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Isolation and culture of cells derived from human cerebral microvessels*

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Summary. Microvessels were isolated from non-neoplastic human cerebral cortical fragments resected for treatment of intractable seizure disorder. The microvessels were incubated in modified Lewis medium with 20 or 30% fetal bovine serum. Within 1–2 weeks, two cell populations emerged from the isolates. One type of cells had polygonal morphology, showed density-dependent contact inhibition at confluence in vitro, showed lectin-binding characteristics of endothelium (but only moderate positivity for factor VIII antigen), demonstrated induction of γ-glutamyl transpeptidase when exposed to astrocyte-conditioned media, and responded to insulin by a pronounced increase in DNA synthesis. The other variety of cells grew in vitro more slowly in irregular strands separated by clear zones, showed ultrastructural features of smooth muscle, and isoelectric focusing of cell proteins revealed the presence of smooth-muscle-specific α-isoactin. Both types of cells could be serially subcultured. The ability to isolate and grow the two cell types, tentatively identified as human cerebral microvascular endothelium and smooth muscle, may facilitate studies of human blood-brain barrier function as well as the pathogenesis of cerebral microangiopathies unique to the human brain.

Key words: Microvessels, cerebral – Endothelium – Smooth muscle – Human – Mouse

A key to understanding cerebral microvascular function and/or abnormalities may lie in the ability to isolate, grow and study elements of walls of microvessels in vitro. Potentially, advances in the study of *micro*angiopathies comparable to those made in elucidating the pathogenesis of *macro*angiopathies, in particular atherosclerosis (Ross 1986), may follow rapidly because interactions between mural components as well as their response(s) to sublethal injury might be assessed in a tissue culture system. Isolation of cerebral microvascular endothelium provides the added advantage of allowing a study of certain blood-brain barrier (BBB) functions in vitro (Beck et al. 1983a, 1984; Vinters et al. 1985a; Bowman et al. 1983) since this endothelium has been characterized as the site of the BBB in vivo (Bradbury 1984; Pardridge 1983, 1984; Reese and Karnovsky 1967).

The isolation of capillaries, venules, and arterioles, as well as separation and growth of the component cells, has now been achieved in the face of considerable methodologic difficulty (De Bault 1982). Cerebral endothelium has been cultured from several species including mouse (De Bault et al. 1979, 1981; De Bault and Cancilla 1980a) and rat (Bowman et al. 1981; Diglio et al. 1982). Microvascular smooth muscle from brain of mice and rats has been cultured (Moore et al. 1984; Spatz et al. 1983) and some of its metabolic properties studied (Beck et al. 1983b). Human endothelium and smooth muscle from several sites, including aorta and vena cava (Fry et al. 1984), vein of umbilical cord (Lewis et al. 1973; Gimbrone et al. 1974, 1975; Maciag et al. 1981; Kan et al. 1985) and other large vascular types (Thornton et al. 1983; Ryan and White 1985) have been grown and occasionally subcultured. Microvascular endothelium from human organs outside the central nervous system (CNS) has been cultured and characterized (Davison et al. 1983; Folkman et al. 1979).

Since studies performed using any of these types of cells may not accurately reflect features of human cerebral microvascular endothelium or smooth muscle (because of variations related to either species or vascular bed of origin), we have undertaken the isolation, growth, and preliminary characterization of cells obtained from microvessels of human brain in vitro. A portion of this work has been presented in abstract form (Vinters et al. 1985c).

Materials and methods

All cerebral tissue from which isolations were performed was obtained from lobectomy specimens removed at surgery on patients with the clinical diagnosis of intractable seizure disorder. Age range of the patients was 15–54 years (mean 26 years). A total of 16 specimens (7 from males, 9 from females) was obtained and used for preparation of isolates. The final pathologic diagnosis in lobectomy specimens included focal gliosis, cytoarchitectural abnormalities, hippocampal sclerosis and occasional patchy perivenous lymphocyte cuffing. In one case, a clinically unsuspected low grade astrocytoma was found on histologic sections. Fragments of cortex (approximately 1 cm^3) were obtained and immediately placed in a sterile container. The

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tissue was washed with physiological saline and leptomeninges were stripped and discarded. The remaining fragments were washed with Hank's balanced salt solution (HBSS), chopped and minced. The procedure thereafter was identical, with minor modifications, to that described for the isolation of microvessels from weanling mouse forebrain (De Bault et al. 1979). The tissue was homogenized (10 strokes of a loose-fitting dounce homogenizer) in HBSS, the homogenate was filtered through a 210 μm mesh screen in a millipore filter holder, and the vessels rinsed with HBSS. The screen was placed upside down in an empty 60 mm (P60) culture dish (dish A), HBSS was added, and the microvessels agitated free of the screen, which was transferred to a second P60 dish (dish B). The remaining contents of dish A were rehomogenized (4–5 strokes of a tight-fitting dounce homogenizer) and the homogenate filtered through a 153 μm mesh filter, which was then rinsed with HBSS as above. This 153 μm screen was placed upside down in a P60 dish (dish C). Serum-free modified Lewis medium (SFMLM) (DeBault et al. 1979) was added to dishes A–C (3 ml/dish) and they were incubated at 37° C in a CO_2 incubator for 1–2 h, after which time most microvessels had attached to the bottoms of the dishes. At the end of this time, all dishes were treated for 2–3 min with collagenase (1 mg/ml in SFMLM), the meshes removed, and all dishes re-fed with MLM supplemented with 20% or 30% heat-inactivated fetal bovine serum (FBS), penicillin and streptomycin. This first change of medium was left on the preparations for 5–10 days, after which medium was routinely changed twice weekly. Growth of cells from all sizes of microvessels was optimal in the 'B' and 'C' dishes, and both types of cells to be described grew well in these dishes. Virtually all isolates have resulted in outgrowth of cells, though proportions of endothelium and smooth muscle have varied from experiment to experiment. Cells were transferred from primary isolates to subculture plates by use of a cloning ring technique. Cells (of uniform morphology) were isolated by scraping away any 'contaminating' cell types with a sterile rubber policeman, placing a glass cloning ring (dipped in silicone grease) around the desired cells, and replacing the ambient medium with trypsin (0.25% solution with 0.02% ethylenediaminetetraacetic acid, EDTA), then gently agitating contents of the ring with a sterile Pasteur pipette until the cells were roughly in a single cell suspension and could be transferred to another culture dish or flask. Cell counts were performed with a hemocytometer.

Light-microscopic, immunofluorescent, and ultrastructural techniques

Microvessels and emerging cells were routinely observed during isolation and growth on an inverted phase microscope. For demonstration of cytologic and nuclear detail, cells were grown on glass coverslips, fixed in acetone or methanol, and stained with Giemsa stain for 20 min. Immunofluorescent staining of cultured cells for the presence of factor VIII was performed by growing cells on coverslips, fixing with acetone, staining initially with rabbit immunoglobulin to human factor VIII antigen (Dakopatts) as the primary antibody in a 1/10 dilution (37° C overnight), washing with phosphate buffered saline (PBS), then applying an appropriate dilution of fluorescein isothiocyanate (FITC)-labeled swine anti-rabbit immunoglobulin (Dakopatts) for 30 min as the secondary antibody. Alternatively, an FITC-conjugated anti-human factor VIII antibody was used in a one-step procedure. Staining for the presence of endothelial-specific lectin receptors was carried out by first fixing cells on coverslips (30 min) using methanol, washing with PBS (2 changes of 8 min), soaking coverslips in 20% FBS in 0.05% Tris for 30 min, then incubating them at 37° C with Ulex europaeus-I lectin (UEA-1) conjugated to FITC (1 h or overnight, 100 μl/coverslip), followed by 2 changes of 10 min washes with PBS. Controls included coverslips incubated under identical conditions, but with 0.2 M fucose (final concentration) added to the UEA-1 as a lectin blocker.

Ultrastructural studies of cells were carried out after either: (a) growing cells to a confluent monolayer in P60 plastic dishes, scraping the cells with a rubber policeman, fixing with 2.5% glutaraldehyde, pelleting them by centrifugation (1000 RPM × 10 min) and processing the cell block; or (b) growing the cells as a monolayer on Lux thermanox tissue culture coverslips (in Nunclon multi-plates), fixing them with 2.5% glutaraldehyde, and processing portions of the monolayer in situ on the coverslips or in plastic dishes. Post-fixation (with 2% OsO_4), followed by dehydration and embedding in Spurr medium was done by established methods (Hunter 1984). Ultrathin sections stained with uranyl acetate and lead citrate were examined. Assessment of uptake of acetylated-low density lipoprotein (Ac-LDL) by cultured cells was done on HBE, mouse-brain endothelium, and human umbilical vein endothelial cells (supplied by Dr. J.A. Berliner) as described by Voyta et al. (1984).

Biochemical studies of 'smooth muscle'

Cells with the morphologic and growth characteristics of smooth muscle (see below) were incubated for 8 h at 37° C with methionine-free Dulbecco's modified Eagle medium (DMEM) containing 10% FBS and 120 μCi/ml of L-[^{35}S]-methionine. Isoelectric focusing (IEF) of cell proteins on polyacrylamide tube gels was carried out as previously described by Rubenstein and Spudich (1977). Selected gels were electrophoresed in a second dimension on sodium dodecyl sulfate (SDS), 10% polyacrylamide slab gels. Autoradiograms were made of both IEF and SDS gels.

Further studies of 'endothelium'

Effects of astrocyte-conditioned media on γ-glutamyl transpeptidase (γ-GTP) synthesis were tested using the method of Maxwell et al. (1986). Primary isolates of mouse-brain astrocytes (provided by Dr. K. Maxwell) were initially isolated according to the method of McCarthy and DeVellis (1980) and maintained in Ham's F12/DMEM media supplemented with 10% FBS, 15 mM HEPES, and antibiotics as described (Maxwell et al. 1986). Prior to conditioning, cultures of astrocytes were washed with sterile PBS. Media to be conditioned was added to cultures (in T-75 culture flasks) for 72 h, after which it was harvested and centrifuged (1400 × g for 10 min) to remove particulate debris, then stored (when necessary) at −20° C until use. Effects of astrocyte-conditioned media (astro-CM) on endothelium were tested by adding these media (or control nonconditioned F12/DMEM media) to confluent endothelial monolayers for 72 h, then determining levels of cellular (endothelial)

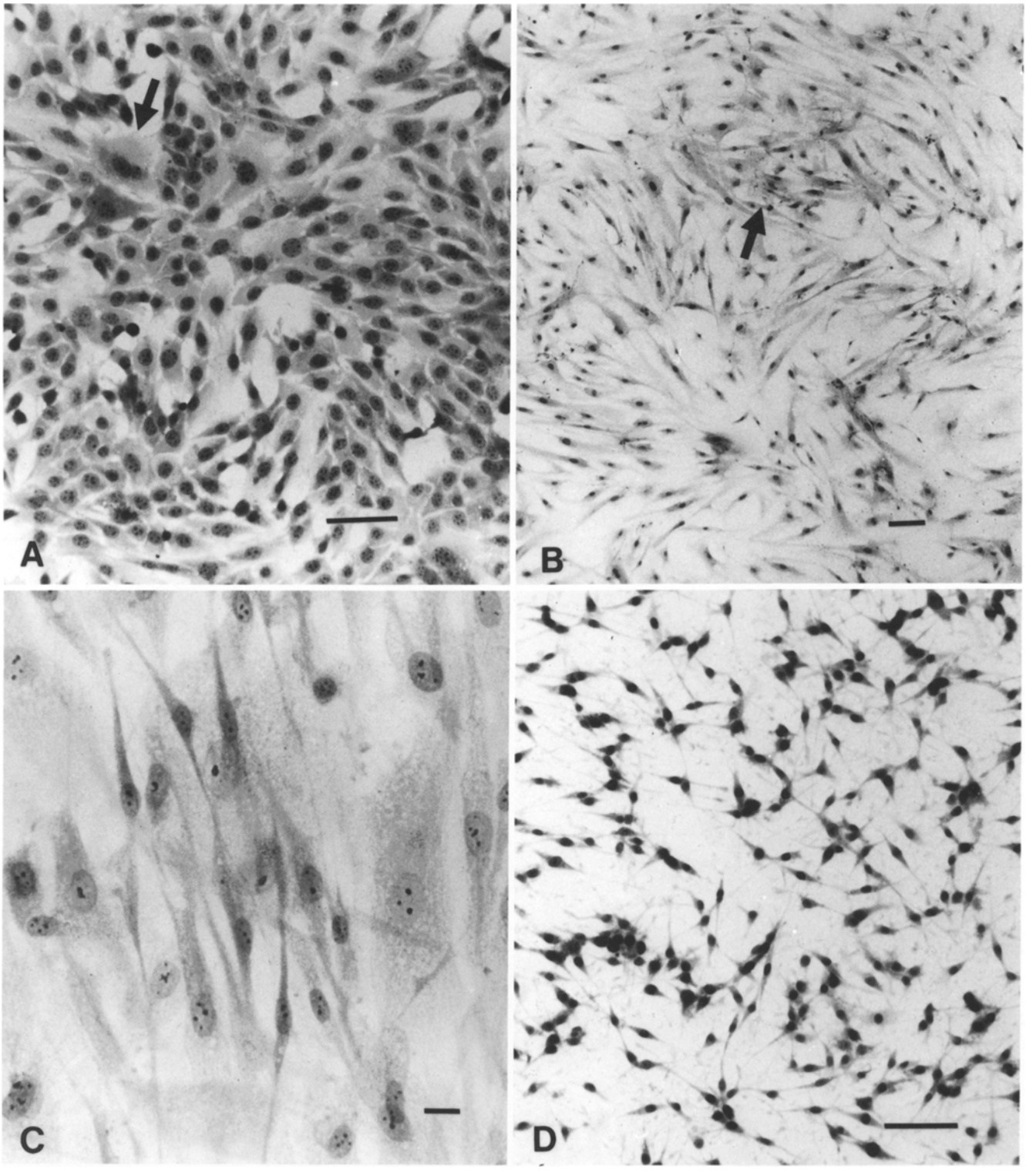


Fig. 1 A–D. Morphology of cell types isolated from human brain cortical microvessels. **A** Giemsa-stained preparation of subconfluent human microvascular cells, tentatively identified as endothelium, after subculture from primary microvessel isolate. Note polygonal (epithelioid) morphology and well-defined cell borders. Occasional multinucleate forms (*arrow*) are seen ×120 Bar = 100 μm. **B, C.** Similar preparation of human brain smooth muscle shows elongated cells with tapering processes, with a tendency to overgrow, even at relatively low cell densities, into a hill-and-valley pattern (*arrow*) (B ×30, Bar = 200 μm; C ×300, Bar = 20 μm. **D** Rat C6 glioma cells prepared and stained under identical conditions show stellate appearance of cells, distinct from both endothelium and smooth muscle. ×120. Bar = 100 μm

γ-GTP as described (Maxwell et al. 1986). Values were expressed per mg cell protein in the monolayer, the latter determined by the method of Lowry et al. (1951). Effects of astro-CM were simultaneously tested on rabbit aortic endothelial cells (provided by Dr. J.A. Berliner) initially maintained in DMEM with 10% FBS on dishes coated with 0.1% gelatin and 5 μg/ml human fibronectin.

Testing for a possible mitogenic effect of insulin on endothelium was carried out using a thymidine incorporation assay identical to that previously described (Vinters et al. 1985b) with mouse brain endothelium and smooth muscle.

All immunocytochemical, biochemical, and physiological tests on cells were carried out on pure subcultures, usually below the tenth passage.

All biochemicals were purchased from Sigma (St. Louis, MO) or other commercial suppliers. Tissue culture supplies, media, and sera were obtained from GIBCO (Grand Island, NY). UEA-1 was from Polysciences (Warrington, PA). L-[^{35}S]-methionine was purchased from Amersham, Arlington Heights, IL.

Results

Using the isolation technique as described, relatively pure preparations of microvessels were consistently obtained. The population of microvessels included vessels of capillary size and a mixture of venules and arterioles. The segments observed corresponded closely to those identified when a similar procedure was applied to forebrain of mouse (De Bault et al. 1979). Occasional amorphous fragments of tissue, probably representing clusters of glial and neuronal origin, were seen, but did not survive in vitro for longer than 2–3 days. The purest preparations of microvessels were present in dishes B and C of each isolation. Outgrowth of cells from vessels was noted within 7–10 days. Often, this took the form of plaques of cells. Cells with one of two primary morphologic types were identified, although mixed cytologic populations were seen frequently. However, groups of cells with uniform appearance could be isolated successfully, trypsinized (using the cloning ring technique), transferred into T25 culture flasks, and subsequently passed serially in T75 culture flasks.

One of the cell-types thus isolated (Fig. 1A) consisted of polygonal or epithelioid cells with well-defined cell margins and prominent centrally-placed oval nuclei containing multiple (usually 2–6) nucleoli. Occasional large multinucleate forms of cells were seen. Although, when sparse in a culture flask or plate, cells put out short processes, these processes were no longer visible after cells had grown to a near confluent state. At confluence, cells exhibited the characteristic contact-inhibited "cobblestone" morphology seen in cultured endothelia isolated form other species and sources (De Bault et al. 1981; Folkman et al. 1979; Maciag et al. 1981; Gimbrone et al. 1974). Furthermore, growth curves for this cell-type (tentatively designated as endothelium of human brain) showed that when cells were initially plated at low density, they rapidly (within one week) grew to confluence, even with replenishment of fresh medium on every second or third day in culture (Fig. 2). The cells have been grown in MLM supplemented with 20% FBS, penicillin and streptomycin, i.e. the medium used in the initial microvessel isolation procedure, without defined extracellular matrices. The endothelial cells have been routinely passaged once or twice weekly (1:2 split ratio) for up

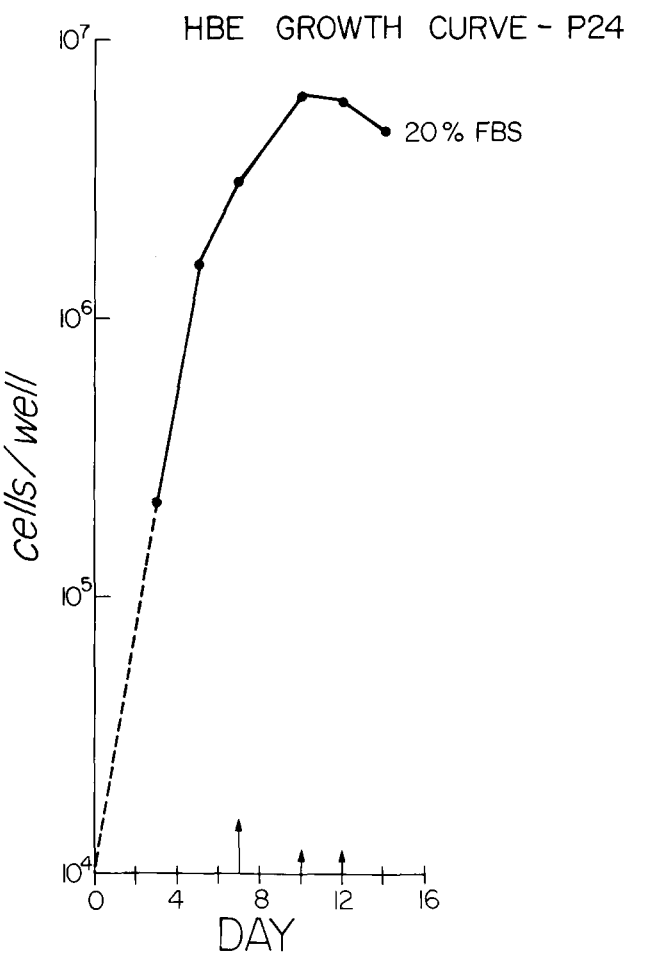


Fig. 2. Growth curve for human brain endothelial cells, passage 24. Cells were trypsinized from confluent T75 flasks and seeded at 10^4 cells/P60 culture dish (day 0). Cell counts were made (after trypsinizing cells) every 2–3 days. Complete medium replenished (↑) until cells counted

to 25–30 passages. Around this passage number, the cells spontaneously transformed as judged by simple phenotypic criteria, i.e. cells lost contact inhibition, piled up and overgrew in the "hill-and-valley" pattern more characteristic of smooth muscle.

Endothelial cells have shown weak or moderate positivity with a fluorescent antibody technique designed to show the presence of factor VIII antigen. Simultaneously-stained human umbilical vein endothelium (HUVE) showed bright granular cytoplasmic positivity. Moderate to strong staining for *Ulex europaeus* surface lectin (UEA-1) was found (Fig. 3), although the intensity of staining varied from culture to culture. Positive staining of the other cell-type isolated (see below) was never observed. Cultured cells showed insignificant uptake of Ac-LDL, whereas appropriate control cells (HUVE) were positive for this marker. Ultrastructural features of endothelial cells (Fig. 4) included: multiple thin cytoplasmic projections; closely apposed cytoplasmic membranes; abundant intracytoplasmic mitochondria and rough surfaced endoplasmic reticulum; and scattered membranous cytoplasmic and multivesicular bodies. Pinocytotic vesicles were sparse near the cell membrane. Although a fine filamentous and microtubular meshwork was identified within the cytoplasmic matrix, well-organized clusters of

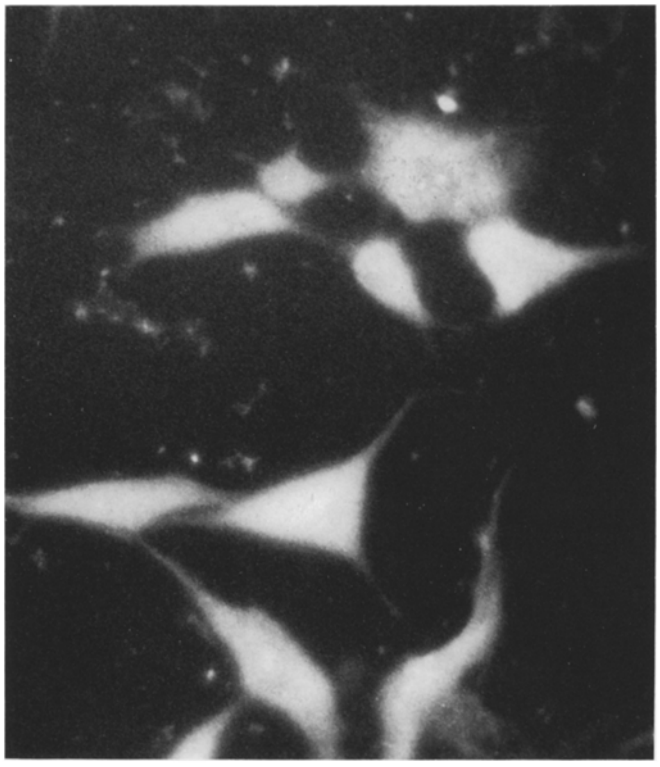


Fig. 3. Immunofluorescent staining of endothelial cells (passage 9) with *Ulex europaeus*-1 lectin (UEA-1) conjugated to fluorescein isothiocyanate (*see methods*). Co-incubation with fucose effectively blocked the staining. ×450

Table 1. Induction of γ-GTP in endothelial cells by astrocyte-conditioned media

Cell type	Media	Units γ-GTP/mg cell protein[1]
HBE	control	166.8
	astro-CM	233.6[2]
RAE	control	388.8
	astro-CM	324.4[3]

[1] Values represent mean of duplicate determinations HBE = human brain endothelium; RAE = rabbit aortic endothelium; astro-CM = astrocyte-conditioned media
[2] Significant ($p < 0.05$) by Student's *t*-test, astro-CM vs. control
[3] Not significant by Student's *t*-test, astro-CM vs. control

Table 2. Effect of insulin on [^{3}H]-thymidine incorporation into endothelial cells

Insulin concentration (ng/ml)	[^{3}H]-thymidine incorporation (CPM)	Ratio $\left[\frac{\text{CPM insulin}}{\text{CPM control}}\right]$
10	824 ± 28	4.4
control	187 ± 33	
100	2361 ± 304	11.1
control	212 ± 49	
100	4696 ± 1137	21.7
control	217 ± 29	
10000	1431 ± 136	6.7
control	215 ± 18	

Human brain endothelial cells were seeded at low density (approximately 10^5 per P35 well), washed and placed on SFM while still subconfluent in order to arrest growth. After 24–48 h, bovine pancreas insulin was added at the stated concentrations. After a further 24 h, cells were pulsed for 6 h with 0.5 μCi/well [Methyl-^{3}H]-thymidine (New England Nuclear, 6.7 Ci/mmol) and trichloroacetic acid precipitable ^{3}H-thymidine incorporation was measured after appropriate PBS washes

fibrils or filaments were not found. Tightly-apposed cell membranes were present (Fig. 4B), but pentalaminar tight junctions characteristic of cerebral mv endothelium (i.e. BBB) in vivo (Bradbury 1984; Reese and Karnovsky 1967) were not seen. Desmosome-like intercellular junctions were noted (Fig. 4A). Typical cytoplasmic Weibel-Palade bodies (Weibel and Palade 1964) were not identified, though occasional membrane-bounded rod-like bodies with a dense matrix were seen (Fig. 5).

Pure cultures of endothelial cells showed two other physiological responses highly characteristic of brain endothelium, i.e. significant stimulation of γ-GTP levels when exposed to astrocyte-conditioned media (Table 1) and a prominent stimulation of DNA synthesis when exposed to insulin (Table 2). γ-GTP levels in the cells increased by 40% above control, whereas a large-vessel endothelium from rabbit aorta actually showed slight diminution of γ-GTP levels after exposure to astro-CM.

The second major type of cell identified (Fig. 1B, C) showed predominantly spindle-cell morphology, with long tapering processes emanating from the cell bodies and generally indistinct cytoplasmic borders. Elongated or oval nuclei contained 1–4 nucleoli. The cells grew over one another and cellular processes frequently overlapped, leading to a "hill-and-valley" pattern of growth at high cell density, even at early subculture passage. Ultrastructurally the cells showed the following (Fig. 4): prominent rough endoplasmic reticulum; abundant pinocytotic vesicles at the cell membrane; patchy extracellular deposition of electron-dense basement membrane material; occasional 'coated pit' formations indenting the cell membrane; and bundles of thin filaments with associated dense bodies beneath the plasmalemma. These cells (hereafter designated as smooth muscle), grew more slowly than endothelium, and routinely were trypsinized and passed once each week or two weeks. A slow rate of growth for cerebral smooth muscle, especially at early passage, has also been observed in preparations from mouse brain (Moore et al. 1984). The cells were cultivated in Dulbecco's modified Eagle medium (DMEM) with 10% heat inactivated FBS (Moore et al. 1984). Cultures were retained for as many as 15–20 passages, although criteria for cellular transformation in this cell-type were more difficult to ascertain than for endothelium. Isoelectric focusing (IEF) of cellular proteins revealed three bands (Fig. 6) migrating in the region of the IEF gel pH gradient where actins are known to focus (Garrels and Gibson 1976; Rubenstein and Spudich 1977). These bands comprised the major protein product of the cells and correspond to α, β and γ-isoactins in order of decreasing acidity. These three putative actin components migrated together in the 43000 dalton region on SDS gel electrophoresis, as would be expected for any of the isoactins. Vimentin intermediate filament protein and its three characteristic breakdown products were also identified (Fig. 6).

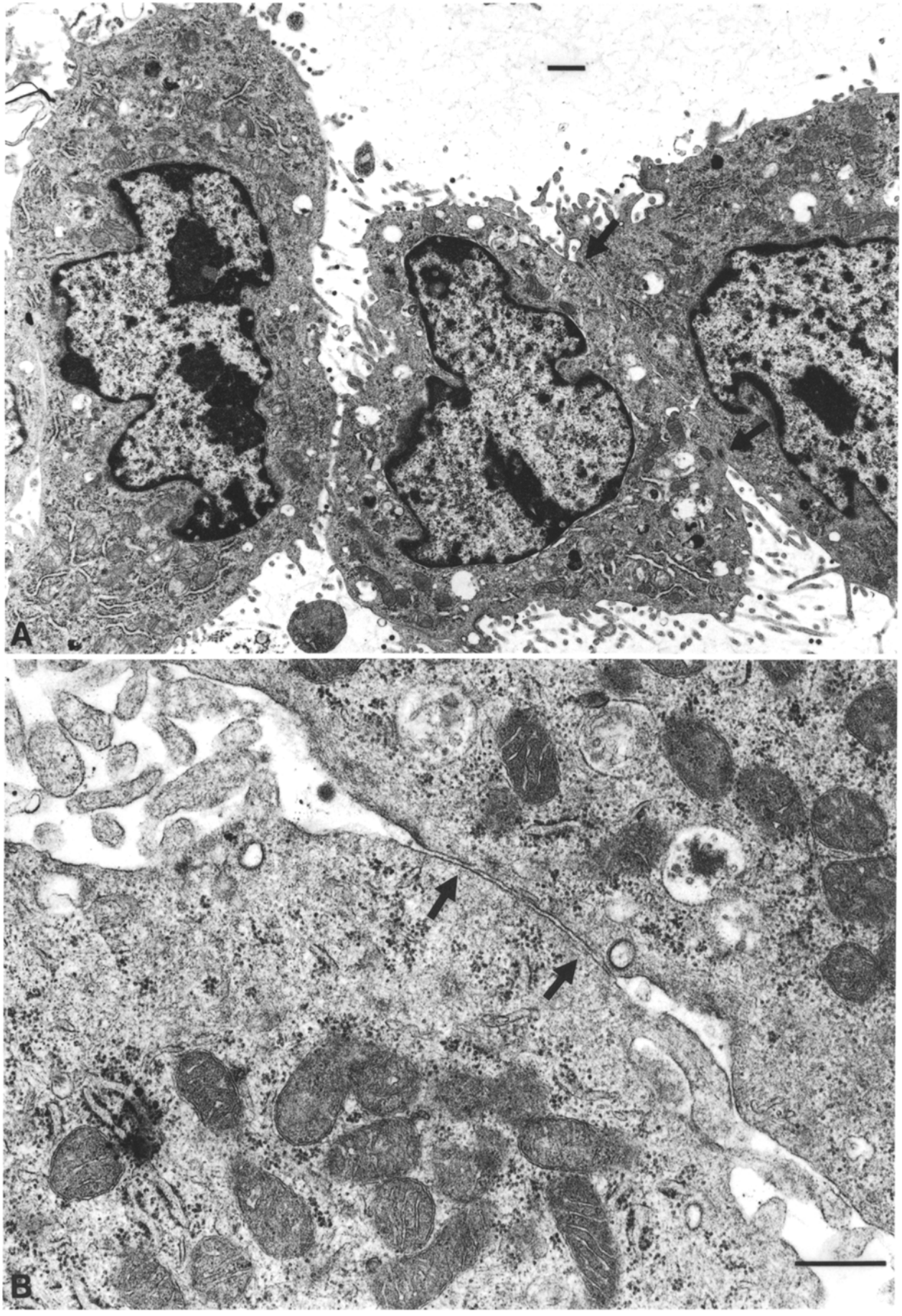
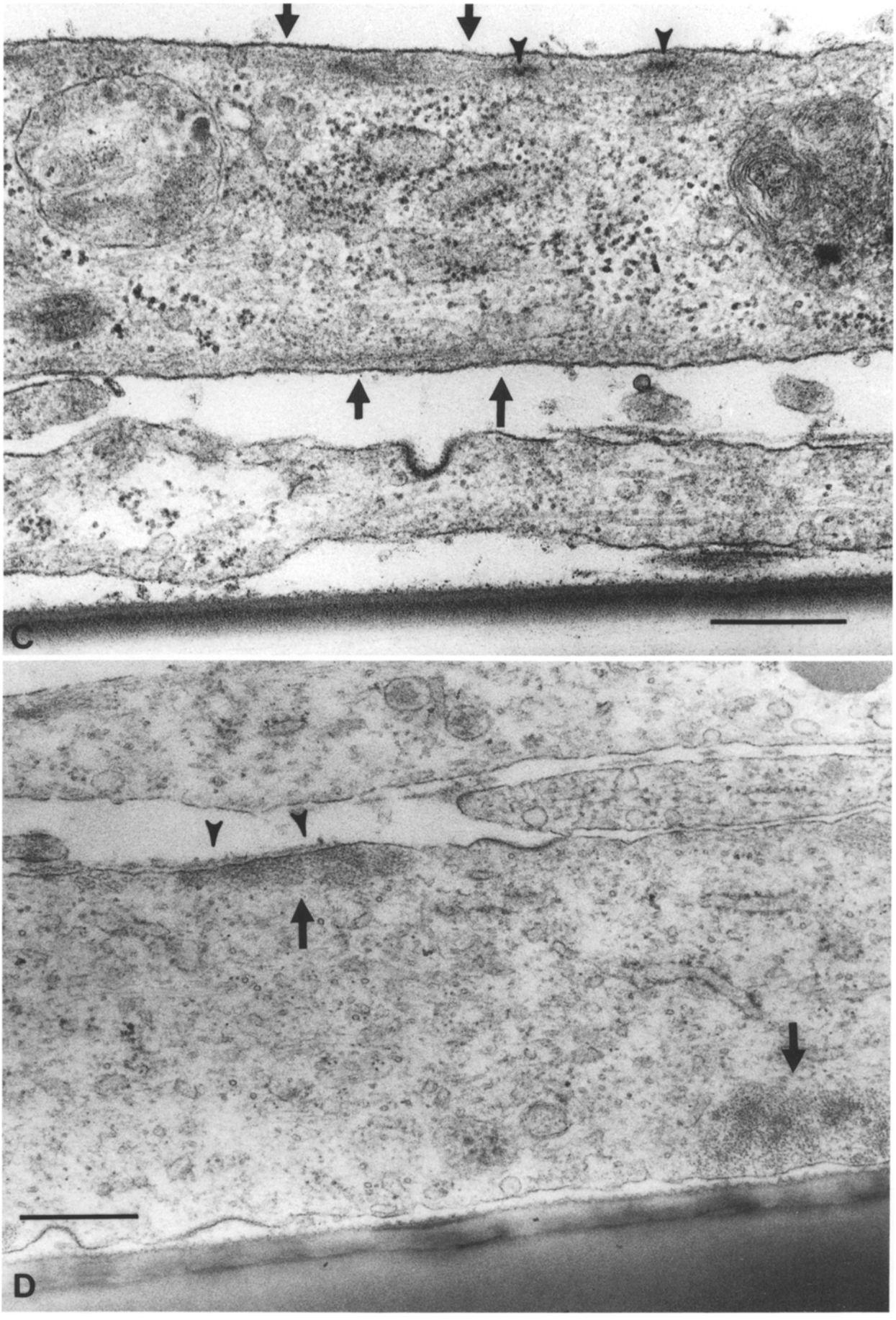


Fig. 4 A–D. Ultrastructure of cells subcultured from human brain microvessels. Endothelial cells (**A, B**) show cytoplasmic projections, abundant mitochondria and rough endoplasmic reticulum. Desmosome-like junctions are seen between cell membranes (*arrows in* **A**) and elsewhere membranes are closely apposed (*arrows in* **B**). Smooth muscle (**C–D**) shows overlapping cytoplasmic processes of variable thickness, prominent rough endoplasmic reticulum and pinocytotic activity, clusters of thin filaments cut in longitudinal (*arrows in*



C) or cross-section (*arrows in* **D**), some with interspersed dense bodies (*arrowheads in* **C**). A coated pit formation is noted in **C**, and scattered deposits of electron-dense extracellular material are present, as in **D** (*arrowheads*) between the cell processes or between processes and underlying plastic. A ×6600, B ×32600, C ×49400, D ×43000. Bars: A = 1.0 μm, B–D = 0.5 μm

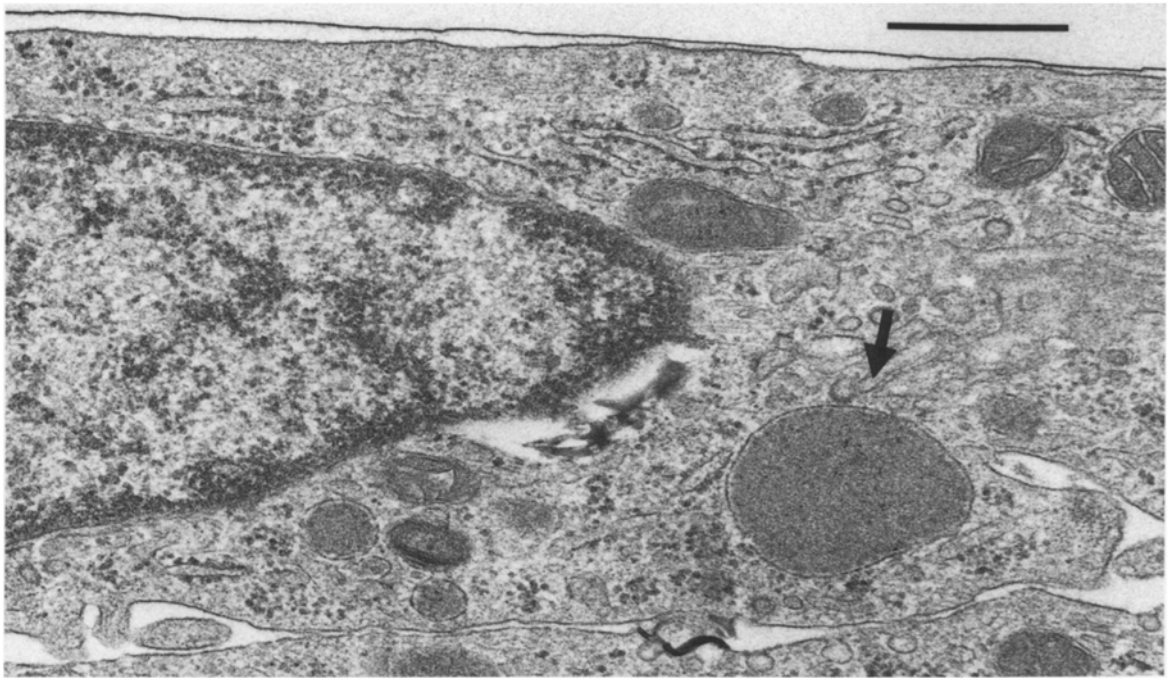


Fig. 5. Cytoplasm of endothelial cell shows elongated membrane-bounded inclusion that resembles Weibel-Palade body (*arrow*) ×47000 Bar = 0.5 μm

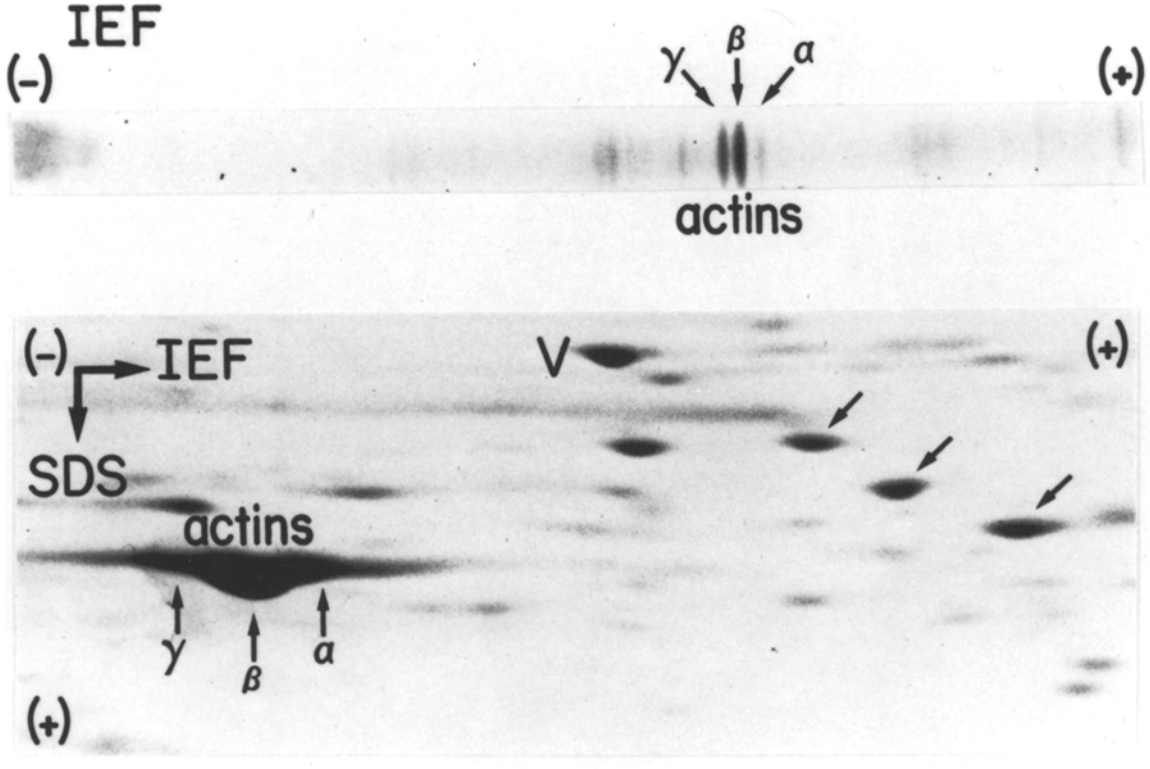


Fig. 6. Actin analysis of human brain smooth muscle cells by IEF and SDS-PAGE autoradiography revealed active production of three separate isoactins [α, β, and γ (most acidic to most basic)] which migrate in the 43 kilodalton range. Vimentin (*V*) and its degradation products (*arrows*) are marked for comparative purposes on the SDS-PAGE autoradiogram

Discussion

In this paper, we report the isolation, culture, and serial passage of cells derived from human cerebral (cortical) microvessels. Brief anecdotal mention of the achievement has appeared in other reports (Krause et al. 1983; Goetz et al. 1985). The method used to isolate the microvessels is similar to that used in preparing mouse forebrain microvessels (De Bault et al. 1979, 1981; De Bault and Cancilla 1980a; De Bault 1982). The two resultant cell-types show morphologic

similarity to cerebral endothelium and smooth muscle of mouse. The type of cell we have designated as endothelium has in vitro growth properties and cobblestone morphology in subculture (Striker et al. 1980) that suggest endothelial origin. The cells in serial passage express small amounts of factor VIII antigen. Cerebral endothelium of mouse (De Bault et al. 1981) fails to show factor VIII surface antigen in serial subculture, though the antigen is present in vivo, during cell outgrowth from mv's, and when endothelial cells proliferate into a denuded area in vitro (De Bault and Cancilla 1980a). These same cells demonstrate many other endothelial and BBB-specific features (DeBault and Cancilla 1980b). Clearly, however, antibodies to factor VIII appear to label virtually all types of large vessel endothelium and many microvascular endothelia, even those derived from brain (Striker et al. 1980; Goetz et al. 1985; Goldstein et al. 1984). However, recent data indicate that factor VIII mRNA *cannot* be detected in cultured endothelial cells, but appears to be localized in hepatocytes, suggesting the latter as the site of factor VIII antigen synthesis (Wion et al. 1985; Zelechowska et al. 1985).

Recently, the claim has been made that the *Ulex europaeus* I lectin may be a more sensitive endothelial marker than factor VIII (Miettinen et al. 1983; Hormia et al. 1983), especially within angiogenic tumors (Böhling et al. 1983) and the vasculature of primary and metastatic brain tumors (Weber et al. 1985). This marker is particularly sensitive for the demonstration of microvascular endothelium (Weber et al. 1985). It is not a marker specific for endothelium (Sato et al. 1984; Lee et al. 1983). Of blood vessel-derived cells, however, endothelium is the only one that has binding sites for UEA-1. Cultured human brain endothelial cells are effectively labeled with this marker. Ultrastructural studies have demonstrated features of the endothelium that are consistent with endothelial origin, yet in themselves relatively non-specific. Pentalaminar tight junctions between cells were not apparent, though desmosome-like junctional complexes and close apposition of cell membranes were identified. Structures resembling Weibel-Palade bodies were present in the cytoplasm of a few cells, although the presence of these structures has not been observed in all vascular beds, even in vivo (Weibel and Palade 1964; De Bault and Cancilla 1980a). Weibel-Palade bodies were absent from cultured bovine retinal capillary endothelium (Buzney et al. 1983), functionally analogous to endothelium of brain.

The physiological responses of the cells to astrocyte-conditioned media and insulin are highly characteristic of cultured brain endothelium (Maxwell et al. 1986; Vinters et al. 1985b). These studies highlight the importance of physiological, rather than purely immunohistochemical, markers for highly specialized microvascular endothelia. The effect of astrocyte-conditioned media on γ-GTP levels is of further interest in that it demonstrates a cross-species phenomenon, i.e. astrocytes from neonatal *mouse* brain were utilized in the media conditioning procedure.

In view of the observation that the cells designated as human brain endothelium grew from fragments of microvessels and were distinguished from smooth muscle elements, the only other cell type they may represent is the pericyte. Though pericytes have been grown in culture after isolation from bovine retinal capillaries (Buzney et al. 1983) the identification of the cells was based on the absence of endothelium-specific markers, and differential growth characteristics with respect to endothelium (e.g. in response to tumor-conditioned medium). Pericytes in extracerebral sites contain contractile- and other proteins essential for the contractile process, i.e. they may be closely related to vascular smooth muscle and appear to possess microvascular contractile functions (Herman and D'Amore 1985; Joyce et al. 1984, 1985a, b).

The absence of Ac-LDL uptake by HBE cells again distinguishes these cells from endothelia of large and small vessels found elsewhere (Voyta et al. 1984). Well-characterized endothelium from mouse brain (De Bault 1982) has also consistently shown absence of Ac-LDL uptake in an assay that demonstrated prominent positivity of umbilical vein endothelium (J.A. Berliner, unpublished observations). Human brain endothelial cells grew well without the presence of a defined extracellular matrix. This has been observed in explanted bovine cerebral capillary endothelium (Goetz et al. 1985).

The other cell type described has light microscopic, ultrastructural, and biochemical characteristics of smooth muscle. The exact electron microscopic appearance of smooth muscle from various sources in vitro can vary significantly, depending on the time interval between explantation and examination, state of modulation or 'dedifferentiation' of the cells, plating density, and stage of growth of the cultures (Chamley-Campbell et al. 1979). However, the presence of bundles of thin myofilaments with associated dense bodies, abundant pinocytotic activity, and electron-dense extracellular material are seen in smooth muscle isolated and subcultured from mouse brain and umbilical cord vessels (Moore et al. 1984; Gimbrone and Cotran 1975). Furthermore, these cells actively produce α-isoactin, one of the actin isoforms made solely by muscle cells (Vandekerckhove and Weber 1978, 1981). Since no skeletal, cardiac or visceral smooth muscle would be expected in these cultures from brain microvessels, the α-isoactin identified, and thus these tissue culture cells, must be of vascular smooth muscle origin.

The capability of isolating and cultivating microvascular wall component-cells from brain that itself shows minimal structural abnormality provides several unique opportunities for further investigation. Although samples of relatively uninvolved brain resected at the time of tumor surgery might be used for a similar isolation procedure, one questions whether normal microvessels are being harvested in that situation, given the propensity of many types of (primary) brain tumor to infiltrate brain parenchyma and produce significant microvascular proliferation in adjacent cerebral tissue (Rubinstein 1972). The ability to culture human microvascular endothelium might allow study of some human BBB properties in vitro, by the use of techniques that have been successfully applied to cultured cerebral endothelium from other species (Beck et al. 1984; Bowman et al. 1983; Goldstein et al. 1984; Goldstein and Betz 1983). Though there is no reason a priori to suspect that these would vary among mammalian species, one assumes that cultured human endothelium would, even after isolation from other brain elements, more accurately reproduce the in vivo situation in man. The fact that the brain endothelial cells described are derived from segments of capillaries, venules, and arterioles is of minor concern, since arterioles in vivo clearly have significant barrier properties (Petito and Levy 1980).

The ability to grow these cells with relative ease also suggests that insights into atherosclerotic disease, recently gained from experimental manipulation of endothelium and

smooth muscle derived initially from large vessels, may soon be paralleled by similar work with microvessel-derived cell cultures (Schwartz 1984; Weinstein et al. 1981). Microvascular endothelia differ significantly from their large vascular counterparts in other species (Zetter 1981; Vinters et al. 1985b; Berliner et al. 1983). Growth factors secreted by endothelium of large vessels (Gajdusek and Schwartz 1982; DiCorleto et al. 1983) may have counterparts in microvascular endothelium. Culture of human brain microvascular cells may lead to insights into microangiopathies that are found exclusively or predominantly in human brain (Vinters and Gilbert 1983; Monteiro et al. 1985). Whether factors that initiate these microvascular lesions include endothelial injury and/or an abnormal response to growth stimulants is currently not known, but using an in vitro assay system with cells derived from human brain, these and related hypotheses are amenable to experimental investigation.

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