displayed a relative paucity of pericytes. Retinal neovascularization no doubt reflects the interplay of multiple local and systemic processes; initial attempts to understand these phenomena must begin with a basic understanding of the biochemistry and growth characteristics of retinal vascular cells. Our tissue culture model and that of Frank et al. may be quite useful toward achieving this goal.

Using our present culture conditions, we regularly observed endothelial cell growth from fetal calf eyes but rarely from adult eyes; pericyte and smooth muscle cells grew easily from young and mature tissues. Accordingly, consideration must be given to conditions and agents capable of stimulating endothelial growth from adult retinal vessels in vitro and the implications such means may have in understanding retinal vascular pathophysiology.

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In vitro proliferation of endothelial cells from kitten retinal capillaries. ROBERT N. FRANK, V. EVERETT KINSEY,† KARIN W. FRANK, KEVIN P. MIKUS AND ANN RANDOLPH.

When microvessel (predominantly capillary) fragments freshly isolated from the retinas of young kittens are in-

cubated in tissue culture medium, we observe the slow proliferation in various diseases and of factors that mod-
ify cell junctions and capillary permeability in the retina. Since we have previously demonstrated proliferation in culture of intramural pericytes ("mural cells") from reti-
nal capillaries, it should now be possible to carry out comparative studies of biochemical and functional prop-
erties of retinal capillary pericytes and endothelial cells.

Vascular endothelial cells are known to perform a wide range of functions. These cells limit the permeability of the blood vessel wall, synthesize a number of substances including basement mem-
brane, factor VIII, and prostaglandins; play an active role in hemostasis, and provide a smooth surface for the vascular lumen that permits blood to flow without turbulence. The endothelial cells of large and small vessels in many organs including the retina divide infrequently under normal cir-
cumstances, but their ability to replicate may in-
crease substantially in certain physiologic condi-
tions and as a result of injury or disease. Vascular endothelial cells from various locations have im-
portant anatomic and functional differences. For ex-
ample, by contrast with capillaries from many organs, the capillaries of the brain and retina are normally impermeable to most ionic and molecu-
lar species, a feature that has been called the "blood-brain barrier" or "blood-retinal barrier". One probable anatomic correlate of this behavior is the extensive system of zonulae occludentes, or tight junctions, that join endothelial cells in retinal and cerebral vessels but are less extensive in other vascular endothelia.

Because many features of endothelial cell func-
tion are still poorly understood, and because ab-
normalities of these cells are prominent in several human diseases, there is considerable value in having preparations of isolated endothelial cells for studies of their biochemistry, growth potential, and physiologic function. Recently, populations of well-differentiated endothelial cells from large vessels such as human umbilical vein and bovine aorta have been grown in culture. Culture of capillary endothelial cells from vessels of the cen-
tral nervous system has not yet been accom-
plished, although Buzney et al. were able to
obtain in vitro proliferation of "mural cells" (in-
tramural pericytes) from bovine and rhesus mon-
key retinal capillaries. We now report the growth in culture of colonies of endothelial cells derived from the retinal capillaries of young kittens. These cultures should prove useful for studying biochemical and functional properties that may be unique to endothelial cells from capillaries of the retina as opposed to endothelial cells from larger vessels, and from sources outside the central nervous system.

**Methods.** Kittens 3 to 4 weeks of age were em-
ployed for these experiments. Animals were sacri-
ficed by decapitation, the eyes were rapidly re-
moved, and retinal microvessel fragments (pre-
dominantly capillaries) were isolated under sterile conditions with slight modifications of methods described previously. Because eyes from small numbers of kittens (one to six) were used for each isolation and because the retinas are small, we used only a single homogenization step, consisting of 10 strokes of a Teflon pestle in a hand-held glass homogenizer (Arthur H. Thomas Co., Philadel-
phia, Pa.), to fragment the tissue. This minimizes loss of material, although it increases the propor-
tion of noncapillary material in the final vessel iso-
late. Homogenization was followed by a single fil-
tration step in which the suspension was decanted onto a 210 \( \mu \)m nylon mesh filter mounted on a metal ring supported on a ring stand. The filtrate passed onto an 85 \( \mu \)m mesh filter mounted with a rubber band on a beaker. The material collected on the 85 \( \mu \)m filter was washed into a fresh recep-
tacle, centrifuged, and cultured as described pre-
viously. More recently, we have substituted 53 \( \mu \)m mesh filters for the 85 \( \mu \)m mesh with im-
proved yields, presumably because the finer filters retain smaller vessel fragments that are produced during homogenization of these immature vessels. Cells were grown in Ham’s F-12 medium contain-
ing 2 mM glutamine, with the addition of 10% fetal calf serum and antibiotics (penicillin 150 U/ml and streptomycin 150 \( \mu \)g/ml). Medium, serum, and antibiotics were purchased from Grand Island Biological Co., Grand Island, N. Y. Culture vessels were 35 mm diameter plastic Petri dishes (Falcon Plastics, Oxnard, Calif.). The cul-
ture medium was changed twice weekly. We have found no apparent difference in retinal capillary pericyte or endothelial cell proliferation and sur-
vival when other enriched media (e.g., TC-199, Waymouth’s) are substituted for F-12, although detailed quantitative studies of this point have not been conducted.

For electron microscopy the cells were fixed in situ in the culture dishes using 2.5% glutaralde-
hyde buffered with 0.1M sodium cacodylate, pH
Fig. 1. A, Kitten retinal capillary endothelial cells following 1 month in culture. (Phase contrast, ×200.) B, Calf retinal capillary pericytes following 2 weeks in culture. (Phase contrast, ×200.)

Fig. 2. Electron micrograph of portions of two kitten retinal capillary endothelial cells overlapping one another and joined by an extensive zonula occludens (tight junction). The cytoplasm shows ribosomal particles, rough endoplasmic reticulum, and a single endocytic vacuole (arrow). (×86,000.) The inset shows a high magnification view of a tight junction from these cultured endothelial cells and demonstrates fusion of the outer leaflets of the adjoining plasma membranes. (×261,000.)

7.3, for 1 hr at 20° C. They were then post fixed with 1% OsO₄, for 1 hr at 20° C, "block" stained with 0.1% aqueous uranyl acetate for 30 min, dehydrated in graded ethanols, and embedded in Epon. Thin sections were stained on the grids with uranyl acetate and lead citrate and viewed with a Philips 301 electron microscope.

Results. Cells with morphologic characteristics different from those of the intramural pericytes described earlier were observed to proliferate extremely slowly from capillary fragments that had become adherent to the floor of the culture vessel. One month after isolation of the microvessels, these cells had only filled an area approximately 3 to 4 medium-power (200×) microscope fields in diameter in a 35 mm Petri plate. Several features of these cells strongly suggested that they derived from capillary endothelium. The proliferating cells were polygonal, although not regularly hexagonal, and they formed a mosaic-like array with all of their sides closely apposed to neighboring cells (Fig. 1A). In contrast, pericytes cultured from calf retinas were more irregular in shape, sometimes with protruding cytoplasmic processes, and they formed a monolayer in which neighboring cells did not form a closely packed array, but may have had spaces between them or they may have partially overlapped one another (Fig. 1, B). Although cells with this "pericyte" growth pattern were the predominant elements that we observed in our cul-
Fig. 3. A portion of another kitten endothelial cell culture showing a tonguelike protrusion from one cell extending above and joined to the cell underneath by a tight junction. (x118,000.)

Electron microscopic examination of cultured kitten retinal microvessel cells revealed less abundant and varied cytoplasmic organelles than we had observed in pericytes growing in vitro. There were moderate numbers of free ribosomes and a well-developed rough endoplasmic reticulum, but only occasional microvilli, vesicles, and lysosomes. These cells were distinguished by the presence of lengthy intercellular junctions, which, with high magnification (Figs. 2 and 3) were seen to be pentalamainar with fusion of the outer leaflets of the adjoining plasma membranes. We observed no other types of junctions in these cultured cells. Although intercellular junctions have been described in human umbilical vein endothelial cells in culture, they appeared to be desmosome-like contacts, quite unlike the "tight" junctions that we report here.

The mosaic-like growth pattern and the presence of extensive tight junctions was strong evidence that the cultured cells derived from capillary endothelium. We have not observed "specific endothelial organelles" (Weibel-Palade bodies), which are claimed to be characteristic of vascular endothelium. These organelles have been observed infrequently in capillary endothelium and, with the exception of one report, they have not been described in the endothelial cells of ocular blood vessels, including those in the retina.

In work that is now in progress, we are beginning to investigate other endothelial cell "markers" in these cultures. Although the data are still preliminary, presumed retinal capillary endothelial cells in culture appear to possess angiotensin-converting enzyme activity. The specific activity is less than that in cultures of aortic endothelium, but definitely more than that present in pericyte cultures, which demonstrated no detectable angiotensin-converting enzyme activity. Localization of factor VIII by immunofluorescence is unlikely to be satisfactory in these cultures since an antibody to feline factor VIII has not been prepared and, in an experiment performed...
with Dr. Barry S. Coller of the Division of Hematology, State University of New York at Stony Brook, factor VIII in adult cat plasma did not cross-react with rabbit anti-human factor VIII antibody.

Discussion. Both our own results and those of Buzney and Massicotte13 in the accompanying report demonstrate that endothelial cells proliferate from retinal capillaries whose component cells are relatively immature. In its state of maturation, the vascular system of the kitten retina at birth resembles that of the human fetus early in the third trimester of pregnancy.14-16 The retinal vessels have not yet extended all the way to the ora serrata, and, although some of the capillary cells in the posterior pole of the eye have differentiated into endothelial cells and intramural pericytes, many of the smallest vessels are composed of cords of undifferentiated cells.16 Growth of the retinal vessels toward the periphery of the retina and differentiation of the primitive capillary cells into mature forms occurs over the first few months of life. Our failure to obtain proliferation of well-differentiated endothelial cells from microvessel fragments from the retinas of older animals of several species (calves, lambs, and adult dogs and rhesus monkeys) suggests that the endothelial cells of these more mature retinal vessels have a greatly reduced ability to replicate, except perhaps in the presence of unusual stimuli such as trauma or disease. Pericytes appear to behave in the opposite fashion. Few cells with pericyte morphology survive in our cultures from kitten microvessel fragments, but pericytes compose nearly the entire population of cells growing from the microvessels that we obtain from calves as well as from adult animals of several species.

Our results open a number of possibilities for investigations that have been difficult to carry out with in vivo preparations. These include studies of factors that promote either the selective proliferation or the degeneration of pericytes or endothelial cells in the retinal capillaries, of factors that affect retinal capillary permeability either by modifying the structure of intercellular junctions and/or by altering pinocytic function, and of differences in the biochemical and physiologic function of retinal capillary pericytes and endothelial cells under normal conditions as well as those that simulate disease states, such as diabetes mellitus.

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From the Kresge Eye Institute of Wayne State University, School of Medicine, Detroit, Mich., and the Institute of Biological Sciences, Oakland University, Rochester, Mich. This work was supported in part by grant RO 1-ET-01857 (Dr. Frank) from the National Eye Institute and by a grant to Dr. Frank from the Juvenile Diabetes Foundation. Submitted for publication April 27, 1975. Reprint requests: Dr. Robert N. Frank, Kresge Eye Institute, Wayne State University School of Medicine, 3994 John R. St., Detroit, Mich. 48201.

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A severe disorganization of the lattice arrangement of tapetal rods occurs in cats nutritionally deprived of taurine. This is the first reported example of a nutritionally related degeneration of tapetal cells. The tapetal cell degeneration, along with the previously noted photoreceptor degeneration in taurine-depleted cats, suggests that taurine plays a vital role in maintaining the structural integrity of some biological structures. The visual evoked potentials were severely reduced in taurine-depleted cats, suggesting degeneration, along with the previously noted photoreceptor degeneration in taurine-depleted cats, indicating that they are deficient in light processing. These results also emphasize the importance of dietary taurine for the maintenance of normal structure and function of the eye, at least in the cat.

Taurine is distributed ubiquitously in animals and is present in especially large concentrations in retina and in developing brain. A series of studies demonstrated that cats fed a synthetic diet containing partially purified casein as the only source of protein developed abnormalities in retinal structure and function. It was subsequently shown that a deficiency of taurine was responsible for these changes. These studies confined themselves to structural alterations in retina, concurrent changes in electroretinogram, and concentration of taurine in retina and plasma. More recent studies have demonstrated that taurine depletion is far more exaggerated in other tissues, including brain tissues, than obvious neurologic consequences were noted. In addition, all previous cats, with a single exception, were maintained on a taurine-free diet for a maximum of 10 months. The present investigation reports the changes in taurine concentrations, electrophysiological parameters, and eye structures in cats maintained on a taurine-free diet for 18 months.

Materials and methods. Four cats (two pairs of littermates, one control and one experimental) were maintained for 18 months on a synthetic diet containing casein as the protein source alone or supplemented with 0.4% taurine. All four cats were examined under nembutal anesthesia for visual evoked potentials (VEP) as follows: three small scalp electrodes were fixed along the midline in occipital, central, and frontal sites. Occipital and central electrodes were referred to the frontal electrode, and an ear clip served as ground. Both eyelids were retracted with masking tape. A photoflood lamp connected to a Grass photostimulator was positioned 25 cm in front of the nose. Light stimulation was presented with the photostimulator at medium intensity (I = 4), at an average rate of one flash per second, for a total of 100 trials per condition. The data were digitized at 1000 points per second with a Hewlett Packard 2115 computer, and 0.5 sec samples were displayed on a Versatec plotter.

Electroretinogram (ERG) measurements were performed on both eyes of each cat under nembutal anesthesia after dark-adapting for an hour and after maximally dilating the pupils with cyclogyl. One-millisecond light flashes were generated by a Grass photostimulator behind a ground glass screen placed 12 cm from the eyes. Each testing session included 0.5, 1, 5, 10, 20, and 40 Hz at two intensities (log I = −0.3 and log I = 0).

All cats were anesthetized with nembutal. From one pair of littermates (one control and one experimental), one eye was removed for perfusion and fixation, the cats were killed, and tissues were dissected and frozen in liquid nitrogen. From the other pair of littermates, samples of visceral tissues (liver, kidney, and heart) were taken, and one eye was removed and dissected into retina, lens, and vitreous fluid and frozen in liquid nitrogen prior to killing by intracardiac perfusion (1% paraformaldehyde and 2% glutaraldehyde in 0.1M Sorenson's phosphate buffer, pH 7.4, for 2 min followed by 4% glutaraldehyde in the same buffer for 15 min). Retina and tapetum were prepared for examination by light and electron microscopy as described previously. Each retina was divided into 18 areas for exact mapping. Results reported in this communication are taken from the center.

Concentration of taurine in tissue samples was measured as described previously. Results. The tapetum of the taurine-depleted cats showed a remarkable reduction in size. The surface area was half that in the taurine-supplemented cats and that found in chow reared cats (approximately 45 mm²); the thickness was one-fifth that found in the taurine-supplemented cats and in chow reared cats (approximately 65 μm), and...