Schwann Cell Myelination: Induction by Exogenous Basement Membrane–like Extracellular Matrix

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Abstract. Exposing rat Schwann cells co-cultured with nerve cells to a reconstituted basement membrane induced the formation of myelin segments by Schwann cells. This occurred in a serum-free culture medium in which, in the absence of this matrix, Schwann cells proliferate but fail to differentiate. This reconstituted basement membrane was prepared from solubilized extracellular matrix proteins synthesized by a basement membrane–producing murine tumor. The major constituents of this reconstituted matrix are collagen type IV, laminin, heparan sulfate proteoglycan, entactin, and nidogen. The matrix also elicited striking morphological changes in Schwann cells, inducing them to spread longitudinally along the nerve fibers (a necessary early step in the process of ensheathment of nerve fibers). Several observations indicated that the effect of the matrix was exerted directly on Schwann cells and not indirectly through an effect on nerve cells. First, the matrix-induced cell spreading occurred only in areas in which Schwann cells directly contacted the matrix; Schwann cells that were associated with the same nerve fibers but that did not themselves directly contact the matrix did not exhibit spreading. Second, the matrix-induced alteration in Schwann cell morphology was observed in cultures in which the nerve cells were removed. These results provide direct evidence that basement membrane contact induces normal Schwann cell differentiation, and support the idea that Schwann cell differentiation in vivo may be regulated by the appearance of the basement membrane, which normally envelops terminally differentiating Schwann cells.

THE extracellular matrix has been shown to influence the behavior of many cell types, including cells of neural origin, during embryonic development. Cellular functions affected by extracellular matrix include proliferation, migration, adhesion, morphology, and terminal differentiation (reviewed in references 14–16, 26, and 28). In most cases the molecular mechanisms responsible for these effects are not known.

We have been studying the development in culture of Schwann cells and nerve cells obtained from sensory ganglia of rat embryos. Under appropriate culture conditions these Schwann cells produce an extracellular matrix consisting of a basement membrane and associated collagen fibrils that ultrastructurally and biochemically is similar to the extracellular matrix of the peripheral nerve endoneurium (7, 9, 11). Under certain conditions, however, the formation of this extracellular matrix by Schwann cells is blocked. This occurs, for example, in cultures treated with cis-hydroxyproline, an analogue of proline that inhibits collagen hydroxylation (10), and in cultures fed the serum-free medium N2 (8, 21). It has been shown that in these cultures the Schwann cells fail to differentiate normally, as demonstrated by their inability to provide ensheathment and myelination for sensory nerve fibers (10, 21). These observations, and others (4), have led to the suggestion that the presence of a suitable extracellular matrix is necessary for normal Schwann cell development (4, 21).

Until recently, however, it was not possible to demonstrate a direct effect of exogenously added extracellular matrix on Schwann cell development in the absence of de novo matrix production. Thus, it could not be ruled out that the culture conditions that prevented extracellular matrix production by Schwann cells were also affecting other cellular functions important for Schwann cell differentiation. In this paper we present evidence that an exogenous basement membrane–like extracellular matrix, under conditions in which extracellular matrix production by Schwann cells does not occur, elicits normal Schwann cell development, as evidenced by the spreading of Schwann cells onto sensory nerve fibers and the formation of myelin segments around sensory nerve fibers.

Materials and Methods

Cell Culture

Primary cultures containing only nerve cells and Schwann cells were prepared from rat embryo dorsal root ganglia by previously published methods (7, 8, 31). Both dissociated cell cultures and explant cultures were used. For dissociated cultures the ganglia were dispersed by incubation in a solution containing trypsin (21) and seeded onto 2-cm diameter Aclar mini-dishes (5) coated with

rat tail tendon collagen (see below). The cultures were fed initially antimitotic medium (Eagle's minimum essential medium containing 10% fetal calf serum, 1×10^{-5} M 5-fluorodeoxyuridine, and 1×10^{-5} M uridine). After 7–10 d the cultures were switched to the serum-free medium N2 (3). For explant cultures ganglia were grown initially in antimitotic medium on a substratum of rat tail tendon collagen. After 10–14 d the ganglionic explants were excised with sterile razor blade fragments and transferred to dishes (Aclar mini-dishes or four-well tissue culture plates, Irvine Scientific, Santa Ana, CA) coated with rat tail tendon collagen or reconstituted basement membrane. After transfer the ganglia were fed N2 medium. The purpose of the antimitotic treatment is to kill the fibroblasts present in the ganglia; any fibroblasts remaining after the antimitotic treatment do not proliferate in the serum-free N2 medium. Schwann cells, which are resistant to the antimitotic treatment, proliferate vigorously in N2 medium but do not produce extracellular matrix (8, 21). Thus, using these procedures one obtains cultures that essentially contain only Schwann cells and nerve cells (7, 31).

To prepare cultures containing only Schwann cells (without nerve cells) the ganglion explants were excised from explant cultures that had been grown in N2 medium for several weeks as described above. Ganglion excision removes the cell bodies of all the nerve cells in the cultures. Within 2–3 d after ganglion removal the amputated neurites degenerate and are phagocytosed by Schwann cells (6, 31).

Basal media components and sera were obtained from Gibco (Grand Island, NY). Medium additives were from Sigma Chemical Co. (St. Louis, MO). The composition of the serum-free N2 medium was as described by Bottenstein and Sato (3) except that Hepes buffer was omitted and nerve growth factor (20 ng/ml) was added.

Culture Substratum Preparation

Collagen type I was prepared by acetic acid extraction of rat tail tendons. To prepare culture substrata a solution of collagen in H_2O was pipetted onto the culture dishes, spread with a glass rod, and gelled by exposure to ammonia vapors (2).

The reconstituted basement membrane was prepared from a soluble extract of the basement membrane produced by the murine tumor known as the Englebreth-Holm Swarm (EHS) sarcoma (22). The procedures for the preparation and use of this extract (17) were developed in the laboratory of Drs. Hynda Kleinman and George Martin, and they generously provided the matrix extract used in the experiments reported here. To prepare culture substrata, aliquots of frozen matrix extract were thawed and mixed with an equal volume of ice-cold N2 medium. The diluted extract was pipetted onto the culture dishes (0.1–0.15 mg/cm^2) and spread with a glass rod. The dishes were then placed into a tissue culture incubator for 5–10 min. The matrix extract, which is a viscous liquid when cold, becomes a semirigid gel at physiological temperatures.

For experiments with dissociated cell cultures the matrix was polymerized onto the surface of already established cultures that had been grown for several weeks in N2 medium on rat tail tendon collagen. The medium was removed and matrix extract was pipetted onto the dishes. After warming to allow the matrix to polymerize, fresh N2 medium was added to the dishes.

Light Microscopy

Cultures were fixed with glutaraldehyde, postfixed with osmium tetroxide, and stained with Sudan black B (Fisher Scientific, Pittsburgh, PA) as described by Wood (31). To visualize myelin segments in living cultures cells were examined by bright field microscopy with the 20 or 40× lens of a Nikon Diaphot inverted microscope. Light microscope autoradiography was performed as described by Salzer and Bunge (24). Methyl-[^{3}H]thymidine (80.2 Ci/mmol) was purchased from New England Nuclear (Boston, MA).

Immunofluorescent visualization of myelin segments was carried out with a rabbit antiserum specific for myelin basic protein (kindly supplied by Dr. B. Riederer). The methods used for the preparation of this antiserum and evidence of its specificity have been described (23). For immunofluorescent labeling with this antiserum the cells were first fixed in 5% acetic acid in ethanol and then exposed to antiserum. Bound antibodies were visualized using fluorescein-conjugated goat anti-rabbit IgG (affinity purified, from Sigma Chemical Co.).

To visualize the distribution of polymerized basement membrane, cultures were immunostained with an antiserum to the basement membrane protein laminin (27) (kindly provided by Dr. H. Kleinman). Staining of extracellular laminin was carried out on living, unfixed cultures as previously described (11).

Gel Electrophoresis and Radiolabeling

SDS gel electrophoresis was carried out in polyacrylamide slab gels using the method of Laemmli (18). For immunoprecipitation with antimyelin basic protein, antiserum cultures were labeled overnight with [4,5-^{3}H]-leucine (50 Ci/mmol, ICN Radiochemicals, Irvine, CA) as previously described (8, 9). At the end of the labeling the cultures were extracted with immunoprecipitation buffer (1% NaCl, 1% deoxycholate, 1% Triton X-100, 0.1% SDS, 5 mM EDTA, 20 mM Tris-Cl, pH 7.4, 1 mM phenylmethylsulfonyl fluoride) for 30 min at 4°C. 100-μl aliquots of labeled extract were mixed with 2 μl of either antimyelin basic protein antiserum or control serum from a nonimmune rabbit. After overnight incubation at 4°C 50 μl of a 10% suspension of Pansorbin cells (Calbiochem-Behring Corp., San Diego, CA) was added to each sample. The samples were gently mixed for 1 h at room temperature, and the immunoprecipitates were harvested by centrifugation at 14,000 *g* for 2 min. The pellets were washed three times with immunoprecipitation buffer. Bound proteins were solubilized in gel sample buffer and aliquots were loaded onto a 12% acrylamide slab gel. After electrophoresis the gels were processed for fluorography with En^{3}HANCE (New England Nuclear), dried, and exposed to preflashed x-ray film. Densitometric tracings of fluorograms were made using a scanning densitometer (Bio-Rad Laboratories, Richmond, CA).

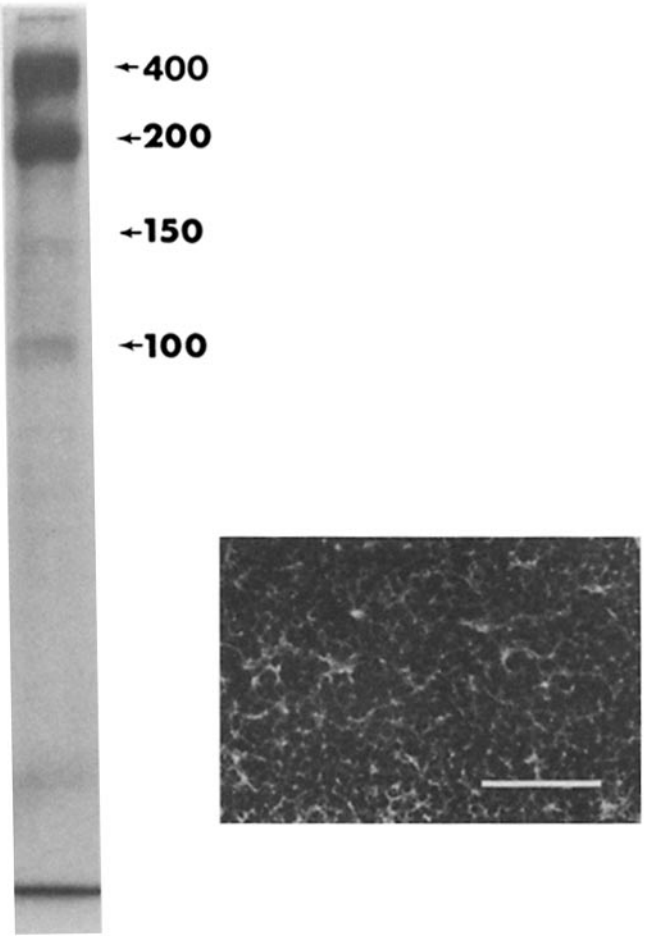

Figure 1. SDS polyacrylamide gel analysis of reconstituted basement membrane. Murine tumor extract was used to prepare the reconstituted basement membrane as described in Materials and Methods. The polymerized basement membrane was washed with tissue culture medium several times and then solubilized with gel sample buffer. An aliquot was subjected to SDS gel electrophoresis in a 7% acrylamide slab gel and stained with Coomassie Blue. Numbers indicate M_r (in thousands). The band at the top of the gel is heparan sulfate proteoglycan; the broad bands at M_r 400,000 and 200,000 are the subunits of laminin; bands at M_r 150,000 and 100,000 are entactin and nidogen; collagen type IV is present but obscured by the small subunit of laminin. The micrograph shows a portion of the polymerized matrix after indirect immunofluorescent staining with antilaminin antiserum. This represents an area where the matrix is fairly thin, allowing the fibrous nature of the matrix to be seen. Bar, 50 μm.

Results

The Tumor Matrix Extract Polymerizes In Vitro into a Basement Membrane-like Structure

Kleinman et al. (17) have shown that a soluble extract of EHS sarcoma extracellular matrix polymerizes into a basement membrane-like structure when warmed to physiological temperatures. Fig. 1 shows an SDS gel electrophoresis profile of the proteins present in basement membrane polymerized in vitro in this manner. The major components are laminin (M_r 400,000 and 200,000), collagen type IV (M_r 190,000, obscured by the small laminin subunit), entactin (M_r 150,000), nidogen (M_r 100,000), and heparan sulfate proteoglycan (does not enter the gel). These molecules are known to be distributed widely in basement membranes (17) including the basement membrane produced by Schwann cells (9, 11, 20). Fig. 1 also shows the polymerized matrix stained with antibodies to laminin. The polymerized matrix gives the appearance of a three-dimensional meshwork of fibers.

Basement Membrane Induces Spreading of Schwann Cells

Schwann cells cultured with nerve cells in the serum free medium N2 proliferate but do not produce basement membrane and do not ensheath or myelinate nerve fibers (8, 21). These cells exhibit abnormal morphologies, appearing as round cells perched upon nerve fiber bundles or as thin, flat cells with round nuclei (Fig. 2, *A* and *C*). We wanted to determine whether normal Schwann cell development in such cultures could be induced by the exogenous basement membrane. To test this we polymerized the mouse tumor basement membrane onto the surfaces of Schwann cells that had been

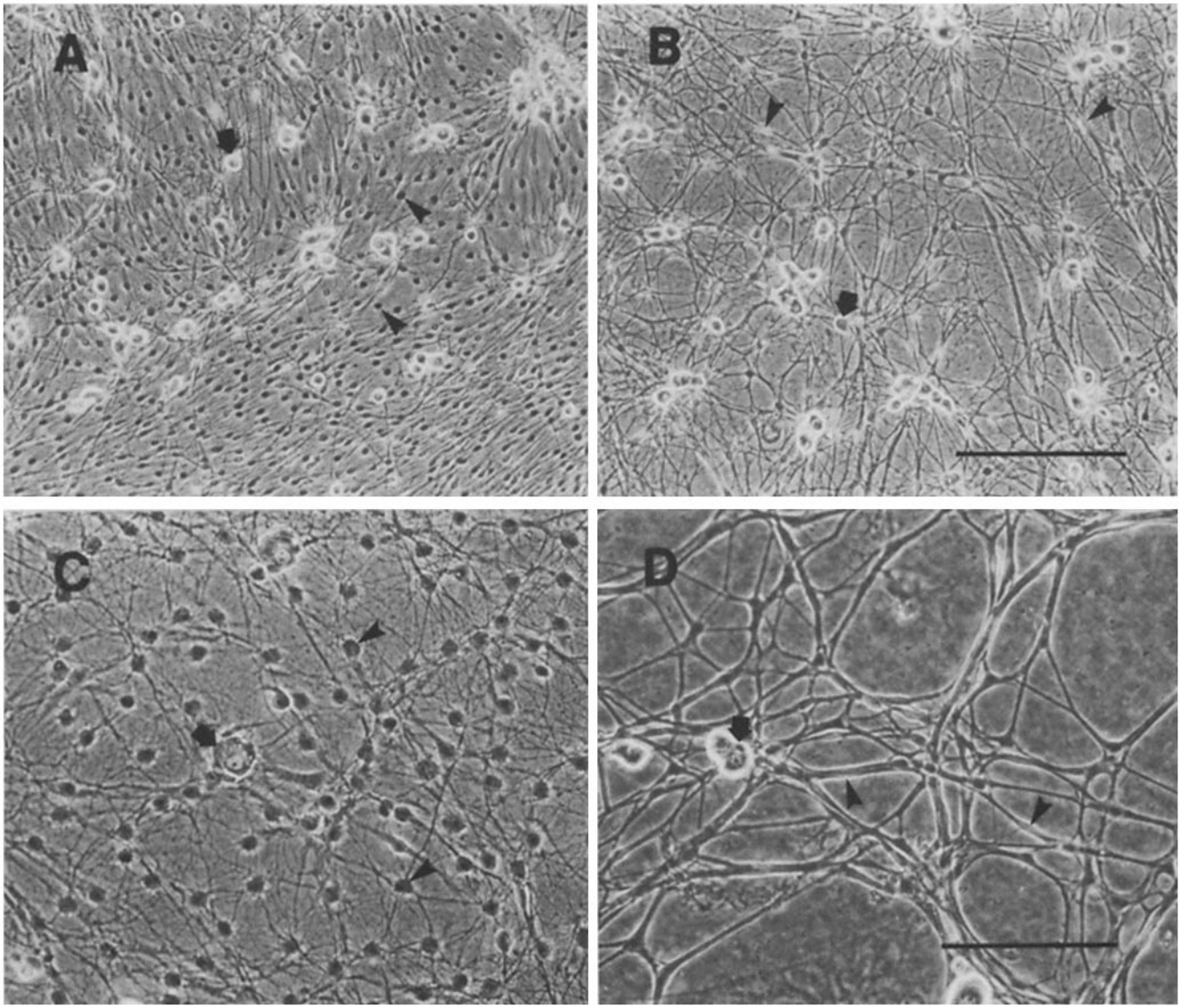

Figure 2. Effect of polymerized basement membrane on Schwann cells. Dissociated cultures of Schwann cells and nerve cells were grown in rat tail tendon collagen in N2 medium for 3 wk. At that time exogenous basement membrane was polymerized onto some of the cultures. (A) Control culture not receiving basement membrane. (B) A sibling culture 18 h after addition of basement membrane. Bar, 200 μm. (C) Control culture 3 d after addition of basement membrane to sibling cultures. (D) Sibling culture 3 d after addition of basement membrane. Bar, 100 μm. Arrows point to cell bodies of nerve cells; arrowheads point to Schwann cell nuclei.

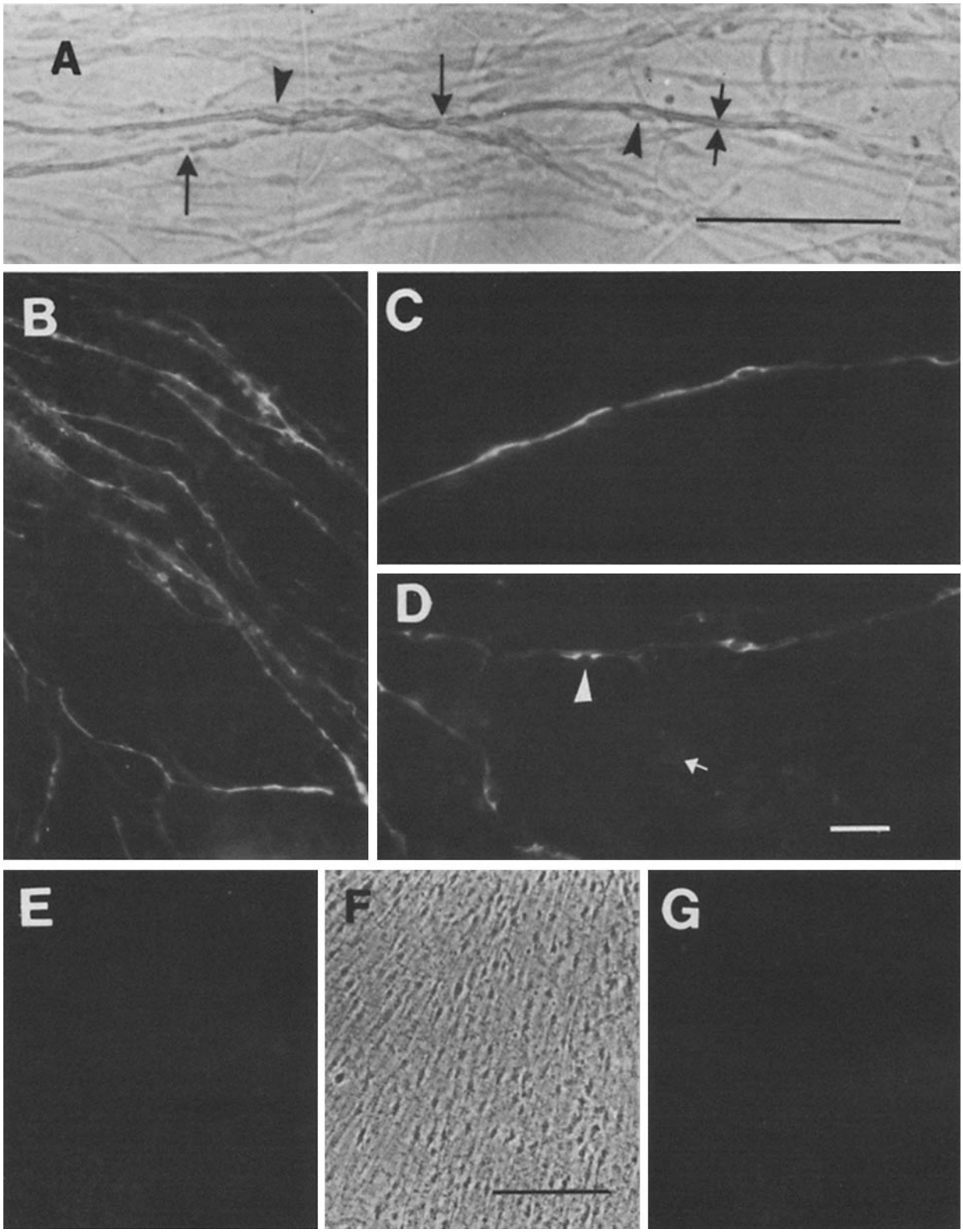

Figure 3. Myelin formation in basement membrane–containing cultures. (*A*) Myelin segments could be visualized in basement membrane containing cultures by bright field light microscopic observation of live cells; arrowheads point to nuclei of myelin-related Schwann cells; paired short arrows delineate lateral margins of a myelin segment; long arrows point to Nodes of Ranvier. (*B–D*) Myelin segments in basement membrane containing cultures were stained with antimyelin basic protein antiserum and fluorescein-conjugated second antibody; in *D* the arrowhead points to the nucleus of a myelin-related Schwann cell; the arrow points to the nucleus of a cell not related to a myelin segment but showing diffuse perinuclear staining. *E* is identical to *B* but stained with a nonimmune rabbit antiserum. (*F* and *G*) Corresponding phase-contrast and fluorescent micrographs of cells growing on rat tail tendon collagen without basement membrane stained with antimyelin basic protein antiserum. Bars: *A* and *F*, 100 μm; *C*, 25 μm.

grown in N2 medium for several weeks. As shown in Fig. 2 polymerization of basement membrane onto the Schwann cells caused them to elongate and spread along the nerve fiber bundles. This change in Schwann cell behavior was evident 18 h after basement membrane addition (Fig. 2*B*) and was complete by 3 d (Fig. 2*D*). This effect on Schwann cells was maintained for at least 2 wk. The elongation and spreading of Schwann cells on nerve fibers represents an early step in the establishment of ensheathment and myelination by Schwann cells (8).

Basement Membrane Induces Myelin Formation by Schwann Cells

The most dramatic demonstration of normal Schwann cell development is the formation of myelin segments around nerve fibers. Schwann cells grown on rat tail tendon collagen in N2 medium do not form myelin (21). As shown in Fig. 3, however, the polymerized basement membrane induced myelin formation by Schwann cells. The myelin segments could be visualized by bright field light microscope observation of living culture (Fig. 3*A*) or by immunostaining with antimyelin basic protein antiserum (Fig. 3, *B–D*). As expected, no myelin was formed in control cultures not receiving basement membrane (Fig. 3, *F* and *G*).

Other experiments indicated that the basement membrane affected not only the assembly of myelin but also the synthesis of a myelin specific protein. Antimyelin basic protein antiserum was used to immunoprecipitate proteins from extracts of [^{3}H]leucine-labeled cultures containing or lacking exogenous basement membrane. This antiserum immunoprecipitated from cultures containing basement membrane a major protein with an M_r identical to that of the major form of pig brain myelin basic protein (M_r 18,500) (Fig. 4) plus other proteins (M_r 21,500 and 17,000) that may represent variant forms of basic protein (23). This antiserum immunoprecipitated only small amounts of basic protein from an equivalent amount of extract from control cultures not given basement membrane (Fig. 4).

An important question raised by these experiments is whether the intact basement membrane was required for myelination or whether individual basement membrane molecules could stimulate myelin formation. To address this question we grew cultures in medium supplemented with purified laminin or collagen type IV (provided by Dr. H. Kleinman). Whereas these proteins bound to the Schwann cell surface, as demonstrated by indirect immunofluorescence with specific antibodies, we never observed myelin formation by Schwann cells in these cultures (not shown). Additional evidence that the intact basement membrane was required for the observed effects on Schwann cells is presented below.

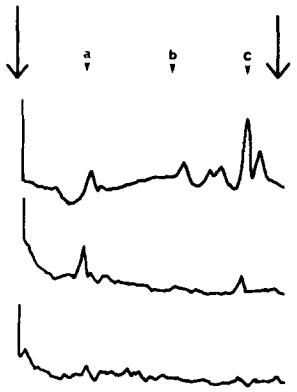

Figure 4. Effect of basement membrane on synthesis of myelin basic protein. Schwann cell–nerve cell cultures containing (top and bottom traces) or lacking (middle trace) exogenous polymerized basement membrane were labeled with [^{3}H]leucine for 18 h; extracts containing equal amounts of radioactivity were immunoprecipitated with antimyelin basic protein antiserum (top and middle traces) or nonimmune antiserum (bottom trace). The precipitates were electrophoresed on a 12% polyacrylamide slab gel and visualized by fluorography. Shown is a densitometer tracing of the fluorogram. Arrowheads indicate migration of standards: (*a*) ovalbumin, M_r 43,000; (*b*) carbonic anhydrase, M_r 29,000; (*c*) pig brain myelin basic protein, M_r 18,500. Arrows indicate the top of the separating gel and position of tracking dye. The direction of migration was from left to right.

Effects of Basement Membrane on Neurite Outgrowth and Schwann Cell Proliferation in Explant Cultures

In other experiments dorsal root ganglion explants that had been treated with 5-fluorodeoxyuridine to suppress fibroblast growth were transferred to dishes coated with basement membrane and fed N2 medium supplemented with nerve growth factor. The behavior of ganglion transferred to collagen type I–coated dishes is well documented (e.g., references 7 and 31). The initial behavior of ganglia placed on basement membrane was very similar (Fig. 5). Within a few hours after transfer the nerve cells extended new axons which grew radially from the ganglia. By the second day after transfer Schwann cells migrated out of the ganglia along the growing nerve fibers and proliferated, presumably in response to contact with axons (24). That Schwann cells were actively proliferating was confirmed by [^{3}H]thymidine autoradiography (not shown). Within a few days the explants produced a rich halo of neuritic outgrowth (Fig. 5). By ~2 wk of growth all of the available neuritic surface was covered by Schwann cells. Light microscopic observation of the outgrowths revealed Schwann cell–nerve fiber bundles growing throughout the thickness of the matrix, with few bundles growing on the matrix surface (Fig. 5). Thus, the matrix could support vigorous neuritic growth, and Schwann cells could proliferate and migrate on the nerve fibers.

The Effect of Basement Membrane on Schwann Cells Requires Direct Contact between the Cells and the Matrix

Schwann cells in explant cultures grown in N2 medium on a substratum of basement membrane were observed to spread and elongate on nerve fibers (Fig. 6, *A–D*) and to form myelin segments (not shown). This was in contrast to the behavior of Schwann cells in explant cultures grown on a substratum of rat tail tendon collagen (Fig. 6, *E* and *F*). This effect on Schwann cells appeared to require direct contact between intact basement membrane and the Schwann cells. This was seen by comparing areas of the cultures where the basement membrane had dissolved (exposing the plastic surface of the culture dish) with areas where the basement membrane was intact (Fig. 6). Dissolution of the basement membrane occurred with time in most cultures, usually near the ganglion. Schwann cells associated with bundles of nerve fibers in areas where the basement membrane had dissolved did not spread along the nerve fibers but instead flattened onto the exposed plastic surface and had round phase-dark nuclei (Fig. 6, *A* and *B*). These were identical in appearance to Schwann cells contacting collagen type I in N2 medium (Fig. 6, *E* and *F*). In areas where the basement membrane was intact the

Schwann cells did not flatten onto the culture surface but instead adhered closely to the nerve fibers and spread longitudinally along the fibers (Fig. 6, *A*, *C*, and *D*). In some cases the presence of Schwann cells was apparent only by slight bulges on the fiber bundles caused by the Schwann cell nuclei (Fig. 6, *C* and *D*). Consistent with the results presented above, no myelin segments were observed in areas lacking basement membrane (not shown).

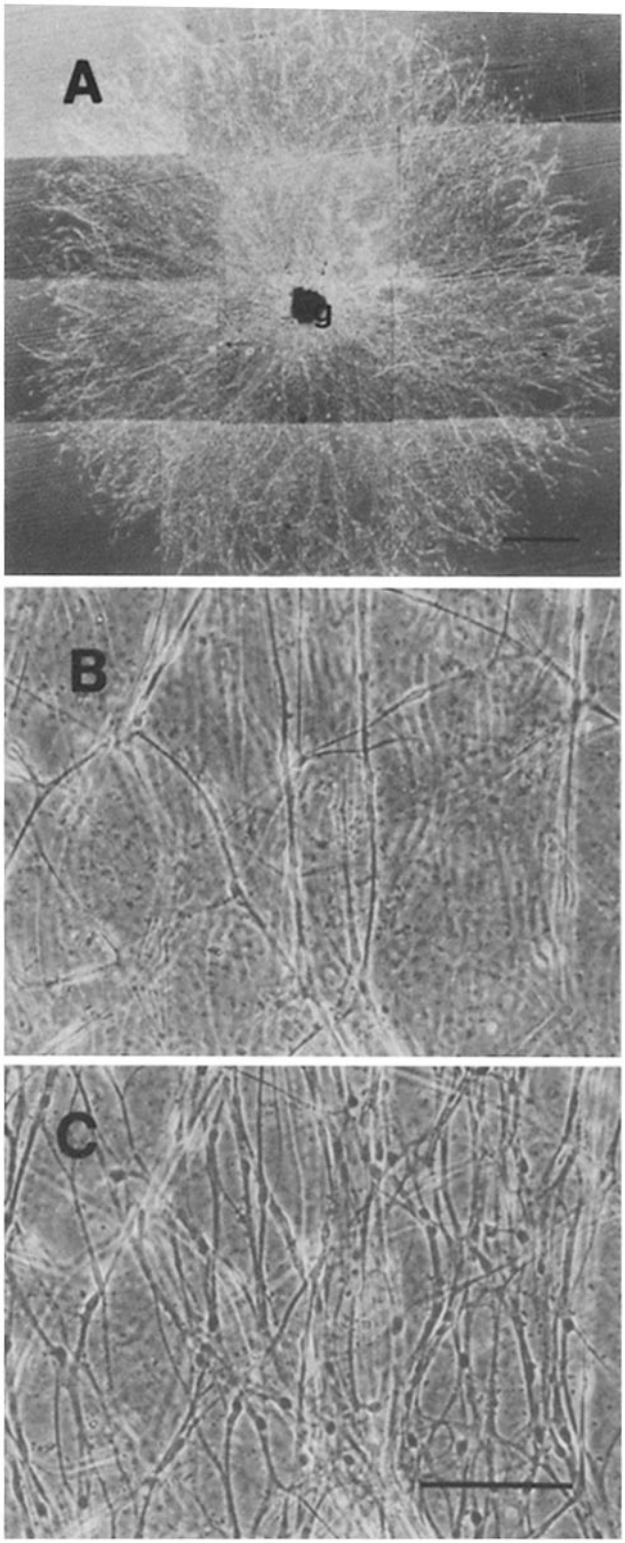

Figure 5. Dorsal root ganglion explant cultured on basement membrane–derived matrix gel. (*A*) Dorsal root ganglion culture grown in N2 medium for 2 wk. Bar, 2 mm. (*B* and *C*) The same field in the outgrowth of the culture shown in *A* was photographed in two different focal planes. *g*, ganglion. Bar, 100 μm.

In the experiments described above the presence of intact basement membrane was deduced by its speckled appearance under the light microscope (compare Fig. 6, *B* and *D*). To demonstrate more rigorously that the difference in Schwann cell behavior was related to contact with intact basement membrane we mapped the distribution of basement membrane by immunostaining with antibodies to laminin. As shown in Fig. 7, the boundary of the intact basement membrane could be easily visualized. When the physical relationship of this boundary was compared with the morphology of the Schwann cells, it was clear that only Schwann cells that were contacting the intact basement membrane were elongated and spread onto the neurites. This occurred in spite of the fact that the flattened Schwann cells not contacting the intact basement membrane were stained by the antilaminin antiserum (Fig. 7).

The Basement Membrane Exerts Its Effects Directly on Schwann Cells

That the basement membrane exerts its effects directly on Schwann cells and not indirectly through an effect on nerve cells is suggested by the observations illustrated by Figs. 6 and 7. Strikingly different behaviors were exhibited by Schwann cells that were related to the same nerve fiber bundles. On these fiber bundles the boundary between the flattened and elongated Schwann cells corresponded to the basement membrane boundary and not to a difference in nerve fibers related to these Schwann cells.

That the effect of the basement membrane was exerted directly on Schwann cells was also indicated by observations of Schwann cells in cultures from which the neurons were removed. Excision of neuronal cell bodies from Schwann cell–nerve cell explant cultures results in degeneration of the neurites and their phagocytosis by Schwann cells. Several days after neuronal excision the cultures contain only Schwann cells (6, 7, 31). In cultures treated in this way and grown on collagen type I the isolated Schwann cells were mostly bipolar. They extended extremely long, thin processes from a small, central region of cytoplasm containing the nucleus (Fig. 8*A*). These cells tended to form long parallel chains, apparently by associating with each other along their processes. The effect of basement membrane on Schwann cells isolated from nerve cells was examined in two ways. First, basement membrane was polymerized onto dishes of Schwann cells grown on rat tail tendon collagen (identical to the cells shown in Fig. 8*A*). Within 24 h the processes on these cells had spread and appeared to form broad sheets of membrane. On these cells the striking difference in thickness between the processes and the central nuclear region, evident in Schwann cells on collagen, was not seen (Fig. 8*B*). In another set of experiments nerve cells were removed from explant cultures that had been grown on a basement membrane substratum for several weeks. These Schwann cells retained the morphology typical of Schwann cells grown on basement membrane (compare Fig. 8*C* with Fig. 6). This morphology was stable in the

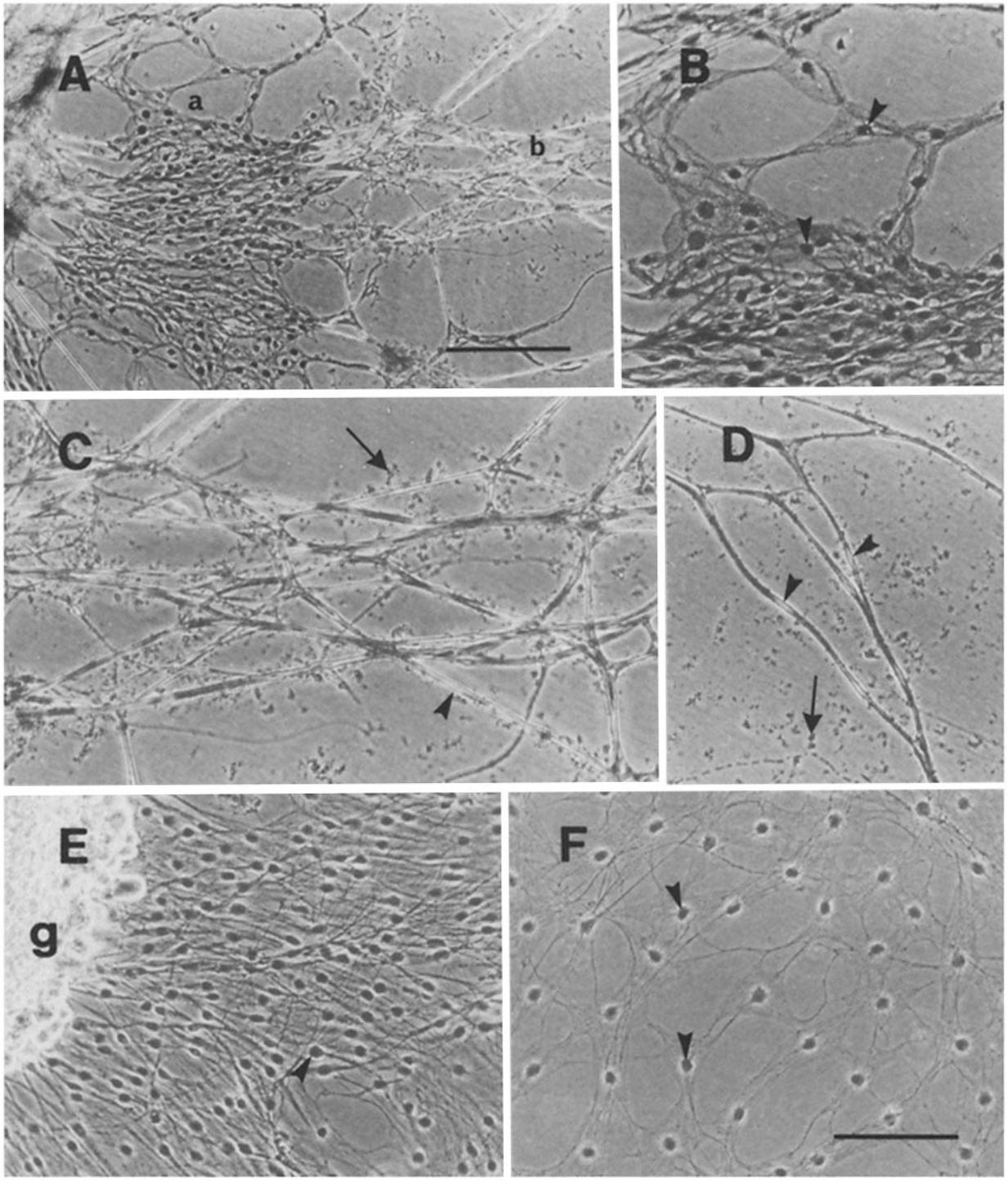

Figure 6. Effect of matrix on Schwann cells. Dorsal root ganglion explant cultures grown for 4 wk in N2 medium on basement membrane-like matrix (*A–D*) or type I collagen (*E* and *F*) were fixed and stained with Sudan black B and photographed with phase-contrast optics. (*A*) A low power view of the outgrowth showing a region from which the matrix has disappeared (left half of field, area *a*) adjacent to a region in which the matrix is intact (right half of field, area *b*); the ganglion is out of view to the left; the nerve fibers have grown across the field from left to right. Bar, 200 μm. (*B*) A higher power view of the area in *A* marked *a*; arrowheads denote Schwann cells nuclei. (*C*) A higher power view of the area in *A* marked *b*; the presence of matrix is indicated by amorphous aggregates of matrix material (*arrows*). (*D*) An area similar to that in *C* but further from the ganglion; note the presence of matrix (*arrows*) and the elongated shape of the Schwann cell nuclei (*arrowheads*). (*E*) Outgrowth of a culture grown on collagen type I close to the ganglion (*g*). (*F*) Similar to *E* but further from the ganglion; in *E* and *F* note the dark ovoid Schwann cell nuclei. (*B–F*) Bar, 100 μm.

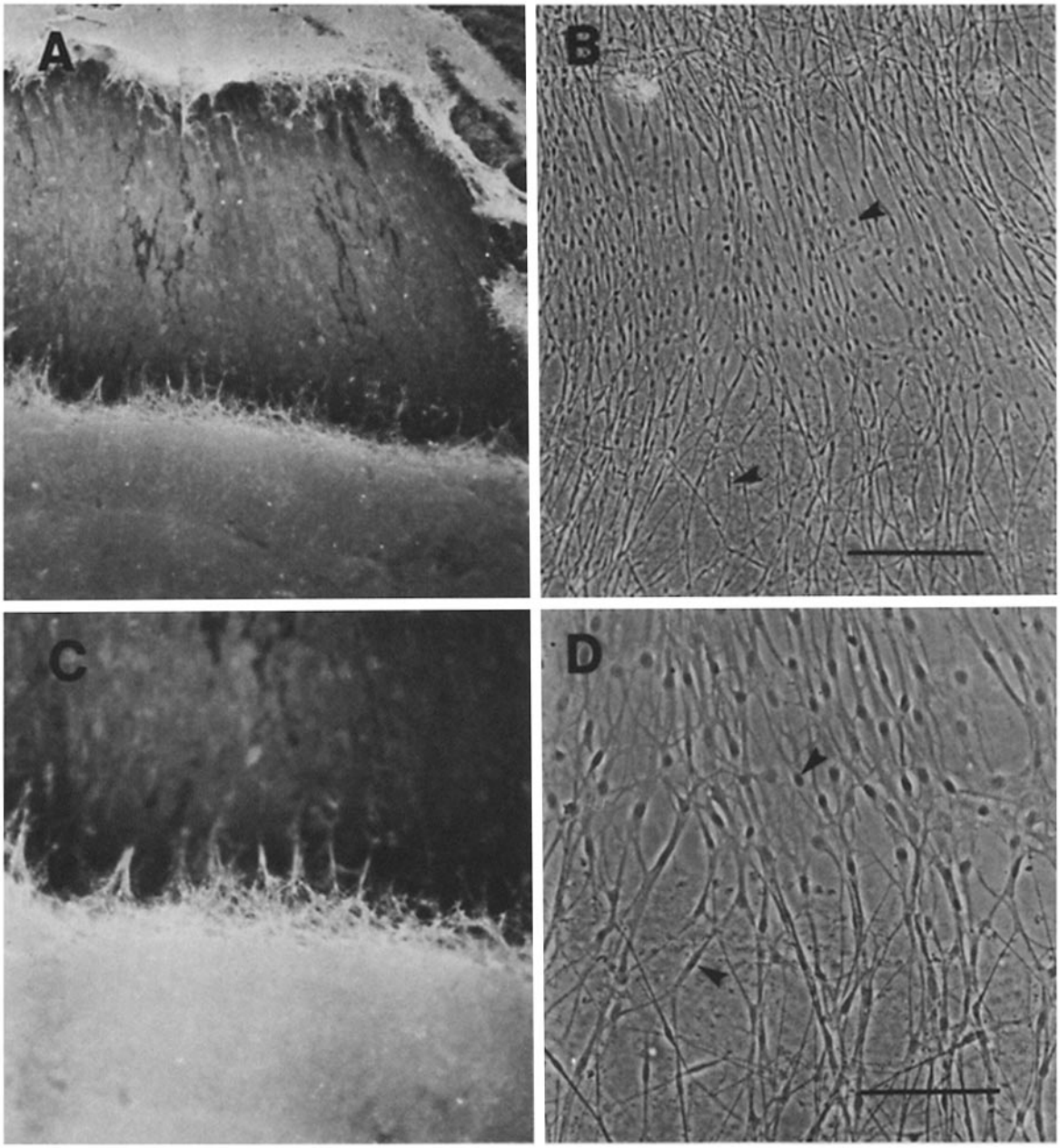

Figure 7. Relationship of basement membrane and Schwann cell spreading. Explant cultures grown on basement membrane for 3 wk were stained with antilaminin antibodies and fluorescein-conjugated second antibody. Selected fields were photographed with both fluorescence (*A* and *C*) and phase-contrast (*B* and *D*) optics. Arrowheads point to Schwann cell nuclei. Bars: *B*, 200 μm for *A* and *B*; *C*, 100 μm for *C* and *D*.

absence of nerve cells for at least 3 wk, the longest period observed.

Discussion

These results provide a direct demonstration that contact with a basement membrane-like extracellular matrix induces Schwann cell terminal differentiation. This effect was manifested as a change in cell morphology, from round or flat to elongated cells, and, in the case of myelin forming cells, as a dramatic change in cell behavior, including the increased production of myelin specific proteins. In the experiments reported here the exogenous matrix appears to be substituting for the basement membrane normally produced in culture (and in vivo) by Schwann cells. The biochemical compositions of these matrices are similar, the major components being collagen type IV, laminin, and heparan sulfate proteoglycans. Previously, McGarvey et al. (19) had demonstrated an effect of purified laminin on the morphology of isolated Schwann cells. Since they did not examine Schwann cell–nerve cell cocultures they could not draw conclusions about the ability of these Schwann cells to form myelin. However, we could not obtain myelination by adding purified laminin to mixed Schwann cell–nerve cell cultures.

Several observations suggest that the exogenous matrix exerts its effects directly on Schwann cells and not indirectly

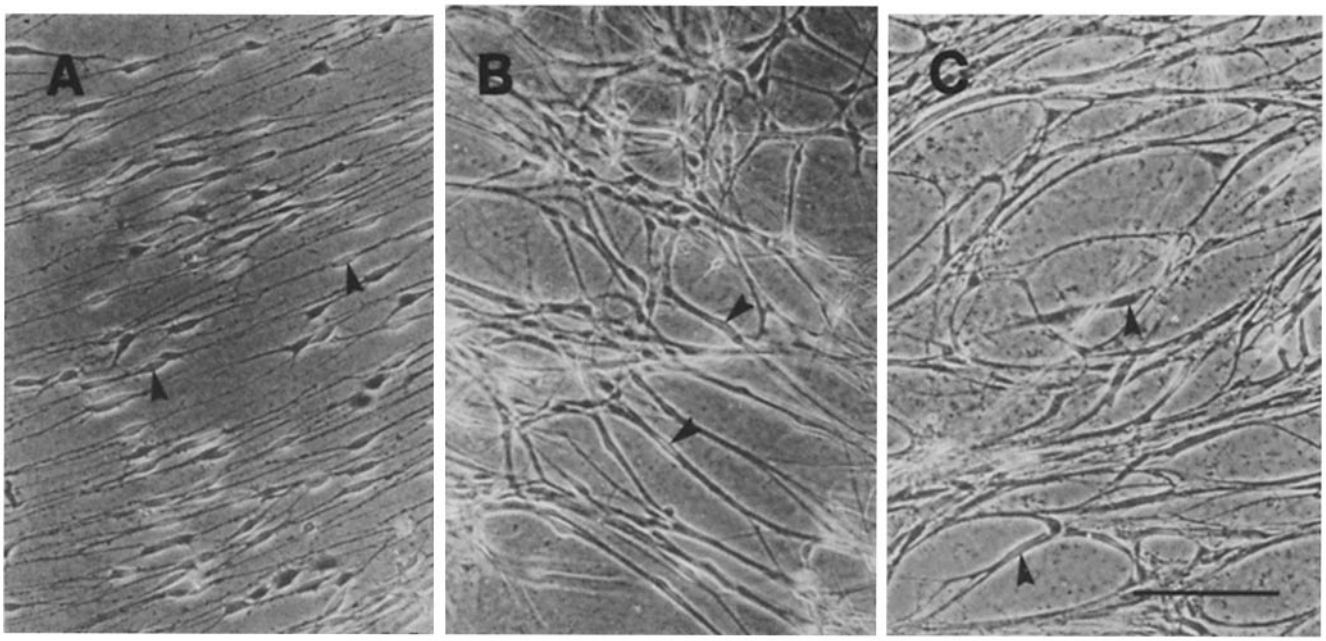

Figure 8. Effect of basement membrane on isolated Schwann cells. Cultures containing only Schwann cells were prepared by excising original explants (containing neuronal cell bodies) from dorsal root ganglion explant cultures (see text) leaving the outgrowths which after several days contain only Schwann cells. These photographs were taken 8 d after ganglion excision. The cultures in *A* and *B* were grown on type I collagen but the culture in *B* was given exogenous basement membrane 5 d after ganglion removal; the culture in *C* was grown on basement membrane from the onset. Arrowheads point to regions of cytoplasm containing Schwann cell nuclei. Bar, 100 μm.

through an effect on nerve cells. Groups of Schwann cells in areas either lacking or containing matrix exhibited very different properties even though they were related to the same nerve fiber bundles and therefore to the same nerve cells. The observation that Schwann cells contacting matrix in the absence of nerve cells also exhibited altered morphologies argues against a local effect of the matrix on axons. A final argument relates to the fact that the reconstituted matrix is similar in composition to the Schwann cell basal lamina (9, 11, 17, 19, 20). In vivo and in primary cultures it is the Schwann cells and not nerve cells that are in contact with this matrix (for an example see reference 7).

The observations reported here bear on the important question of the regulation of Schwann cell differentiation. Peripheral nerve development (both in vivo and in tissue culture) proceeds through an orderly series of steps. After the initial outgrowth of axons Schwann cells migrate along the growing nerve fibers and proliferate in response to contact with a mitogen present in the nerve fiber axolemma (25). At some point during development the Schwann cells become nonproliferative and begin to ensheath and myelinate the nearby nerve fibers (29, 30). It has been suggested from observations made in vivo that the transition from the migratory and proliferative to the nonproliferative and ensheathing state coincides temporally with the appearance of basement membrane around individual Schwann cells (1). A similar situation is seen in cultures of Schwann cells and nerve cells. The results presented in this paper indicate that the basement membrane exerts a direct effect on Schwann cells and that basement membrane contact may trigger this transition.

The paradoxical aspect of Schwann cell differentiation is that terminally differentiated Schwann cells stop proliferating even though they are still contacting axons, and the Schwann cell mitogen is present in axolemma isolated from adult rat sciatic nerve (12). The results presented in this paper indicate that the presence of basement membrane per se is not sufficient to turn off Schwann cell proliferation, since in the presence of the exogenous matrix, which promoted myelination, Schwann cell proliferation proceeded, at least in young cultures. Thus, Schwann cell proliferation may be more directly related to cell density or cell shape, both of which are known to effect proliferation of other cell types (13, 14). The matrix, however, may indirectly modulate Schwann cell proliferation to the extent that the matrix affects Schwann cell morphology.

The biochemical mechanisms responsible for the effects of basement membrane on Schwann cells are not known. We suggest that the basement membrane (exogenously supplied or produced by Schwann cells) provides a three-dimensional scaffold which supports and polarizes the Schwann cell as it undergoes the complex morphological changes associated with ensheathment and myelin formation (29, 30). The importance of the three-dimensional structure of the matrix was apparent from observations of cultures in which the basement membrane had broken down. Also, we have been unable to observe similar effects on Schwann cells after the addition to the medium of purified laminin or collagen type IV. The effect of reconstituted basement membrane is also specific since type I collagen gels do not produce the same morphological or developmental changes.

The matrix-induced changes in Schwann cell function presumably involve specific binding interactions between the Schwann cell membrane and components of the matrix. Whether this involves binding to one of the components of the exogenous basement membrane or to molecules released by Schwann cells that become incorporated into the matrix is being investigated. We have obtained preliminary evidence that a heparan sulfate proteoglycan secreted by Schwann cells

associates with the exogenous basement membrane (unpublished observation). The availability of a culture system in which normal Schwann cell function can be restored by the addition of a rather simple exogenous matrix should allow us to investigate the nature of the Schwann cell–matrix interactions in detail.

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