Modulation of extracellular matrix biosynthesis by bovine retinal pericytes in vitro: effects of the substratum and cell density

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Summary

Bovine retinal pericytes plated on a two-dimensional substratum display a characteristic stellate morphology. In post-confluent cultures these cells aggregate spontaneously to form multicellular nodules. The same cells plated within a three-dimensional collagen matrix display an elongated sprouting morphology. Sprouting pericytes may be embedded within a gel either as individual cells or as multicellular aggregates. We have compared the nature of the matrix proteins synthesised by pericytes displaying these different phenotypes. Stellate pericytes cultured on plastic dishes synthesised predominantly type I collagen, some type III collagen and only traces of type IV collagen. The same collagen types were secreted when nodules had formed in post-confluent cultures on plastic, and by sprouting cells plated as single cells within the collagen gel. By contrast, sprouting pericytes plated as aggregates within the collagen gel secreted increased levels of type IV collagen and reduced amounts of type I collagen. Fibronectin was synthesised by pericytes under all experimental conditions examined; thrombospondin was produced in relatively large amounts by cells grown on plastic dishes, whereas only trace amounts could be detected in the medium when the cells were cultured within a collagen gel matrix. Transmission electron microscopy revealed that pericyte aggregates within a collagen gel contained cells in close apposition surrounded by a dense extracellular matrix. In contrast, cells in the centre of a nodule on plastic appeared to be separated from each other by loose extracellular material. These results suggest that the morphological and biosynthetic phenotypes of retinal pericytes are modulated by cell-matrix and/or cell-cell interactions.

Key words: pericytes, collagen, thrombospondin, angiogenesis.

Introduction

Retinal microvessels are characterised by an unusually thick basement membrane and contain two types of cells: endothelial cells, which rest on the basement membrane and line the vessel lumen, and pericytes, which are embedded within the basement membrane (Cogan et al. 1969). Although there is a considerable body of data regarding the biochemical composition of basement membranes (for a recent review, see Timpl, 1989), the mechanisms involved in the deposition, maintenance and remodelling of the basement membrane under normal physiological and pathological situations are not fully understood. With particular reference to the retinal microvessel basement membrane, it is not clear whether pericytes contribute to its synthesis in vivo, or how this synthesis may be altered under conditions in which there is a preferential loss of either endothelial cells (e.g. ageing) or pericytes (e.g. diabetic retinopathy) (Cogan et al. 1969).

The nature of the extracellular matrix is now recognised to exert a profound effect on cellular biosynthetic activity (for example, see Gibson et al. 1983; Greenburg and Hay, 1986; Bissell and Barcellof-Hoff, 1987). We have previously demonstrated that retinal endothelial cells in culture synthesise predominantly type IV (basement membrane-specific) collagen, although the substratum on which the cells are cultured modulates both the morphology (Schor and Schor, 1986) and the biosynthetic phenotype of these cells (Canfield et al. 1986). Pericytes can also synthesise collagenous proteins in vitro (Cohen et al. 1980; Kennedy et al. 1986; Canfield et al. 1989). However, there is still some confusion in the literature regarding the types of collagen secreted by these cells; initial studies suggested the synthesis of mainly type III collagen (Canfield et al. 1986), whilst Kennedy et al. (1986) have subsequently reported the production of predominantly type I collagen together with some type IV collagen and minor amounts of type III collagen.

In the present study, we have investigated the nature of the matrix macromolecules synthesised by retinal pericytes cultured either on plastic Petri dishes or embedded within three-dimensional gels of native type I collagen fibres. Data are presented indicating that the synthesis of extracellular matrix proteins (in particular, collagens and...
thrombospondin) by these cells may be modulated by both cell-cell and cell-matrix interactions.

Materials and methods

Materials
Culture medium, donor calf serum, sodium pyruvate, glutamine, non-essential amino acids and antibiotics were obtained from Gibco Bio-Cult, Paisley, Scotland, UK. Ascorbic acid was obtained from BDH Chemicals, Poole, Dorset, UK. B-aminopropionitrile (form HI) and [3H]proline were purchased from Amersham International, Amersham, Bucks, UK. Protein A-Sepharose (CL-4B) was purchased from Pharmacia Fine Chemicals, Milton Keynes, UK. Rabbit anti-(human platelet thrombospondin) and rabbit anti-(human plasma fibronectin) were generously supplied by Dr. D. S. Pepper, Scottish National Blood Transfusion Service Headquarters, Edinburgh, Scotland, UK, respectively.

Enzymic digestion procedures

Bovine retinal pericytes were isolated from the adult retina as previously described (Schor and Schor, 1986). The isolated cells were identified on the basis of several criteria: (1) origin (retinal microvascular) and morphology; (2) lack of factor VIII-related antigen; (3) lack of glial fibrillar acidic protein; (4) presence of smooth muscle cell alpha-actin; (5) ability to contract three-dimensional collagen gels; (6) ability to retract and form nodules on a two-dimensional substrate; (7) migratory behaviour in three-dimensional collagen gels; and (8) mineralisation of the extracellular matrix as described elsewhere (Schor and Schor, 1980). Cells were plated at the same densities on plastic dishes and in collagen gels, as we have previously shown that the total radioactivity deposited into the cell layer/matrix by cultured pericytes. Samples were stored frozen at -20°C before analysis.

Cell culture
Bovine retinal pericytes were isolated from the adult retina as previously described (Schor and Schor, 1986). The isolated cells were identified on the basis of several criteria: (1) origin (retinal microvascular) and morphology; (2) lack of factor VIII-related antigen; (3) lack of glial fibrillar acidic protein; (4) presence of smooth muscle cell alpha-actin; (5) ability to contract three-dimensional collagen gels; (6) ability to retract and form nodules on a two-dimensional substrate; (7) migratory behaviour in three-dimensional collagen gels; and (8) mineralisation of the extracellular matrix as described elsewhere (Schor and Schor, 1980). Cells were plated at the same densities on plastic dishes and in collagen gels, as we have previously shown that the total radioactivity deposited into the cell layer/matrix by cultured pericytes. Samples were stored frozen at -20°C before analysis.

Electrophoretic analysis
Discontinuous SDS/polyacrylamide-gel electrophoresis was carried out on samples of conditioned medium with or without reduction by 5% (v/v) 2-mercaptoethanol (Canfield et al. 1986). The same amount of radioactivity was applied to each track of the polyacrylamide gel. Newy synthesized proteins were detected by fluorography (Bonner and Laskey, 1974; Laskey and Mills, 1975). [14C]Methylated M, standards were myosin (M, 200 000), phosphorylase b (a doublet of M, 100 000 and 92 500), bovine serum albumin (M, 69 000), ovalbumin (M, 46 000), carbonic anhydrase (M, 30 000) and lysozyme (M, 14 300).

Electron microscopy
The methodology for the in situ preparation of cells grown on plastic dishes or in collagen gels was as described elsewhere (Allen et al. 1984). Briefly, the cells on plastic dishes or entire collagen gels were fixed in situ by flooding the culture dish or the drained gel with 5% glutaraldehyde in 0.1 M Sorenson’s phosphate buffer for 1 h, followed by three washes with this buffer and post-fixation with 1% OsO4 for 1 h. After three further washes,
areas of the fixed gel were cut out for further processing and flat embedded in an Epon–Araldit mixture after dehydration in ethanol. The cells on plastic dishes were embedded in situ in Lufts Epon after the use of hydroxypropylmethacrylate as intermediate solvent between absolute ethanol and resin. After polymerisation, the embedded resin and cells were snapped free of the plastic substratum and cut either at right angles or parallel to the growing surface. Thin sections were stained with uranyl acetate and lead citrate, and viewed in an AEI 801A transmission electron microscope.

**Results**

**Effect of substrata on the morphology of retinal pericytes**

Bovine retinal pericytes were cultured on plastic tissue culture dishes (referred to as 'on plastic') and within three-dimensional gels of native type I collagen (referred to as 'in gel'). In this latter gel system, the cells were plated either as single cells or as cell aggregates (see Materials and methods). When the cells were plated on plastic they displayed a characteristic stellate morphology with prominent ruffled edges (Fig. 1A). These cells proliferated and at confluence they started to pile up and eventually retracted upon themselves forming large cellular nodules (Fig. 1B). Cells plated in gel as a single cell suspension adopted an elongated tubular morphology (Fig. 1C) that will be referred to as sprouting in analogy with the apparently similar sprouting morphology displayed by endothelial cells plated under the same conditions (Schor et al. 1983; Schor and Schor, 1986). Pericytes proliferate when plated in gel as single cells, and they remain homogeneously distributed throughout the three-dimensional gel matrix (Fig. 1D). This behaviour differs from that of sprouting endothelial cells, which do not proliferate when plated in gel but migrate and self-associate to form multicellular aggregates (Schor et al. 1983; Schor and Schor, 1986). In order to obtain three-dimensional aggregates of pericytes in gel, these cells were allowed to form clumps before plating them in gel, as described in Materials and methods. When pericytes were plated as aggregates or clumps of cells in gel they also elongated into a sprouting morphology (Fig. 1E), and cells then grew out from the

**Fig. 1. Effect of substrate on pericyte morphology.** A. Confluent cells cultured on plastic, exhibiting typical stellate morphologies with ruffled edges. B. The same culture as in A, 6 days later. The cells have retracted upon themselves. C. Cells plated as a single cell suspension in gel, 1 day after plating. Cells have an elongated sprouting morphology. D. The same culture as in C, 5 days later. Cells have proliferated but remain homogeneously distributed in the gel matrix. E. Cells plated as clumps in gel, 1 day after plating. F. Same culture as in E, 5 days later. Cells have proliferated and formed extensive three-dimensional meshworks. Bar, 150 μm.

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clumps in a radial manner forming interconnected networks within the gel matrix (Fig. 1F). These networks are morphologically similar to those formed by sprouting endothelial cells in gel (Schor et al. 1983; Schor and Schor, 1986).

Using transmission electron microscopy, we have compared pericyte nodules spontaneously produced on plastic (Fig. 2) with sprouting pericytes plated as aggregates in gel (Figs 3, 4 and 5). Cells cultured under both conditions were rich in mitochondria, Golgi bodies, rough endoplasmic reticulum and glycogen deposits, suggesting that they were metabolically active (Figs 2, 3 and 4). It is also noteworthy that pericytes appeared to have a characteristically dense nuclear lamina that clearly separated the peripheral chromatin from the nuclear membrane (Fig. 4); this feature has previously been observed in arterial smooth muscle cells (Ghadially, 1988). Varying degrees of cellular associations were seen when pericytes were plated on plastic dishes. In the centre of a nodule the cells were separated from each other by loose extracellular material (Fig. 2); in other areas of the dish (e.g. at the surface of a nodule, and where the cells had become multilayered), two or three closely apposed cells were often observed (not shown). By comparison, aggregates of pericytes plated in gel (Fig. 3) appeared more closely apposed than cells on plastic. When these aggregates were examined at higher magnification (Fig. 4), the cell membranes were found to be intimately associated in some areas with structures reminiscent of gap junctions occasionally observed, whereas in other areas the cells were separated by a thin layer of extracellular material. Amorphous and fibrillar extracellular material was produced by cells cultured on plastic and in gel (Figs 2 and 3). This material appeared to be particularly dense around cellular aggregates plated in gel (Fig. 3), and seemed to be organised at the cell–collagen interface (Fig. 5) in a manner highly reminiscent of the external lamina of pericytes in vivo (Ghadially, 1988). Needle-like crystals of hydroxyapatite were also detected in the ‘in gel’ cultures (Figs 3 and 4) clearly indicating early stages of calcification. This process will be described in more detail (Schor et al. unpublished data).

Protein synthesis by bovine retinal pericytes cultured on plastic Petri dishes or embedded within three-dimensional collagen gels

In order to investigate the nature of the proteins synthesised by retinal pericytes in vitro, cultures were incubated with either [³H]proline or [³⁵S]methionine for 18–24 h and the newly synthesised proteins in the medium and cell layer/matrix were collected as described in Materials and
methods. Most of the data presented in this communication relate to the characterisation of newly synthesised proteins present in the medium, but it should be noted that in all cases the profiles of extracellular matrix proteins in the medium and in the cell layer/matrix were in fact very similar; the only difference being the relative proportions of collagens types I and III (see below).

Fig. 6 shows the total [³H]proline-labelled proteins secreted into the medium by retinal pericytes plated on plastic Petri dishes (tracks 1 and 3), as single cells in gel (track 2) or as aggregates of cells in gel (track 4). Samples in tracks 1 and 2 were from one experiment; samples in tracks 3 and 4 were from a separate experiment. It is interesting that the profiles of proteins secreted by pericytes cultured on plastic dishes were different in these two experiments (compare tracks 1 and 3). Such differences are often seen when one compares samples from different experiments, and may be due to the use of different batches of cells, different plating densities or different times of harvesting. However, it is important to note that these differences appear to reflect alterations in the levels of processing of procollagen molecules rather than a change in the types of collagen secreted by the cells.

Consistent results were always obtained from replicate dishes within a single experiment. The major high M₉ proteins secreted by pericytes grown in monolayer culture on plastic dishes (Fig. 6, tracks 1 and 3) were identified as fibronectin, thrombospondin and type I procollagen (20 experiments); in addition, small amounts of type III collagen could also be identified in the medium of these cultures after pepsin digestion of the samples (see below). This biosynthetic profile was found to be independent of the state of confluency of the cultures (not shown). However, when the pericytes were cultured within three-dimensional collagen gels, the nature of the matrix molecules secreted by these cells was modulated. Thus, cells plated in gel as single cells (3 separate experiments) or as clumps (11 separate experiments) also secreted significant amounts of fibronectin and type I procollagen, but under these conditions the secretion of thrombospondin into the medium was barely detected (Fig. 6, tracks 2 and 4). Furthermore, only pericytes plated as clumps of cells in gel secreted significant levels of type IV procollagen into the medium (Fig. 6, track 4). Fig. 6B shows the results obtained by scanning tracks 3 and 4 of the fluorogram in Fig. 6A. The densitometric traces clearly show the de-
Fig. 4. Transmission electron micrograph showing details of cellular associations in gel. Detail of an area of close cellular packing in a collagen gel culture of pericytes, showing the variation in cellular interfacing. Membranes may be in intimate apposition, with structures reminiscent of gap junctions (1), separated by relatively large (2) or small (3) amounts of extracellular matrix. The thickened nuclear lamina clearly separates the peripheral chromatin from the nuclear membrane, and the actin microfilament bundles show characteristic focal densities (fd). In the region of the gel adjacent to the cells, matrix vesicles and needle-like crystals of hydroxyapatite are apparent. Bar, 0.5 μm.

crease in thrombospondin synthesis and increase in type IV collagen synthesis by pericytes plated in gel as clumps compared with cells plated on plastic. By measuring the areas under the peaks from several different fluorograms it was determined that type IV collagen could represent between 10 and 15% of the total collagenase-sensitive proteins secreted into the medium by pericyte aggregates in gel. These experiments were conducted with seven different batches of pericytes; results identical to those described above were a consistent observation.

Fibronectin, thrombospondin and the procollagens described above were identified by a combination of immunological and biochemical techniques. The effect of bacterial collagenase on the newly synthesised proteins secreted by retinal pericytes is shown in Fig. 7. This figure clearly shows that pericytes cultured on plastic dishes secrete two major high Mr proteins that are resistant to digestion by this enzyme (Fig. 7, tracks 1 and 2). These non-collagenous proteins are identified as fibronectin and thrombospondin by immunoprecipitation with specific antibodies (Fig. 8). Fig. 7 also shows that pericytes cultured in collagen gels (as single cells or as cell aggregates) retain the
ability to secrete fibronectin, but the secretion of thrombospondin by these cells can barely be detected (tracks 3–6) confirming the results shown in Fig. 6. Analysis of the proteins deposited into the cell layer/matrix by these cells indicated that the differences in thrombospondin secretion could not be attributed to a redistribution of the protein to the cell layer, but rather was due to a specific decrease in the synthesis of this protein (results not shown). Pericytes plated on plastic dishes and in collagen gels secreted several high $M_r$, bacterial collagenase-sensitive proteins (Fig. 7). These collagenous proteins were identified by immunoprecipitation as being predominantly type I procollagen and its processed forms (Fig. 8). In addition, when pericytes were plated as aggregates of cells in gel, the presence of two extra collagenase-sensitive bands that migrated just ahead of fibronectin on SDS/polyacrylamide gels were detected (Fig. 7, tracks 5 and 6). These polypeptides were identified as the pro-$\alpha_1$ and pro-$\alpha_2$ chains of type IV collagen by immunoprecipitation (Fig. 8A, tracks 7 and 8, and Fig. 8B).

In order to confirm the nature of the collagen types secreted by pericytes cultured under different conditions, samples of medium proteins were digested with pepsin and the products were analysed by SDS/polyacrylamide gel electrophoresis under non-reducing conditions and after interrupted reduction, as previously described by Sykes et al. (1976) (Fig. 9). The profiles of pepsin-resistant proteins were very similar when pericytes were cultured on plastic or as single cells in gel (Fig. 9, tracks 1–4). The $\alpha_1$ and $\alpha_2$ chains of type I collagen were the major products identified, together with smaller amounts of $\alpha_2$ (III) collagen chains, which were detected after delayed reduction of the samples (tracks 3 and 4); unreduced type III collagen remains at the top of the resolving gel in these fluorograms (tracks 1 and 2). By contrast, when the cells were plated as clumps in gel, the relative levels of $\alpha_1$ (I), $\alpha_2$ (I) and $\alpha_1$ (III) chains were lower than those detected when the same cells were plated on plastic and two bands identified as pepsin products of type IV collagen were seen to increase (Fig. 9, compare tracks 5–8). These results confirm the increased secretion of type IV collagen by pericytes forming three-dimensional aggregates in collagen gels.

As mentioned earlier, the proteins deposited into the cell layer/matrix by the pericytes were very similar to those secreted into the medium. The only difference observed between medium and cell layer/matrix proteins was when the proteins were digested with pepsin and the products were analysed by SDS/polyacrylamide gel electrophoresis as described above (compare Figs 9 and 10). Fig. 10 clearly shows that pericytes plated on plastic dishes (tracks 1 and 2) and in gel as single cells (tracks 3 and 4) deposit an increased proportion of type III collagen into the cell layer/matrix compared to the medium. The relative proportions of these collagen types were determined by scanning the fluorograms using a Shimadzu laser densitometer. In any given experiment, the proportion of type III collagen in the cell layer was between three and six times greater than in the medium. Thus, an average of 7–12 % of the total collagen secreted by pericytes cultured on plastic or in gel consisted of type III collagen, and in the cell layer/matrix this value rose to an average of 38–45 %.

This change in the deposition of collagen types I and III also occurred when pericytes were plated in gel as clumps (results not shown).

**Discussion**

Microvascular pericytes are intramural cells that are defined in vivo by their location within the basement membrane surrounding the endothelial cells. The functional relationship between these two cell types and the basement membrane is, however, not understood. The main objective of this study has been to examine the manner in which cell–cell and cell–matrix interactions influence the biosynthetic activity of retinal microvascu-
Pericytes plated on plastic tissue culture dishes and within three-dimensional collagen gels displayed very different morphologies (Fig. 1). Cells plated on plastic dishes grew as polygonal cells with ruffled leading edges. When these cells were plated as a single cell suspension in gel they elongated into a sprouting morphology that was very reminiscent of endothelial cells plated under the same conditions (Schor and Schor, 1986). However, unlike endothelial cells, which migrate and self-associate to form cords and tube-like structures, pericytes remain homogeneously distributed within the gel matrix. Pericytes cultured under these two conditions synthesised a similar spectrum of extracellular matrix proteins (Figs 6–10); type I collagen was the major collagen type produced together with some type III collagen, and only traces of type IV collagen were ever detected. The synthesis of interstitial collagens by retinal pericytes is in agreement with the biosynthetic study of Kennedy et al. (1986), who showed the secretion of predominantly type I collagen by pericytes maintained in monolayer cultures. Immunolocalisation studies have also demonstrated the presence of collagen types I and III as well as type IV collagen in bovine (Kennedy et al. 1986) and human (Jerdan and Glaser, 1986) retinal vessels. In this regard it is particularly noteworthy that Carlson et al. (1988) have demonstrated that collagen fibrils were restricted to the 'pericytic matrix' of isolated bovine retinal vessels, and that no collagen fibrils were detected in the subendothelial base-
Fig. 8. Identification of fibronectin, thrombospondin and collagen types I and IV by immunoprecipitation. Pericytes were cultured on plastic dishes and as clumps of cells within collagen gels. Cultures were radiolabelled with [3H]proline (plastic) or [3H]proline (in gel) for 24 h. At the end of the incubation period, samples of medium were used for immunoprecipitation experiments as described in Materials and methods. Precipitated proteins were separated by electrophoresis on 6.5% polyacrylamide gels and detected by fluorography. A. Samples in tracks 2–6 are from cells cultured on plastic dishes; samples in tracks 7–9 are from cells cultured as clumps in gel. Samples in tracks 1–9 are as follows: 1, standard [3H]acetylated rat tail tendon collagen; 2, total proteins secreted into the medium; 3, proteins immunoprecipitated with anti-fibronectin serum (aFn); 4, proteins immunoprecipitated with anti-thrombospondin serum (aTs); 5, proteins immunoprecipitated with anti-type I collagen serum (aT1); 6, proteins immunoprecipitated with anti-type IV collagen serum (aIV); 7, total proteins secreted into the medium; 8, proteins immunoprecipitated with anti-type IV collagen serum (aIV); 9, proteins immunoprecipitated with normal rabbit serum (nrs). The migration positions of fibronectin (Fn), thrombospondin (Ts), type IV procollagen and the proα1 and proα2 chains of type I collagen are indicated. B. Densoitometric scan of track 8.

Fig. 9. Identification of collagen types in the medium by pepsin digestion. Cells were incubated with [3H]proline for 20 h. Samples of medium proteins were digested with pepsin (100 μg ml⁻¹) for 4 h at 4°C in 0.5 M acetic acid. The products were separated by electrophoresis on 6.5% polyacrylamide gels and detected by fluorography. Samples in tracks 1, 2, 5 and 6 were electrophoresed without reduction (NR), samples in tracks 3, 4, 7 and 8 were reduced after they had entered the separating gel by approx. 1 cm (interrupted reduction, IR). Tracks 1, 3, 5 and 7 contain samples from cells cultured on plastic dishes (P), tracks 2 and 4 contain samples from cells grown as single cells in gel (IGs), and tracks 6 and 8 contain samples from cells grown as clumps in gel (IGc). The migration positions of α1(I), α2(I), α1(III) collagen chains and the pepinin products of type IV collagen are indicated.

Our results would therefore be consistent with the suggestion that in the normal microvessel wall, endothelial cells synthesise the type IV collagen present in the subendothelial basement membrane (Canfield et al. 1986) and the pericytes synthesise the interstitial collagens present in the pericyte matrix described by Carlson et al. (1988).

The results presented in this paper also demonstrate the secretion of the high Mr, glycoproteins, fibronectin and thrombospondin, by retinal pericytes in vitro. Fibronectin was synthesised by cells cultured either on plastic dishes or within collagen gels; in contrast, the secretion of thrombospondin was dependent upon the matrix on which the cells were cultured. Thrombospondin was synthesised in large amounts by pericytes cultured on plastic dishes, but could barely be detected in the medium when the cells were cultured in collagen gels (Fig. 6). Analysis of the peptidase deposited into the cell layer/matrix indicated that the differences in thrombospondin secretion could not be attributed to a redistribution of this protein to the cell layer/matrix, but rather to a specific decrease in thrombospondin synthesis per se. The synthesis of thrombospondin by cells in vitro has previously been reported to be directly proportional to the proliferative state of the cells (Mumby et al. 1984). This is clearly not the reason for the differences in thrombospondin synthesis observed in the pericyte cultures, since pericytes plated on plastic and in gel proliferate at similar rates (Schor and Schor, 1986). This finding is consistent with our previous studies using retinal endothelial cells (Canfield et al. 1986) and aortic endothelial cells (Canfield et al. 1990) cultured on two- and three-dimensional substrata and suggests that the secretion of thrombospondin may be modulated by cellular morphology and/or the presence of a three-dimensional collagen gel.

Of particular interest was the finding that the synthesis of type IV collagen by pericytes was promoted by plating aggregates of cells within three-dimensional collagen gels. Under these conditions, the pericytes elongated and grew out from the clumps forming interconnected networks (Fig. 1) that strongly resembled those formed by endothelial cells plated in gel (Schor and Schor, 1986). Furthermore, the biosynthetic profile displayed by these pericyte cultures was very similar to that previously described for retinal endothelial cells forming extensive cellular associ-
tracks 1 and 3 were electrophoresed without reduction, samples in polyacrylamide gels and detected by fluorography. Samples in products were separated by electrophoresis on 8 % chains are indicated. The migration positions of Qi(I), o^CD and cv^Ul) collagen by pepsin digestion. Cells were incubated with [ 3H]proline for within the nodules, as they only represent a small pro-

small, localised increase in type IV collagen biosynthesis collagen gel, may not be sufficient for this modulation in presence of large cell aggregates in the absence of a aations in the gel matrix (see Canfield et al. 1986). The presence of large cell aggregates in the absence of a collagen gel, may not be sufficient for this modulation in collagen biosynthesis. Thus, our data indicate that there was no detectable increase in type IV collagen biosynthesis in post-confluent cultures of pericytes on plastic dishes where extensive cellular nodules have been formed. We cannot exclude the possibility that there may be a small, localised increase in type IV collagen biosynthesis within the nodules, as they only represent a small proportion of the total cells present in any given culture. Transmission electron microscopy revealed that although pericytes cultured under these two conditions were ultrastructurally very similar, the cells appeared more closely apposed when plated as aggregates in gel than they were in the centre of a nodule on plastic (Figs 2 and 3). Furthermore, cells cultured on plastic dishes appeared to be separated from each other by loose extracellular material (Fig. 2; and Buzney et al. 1983). In contrast, cells in gel were surrounded by a dense extracellular matrix (Fig. 3). The main localisation of the non-fibrillar 'external lamina' seen around pericytes in gel (Fig. 5) is highly reminiscent of the morphology of peri-
cytes in vivo (Ghaddi, 1988). The finding that this lamina appears preferentially deposited at cell–collagen interfaces rather than cell–cell interfaces suggests a response to the environment in terms of extracellular matrix secretion. Our data therefore indicate that the biosynthesis of type IV collagen by retinal pericytes may be modulated both by the density of the cells and by the nature of the matrix in contact with the cells. The relationship between endothelial cells and pericytes is not fully understood (see review by Sims, 1986). It has been suggested by several workers that these cells have a common mesenchymal origin (Kuwabara and Cogan, 1963; Ashton, 1966; Shakib and de Oliveira, 1986). Moreover, Schoell (1963) noted that in corneal capillaries, cells that first appeared to be periendothelial (e.g. a pericyte) could also be traced to the inner vascular lining (e.g. an endothelial cell). It has also been proposed that pericytes in the adult represent primitive pluripotential mesenchymal cells (Sims, 1986) that are able to differentiate into other cells under certain conditions. In this regard, it is interesting that the results presented in this paper indicate that under certain culture conditions (e.g. cell aggrega-
ges in gel) pericytes resemble table endothelial cells both morphologically and in terms of matrix protein biosyn-
thesis; it may therefore seem reasonable to suggest that during angiogenesis in vivo these two cell types may also be indistinguishable.

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References


Fig. 10. Identification of collagen types in the cell layer/matrix by pepsin digestion. Cells were incubated with [3H]proline for 20 h. Samples of proteins in the cell layer/matrix were digested with pepsin (100 μg ml⁻¹) for 4 h at 4°C in 0.5 M-acetic acid. The products were separated by electrophoresis on 8 % polyacrylamide gels and detected by fluorography. Samples in tracks 1 and 3 were electrophoresed without reduction, samples in tracks 2 and 4 were reduced after they had entered the separating gel by approximately 1 cm. Tracks 1 and 2 contain samples from cells cultured on plastic dishes (P); tracks 3 and 4 contain samples from cells grown as single cells in gel (IGs). The migration positions of α1(I), α2(I) and α3(III) collagen chains are indicated.


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