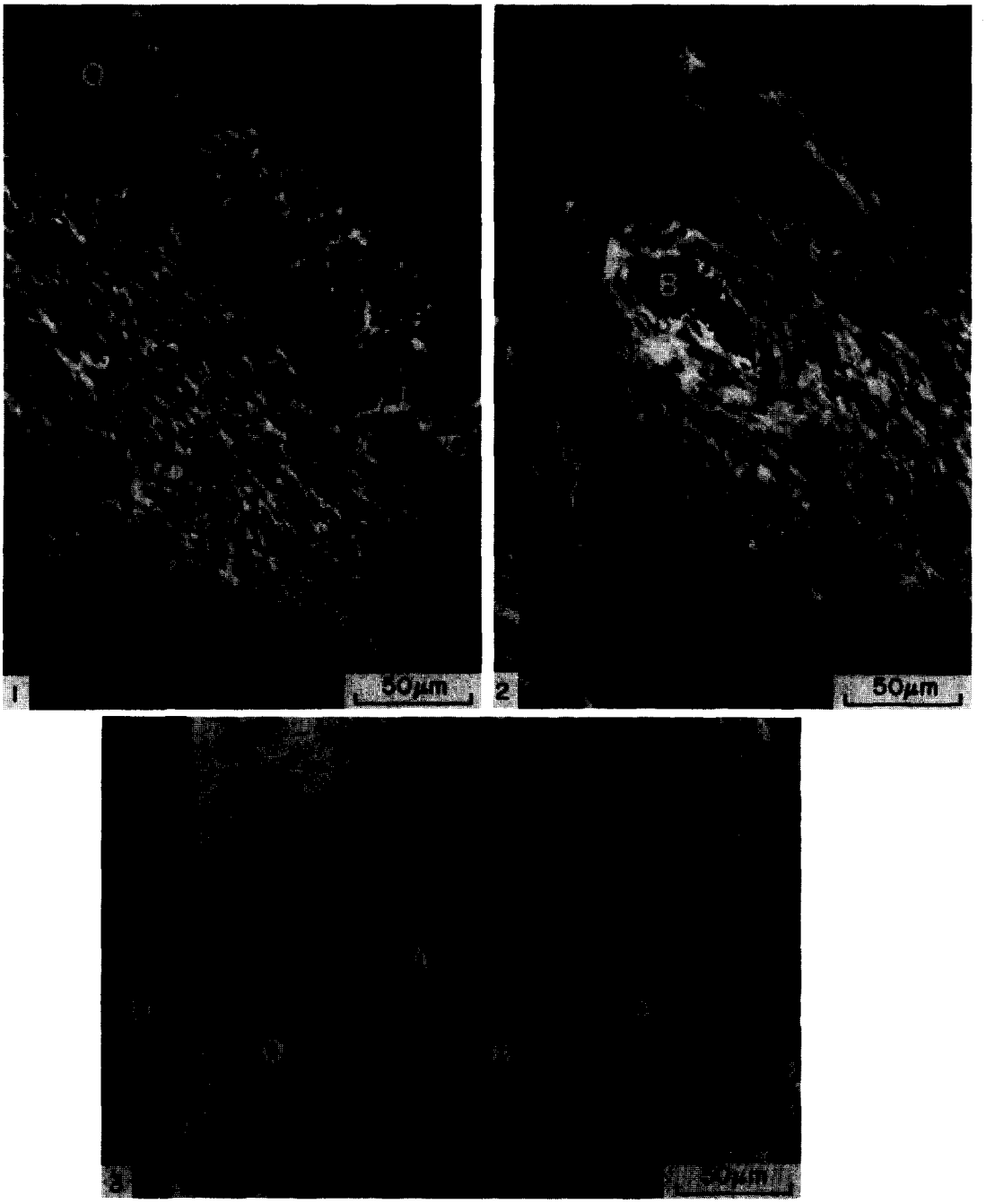
Plate 1.

Sections of young human premolar pulps. O = odontoblast layer and von Korff fibres; A = argyrophilic fibres; B = blood vessel; D = dentine.

Fig. 1. Section showing indirect immunofluorescence with rabbit anti-human fibronectin IgG of surface of human pulp. Note the abundant fibronectin in the odontoblast layer. × 360

Fig. 2. As Fig. 1 but showing central part of the pulp. × 360

Fig. 3. Silver impregnation technique of Gomori on human pulp section. Note the large amount of argyrophilic fibres in the pulp and the presence of von Korff fibres among the odontoblasts. × 360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