*Proc. Natl. Acad. Sci. USA*
Vol. 73, No. 11, pp. 4120–4124, November 1976
Cell Biology

# Clonal growth of bovine vascular endothelial cells: Fibroblast growth factor as a survival agent

(antihemophilic factor/smooth muscle cell/survival factor/cloning)

D. GOSPODAROWICZ, J. MORAN, D. BRAUN, AND C. BIRDWELL

The Salk Institute for Biological Studies, P.O. Box 1809, San Diego, California 92112

*Communicated by Robert W. Holley, August 30, 1976*

ABSTRACT **Bovine vascular endothelial cells do not grow when cultured at low density unless fibroblast growth factor is included in the culture medium. When endothelial cells obtained from the intimal surface of fetal and adult aortas were seeded at low density (8 cells per $cm^2$), they formed small colonies of large, irregular, vacuolated cells. At very low density (0.3 cells per $cm^2$) they did not survive. The addition of fibroblast growth factor to endothelial cells maintained at such low densities resulted in the formation of vigorously growing colonies of small, uniform cells. Electron microscopy showed that the cultured endothelial cells had the fine structure characteristics of endothelial cells. Immunofluorescence microscopy revealed antihemophilic factor (Factor VIII) antigen in the cells. Our results demonstrated that fibroblast growth factor permits the survival of endothelial cells plated at extremely low cell density. With the use of fibroblast growth factor, endothelial cell clones are easily produced.**

Endothelial cells constitute the inner lining of the blood vascular system. Because of their location at the interface between blood and tissue, they are the chief elements involved in the permeability of blood vessels (1, 2). Abnormalities of endothelial cell structure and function are prominent in the pathology of a number of diseases of blood vessel walls such as thromboangiitis (3) and microangiopathy (4).

Since the continuity of the vascular endothelium is essential for the survival of the organism, the elucidation of the factors involved in endothelial cell survival and proliferation is important. Their survival and proliferation can be examined most easily in tissue culture.

Recently, several factors that promote the growth of cells in culture have been identified. One of the most potent is the fibroblast growth factor (FGF) which stimulates the growth of a variety of mesoderm-derived cells (5).

Three observations have led us to examine the effect of FGF on vascular endothelial cells: (*i*) FGF is a potent mitogen for Balb/c 3T3 cells (6). Although these cells are commonly referred to as fibroblasts, their morphology and the fact that they can produce vasoformative sarcomas *in vivo* (7) suggests that they are derived from vascular endothelium. (*ii*) Although FGF was named fibroblast growth factor when first isolated, subsequent studies have shown that it is a mitogen for a wide variety of mesoderm-derived cells (5). Because the vascular endothelium is derived from the embryonic mesoderm, one would also expect endothelial cells to be responsive to FGF. (*iii*) FGF has been shown to promote the development of a regeneration blastema in the stump of amputated frog limbs (8). Because Smith and Wolpert (9) hypothesized that nerves may be necessary for amphibian limb regeneration as they promote vascularization of the injured tissue in the stump, and because vascularization depends on the proliferation of endothelial cells, a mitogenic effect of FGF on endothelial cells (to promote vascularization) in addition to a direct effect on blastema precursor cells, may explain FGF's *in vivo* effect on limb regeneration.

We have examined the possibility that FGF is a survival factor as well as a mitogen for cultured vascular endothelial cells. These studies indicate that FGF can easily be used to maintain and grow endothelial cells in tissue culture for prolonged periods.

Abbreviations: FGF, fibroblast growth factor; DME, Dulbecco's modified Eagle's medium; AHF, antihemophilic factor, Factor VIII.

## MATERIALS AND METHODS

**Materials.** FGF was purified from bovine pituitary glands (10) and bovine brains (5) as described. For reasons of economy, FGF from brain was used in the experiments reported here. However, the same results could be obtained with FGF from pituitary. Cells were routinely cultured at 37° on Falcon dishes in Dulbecco's modified Eagle's medium (DME) supplemented with 10% calf serum (Gibco) and 100 ng/ml of FGF in an atmosphere of 12% $CO_2$ in air to maintain the pH of the medium between pH 7.3 and 7.5. Bovine fetuses of 4 months' gestation (45 cm, crown to rump), bovine adult aortic arches, and bovine umbilical cords were obtained from a local slaughterhouse.

**Endothelial Cell Culture.** The aortic arches were opened lengthwise with a scalpel and the intimal surface was washed with $Ca^{++}$-free phosphate-buffered saline to remove blood. The endothelial cell layer was removed by gently scraping the intimal surface with a grooved director. The grooved director was dipped in 10 ml of DME with 10% calf serum. This technique gave cell populations composed of 99% endothelial cells.

For purer populations, a cotton swab was used instead of a grooved director. By rolling the swab, under gentle pressure, on the endothelium, endothelial cells became trapped in the cotton fibers. The swab was then swirled in culture medium to liberate the cells. Using cotton swabs, we have found no contamination by smooth muscle of the primary endothelial cell cultures.

The medium containing the cells was transferred to a 15 cm tissue culture dish and 20 ml of DME with 10% calf serum and 100 ng/ml of FGF was added to the plates. After a 24 hr incubation, aggregates or single cells started to attach to the bottom of the dishes and at 48 hr the medium was renewed. Cultures maintained with FGF became confluent in about 7 days. In cultures without FGF, the cells proliferated slowly for a time, but eventually deteriorated and died without forming a monolayer. The same technique used for the fetal and adult aortic arches was applied to veins of the bovine umbilical cord with the same success.

**Cloning of Endothelial Cells.** Small aliquots of trypsinized, secondary cultures were transferred to 6 cm dishes containing 5 ml of DME with 10% calf serum, and 100 ng/ml of FGF so that only a few cell aggregates could plate. When the colonies

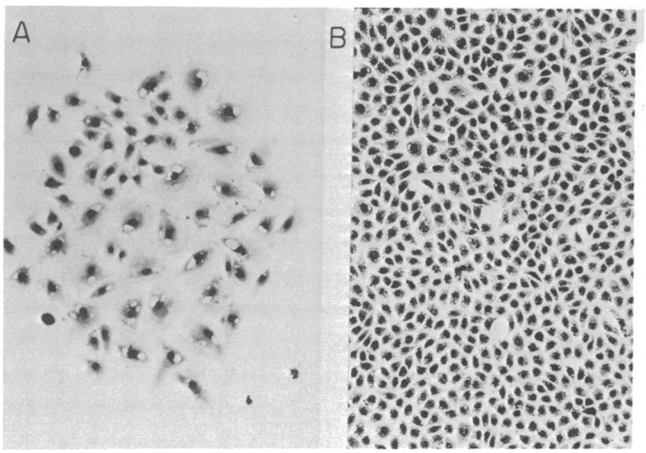


FIG. 1. Primary cultures of fetal bovine aortic endothelial cells maintained in tissue culture with and without FGF. (A) Colony of endothelial cells maintained in 10% calf serum for 6 days without FGF. The cells are vacuolated with a broad and thin peripheral cytoplasm (×50). (B) Monolayer obtained after 6 days in culture in the presence of 10 ng/ml of FGF and 10% calf serum (×50).

were visible to the naked eye, they were trypsinized in a cloning ring. The cell suspension was then diluted and an aliquot was transferred to a 15 cm dish and maintained in DME with 10% calf serum and 100 ng/ml of FGF. When colonies which arose from single cells were visible the colonies were trypsinized in cloning rings and transferred to 6 cm dishes. Since colonies could develop from five cells plated in a 15 cm dish provided FGF was present, the colonies were so far apart that the probability that they arose from more than one cell approached zero. Cloned endothelial cell lines have been continuously passaged in our laboratory for the past 8 months. Adult bovine cells are passaged weekly at a 1 to 10 dilution and placed in DME with 10% calf serum and 50 ng/ml of FGF. The FGF is added every other day until the cells are nearly confluent, then the medium is changed to 5% calf serum without FGF. Fetal bovine cells are maintained as described for adult cells except that 5% calf serum is always used.

**Tissue Culture of Smooth Muscle.** After removal of the intima by scraping with a surgical blade, the media of the aortic arch was dissected with a scalpel and fragments (11) were cultured in DME with 10% calf serum and 100 ng/ml of FGF. Alternatively, small pieces of media were digested with 0.25% collagenase (EC 3.4.24.3) (Worthington) and the resulting cell suspension was cultured as described for endothelial cells. Cloning, as described for endothelial cells, was easily performed with the smooth muscle cells arising from the primary culture. FGF was routinely added to the culture medium to speed up the proliferation of smooth muscle plated at clonal density ($5 \times 10^{-2}$ cells per $cm^2$).

**Fluorescence Microscopy.** The endothelial cells were identified by fluorescence microscopy using antihemophilic factor (AHF, Factor VIII) antigen as a marker. Rabbit antibodies against bovine AHF were a generous gift from E. Davie (University of Washington, Seattle) (12).

The endothelial cells and/or smooth muscle were plated in DME with 10% calf serum and 100 ng/ml of FGF. The cells were washed for 5 min in phosphate-buffered saline, then fixed for 5 min in ethanol:methanol 1:1 (vol/vol) and dried. The dried cells were then stained as described by Jaffe *et al.* (13), with dilutions of either rabbit anti-AHF serum or normal rabbit serum and a 1:50 dilution of fluorescein-conjugated goat antiserum against rabbit IgG.

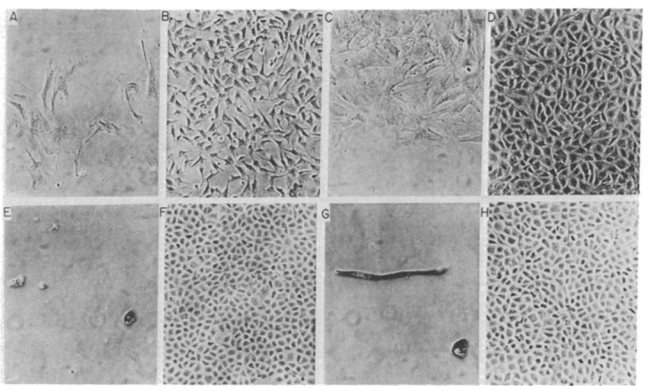


FIG. 2. Effect of FGF on survival of endothelial cells maintained at low density in the presence of serum. Fetal bovine aortic endothelial cells in their third passage (21 generations) were plated at two cells per $cm^2$ in 6 cm dishes in the presence of 10% calf serum. FGF (100 ng/ml every other day) was added to half the dishes. Twelve days later the media were changed and FGF was added to half the dishes that had been maintained without FGF. At the same time, half the dishes that had been maintained with FGF were switched to medium lacking FGF. Colonies were photographed under phase contrast at the time of the medium change (A–D) and the same colonies were photographed again 7 days later (E–H). (A and C) Cells maintained without FGF for 12 days. (B and D) Cells maintained with FGF for 12 days. (E) Same as (A) but after 7 additional days in the absence of FGF; (F) same as (B) but after 7 additional days in the presence of FGF; (G) same as (C) but after 7 additional days now in the presence of FGF; (H) same as (D) but after 7 additional days now in the absence of FGF.

## RESULTS

### Fibroblast growth factor as a survival agent

Primary cultures were started with a few cells and the development of a uniform monolayer of endothelial cells depended on whether or not FGF was present in the medium. When bovine endothelial cells were plated in the absence of FGF, small colonies developed from cell aggregates during the first few days, but the cells looked unhealthy and became vacuolated (Fig. 1A). In contrast, if FGF was present the cells proliferated vigorously and formed a monolayer (Fig. 1B).

When third passage cells were plated at a very low density (2 cells per $cm^2$) and maintained in 10% calf serum without FGF for 12 days, they formed very small colonies of large, irregular cells (Fig. 2A and C). In contrast, cells maintained with FGF formed much larger colonies of rapidly growing cells (Fig. 2B and D). To see whether cells maintained without FGF for 12 days could yet be stimulated to proliferate, we added FGF to the cells shown in Fig. 2C. As shown in Fig. 2G, despite the addition of FGF, the cells all died 7 days later, just as did cells maintained the entire 19 days without FGF (Fig. 2E). To see whether cells grown in FGF for 12 days would die when deprived of it, we put the cells shown in Fig. 2D into medium containing calf serum only. As shown in Fig. 2H, the cells continued to proliferate and were healthy 7 days later; they were very similar in appearance to cells maintained for the entire 19 days in FGF (Fig. 2F).

In contrast with experiments done at low cell densities, cloned endothelial cells plated without FGF at high density (10,000 cells per $cm^2$) survived but proliferated slowly (doubling time 72 hr) and eventually became vacuolated. This observation agrees with that of Gimbrone *et al.* (14) that endothelial cells survive and grow better at high density than at low density.

### Cloned endothelial cell strain characterization

**Light Microscopy.** Cloned endothelial cells maintained in

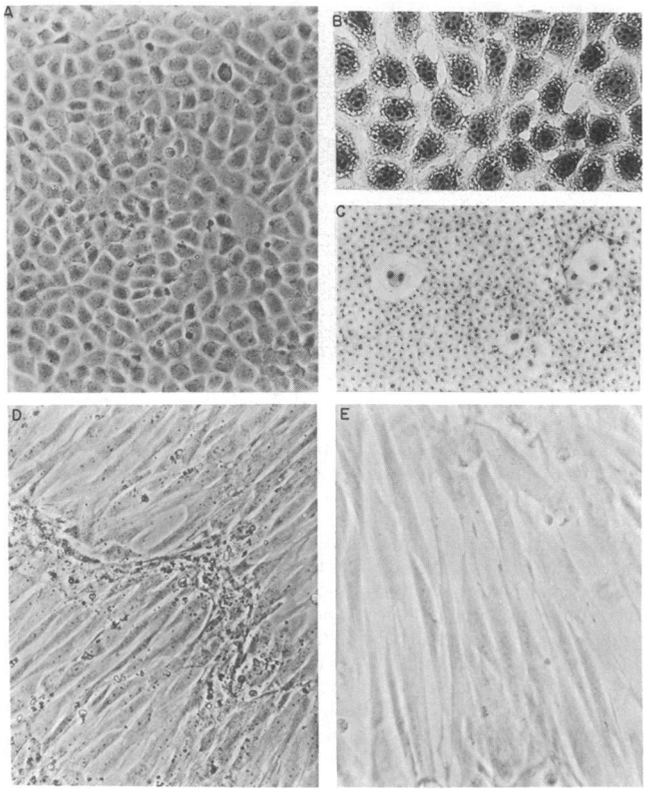


FIG. 3. Appearance of cloned cells and smooth muscle cells from adult bovine aortic endothelium maintained in tissue culture in the presence of FGF. (A) Monolayer of endothelial cells (3 passages, 27 generations) maintained in the presence of 10% calf serum and 100 ng/ml of FGF (phase contrast, ×65). (B) Same monolayer as in (A) but fixed with 10% formalin and stained with 0.1% Giemsa (×172). (C) Monolayer of endothelial cells after 42 generations (6 passages). (D) Two-week-old smooth muscle culture. The cells have grown parallel to one another in multilayers separated by a wavy ridge (phage contrast, ×65). (E) View at a higher power (×129) of the same culture. The cells are elongated with a cytoplasm with longitudinal ridges and ovid nuclei with one or two nucleoli. Some of the cells are binucleated.

the absence of FGF proliferated slowly, and produced small colonies of vacuolated cells similar to those observed in primary cultures (Fig. 1A). In the presence of FGF, the cells reached confluency and assumed the distinct appearance of contact-inhibited cells (Fig. 3A). The cells were homogeneous, closely apposed, with centrally located nuclei containing three to four nucleoli (Fig. 3A and B). The cell borders were not apparent by phase contrast microscopy (Fig. 3A). While in early passage (first to fourth passage) only small and compact cells were seen; in later passages (sixth to twelfth) a number of large mono- and binucleated cells became evident (Fig. 3C). These cells never constituted more than 2–5% of the total number of cells, but their presence was quite noticeable by light microscopy due to the large area they occupied.

Cultures of endothelial cells could be readily distinguished from cultures of smooth muscle. At high density, the smooth muscle cell cultures developed a distinct spatial organization consisting of a patchy monolayer of elongated cells arranged in parallel arrays. The arrays formed irregular bands of cells interspersed with multilayered hillocks. Each band appeared to be separated from the next one by wavy, transverse ridges (Fig. 3D and E).

To prove conclusively that the cells observed in the endothelial cell cultures were not contaminated by smooth muscle

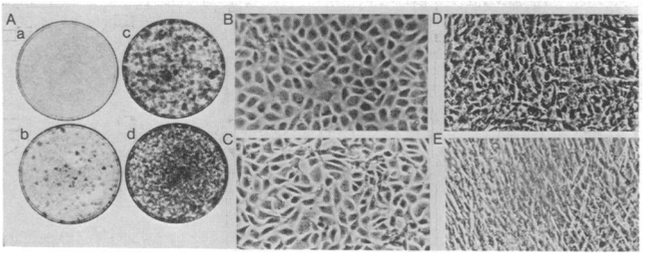


FIG. 4. Appearance of pure cultures of endothelial cells as compared to mixed culture of endothelial and smooth muscle cells. Endothelial cells were plated at an initial density of 20,000 cells per 6 cm dish and deliberately contaminated with either 1%, 10%, or 50% smooth muscle cells. (A) Shows the macroscopic appearance of a dish 7 days later with endothelial cells only (a) and of dishes containing either 1% smooth muscle (b), 10% smooth muscle (c), or 50% smooth muscle (d). (B) Shows the appearance of endothelial cells grown to confluency, (C) endothelial cells containing 1% smooth muscle, (D) 10% smooth muscle, and (E) 50% smooth muscle.

cells, we deliberately mixed cultures of these two cell types. Smooth muscle cells were plated together with cloned endothelial cells so that the smooth muscle cells made up 1%, 10%, or 50% of the total cell population. As seen in Fig. 4A, endothelial cell cultures contaminated with smooth muscle could be readily distinguished macroscopically from normal cultures free of contamination. While Giemsa-stained dishes of endothelial cells showed a uniform distribution of the stain, dishes containing as little as 1% contaminating smooth muscle had a mottled appearance, with colonies of smooth muscle cells growing as hillocks rising above the monolayer of endothelial cells. The difference was even more pronounced when the cultures were examined by phase contrast microscopy. Dishes containing endothelial cells showed the monolayer characteristic of that cell type (Fig. 4B) while dishes containing both smooth muscle and endothelial cells showed elongated cells (presumably smooth muscle) growing on top of the monolayer (Fig. 4C, D, and E).

**Electron Microscopy.** The cytoplasm of the cloned endothelial cells contains rough endoplasmic reticulum, free ribosomes, microtubules 250 Å in width, as well as numerous micropinocytotic vesicles 500–1000 Å in diameter. Small bundles of 60–70 Å diameter filaments were observed at the cell peripheries. Weibel-Palade bodies (15), which have been seen in human umbilical vein endothelial cells (16), were not clearly identified in our cells.

In contrast, the cytoplasm of smooth muscle cells was filled with bundles of 60–70 Å diameter myofilaments interspersed with dense bodies, and contained extensive aggregates of rough endoplasmic reticulum, prominent golgi complexes, free ribosomes, and polysomes. Round osmophilic lipid bodies were present in many cells. These features were characteristic of cultured arterial smooth muscle (13).

**Fluorescence Microscopy.** AHF antigen has been shown to be present only in tissue sections (17), and cultured endothelial cells (13, 18). When cloned bovine endothelial cells were examined by immunofluorescence for the presence of the AHF antigen, the cells showed a perinuclear reaction with distinct foci of fluorescence, suggesting that the AHF was present in small vesicles (Fig. 5A and B). Vascular smooth muscle cells, granulosa cells, corneal endothelial cells, uterine fibroblasts and adrenal cells, all of bovine origin, were unreactive. The presence of AHF antigen in cloned bovine endothelial cells of fetal or adult origin has been a constant feature of all subcultures to date (passage 1 to 20).

**Cell Proliferation.** Adult bovine aortic endothelial cells grew

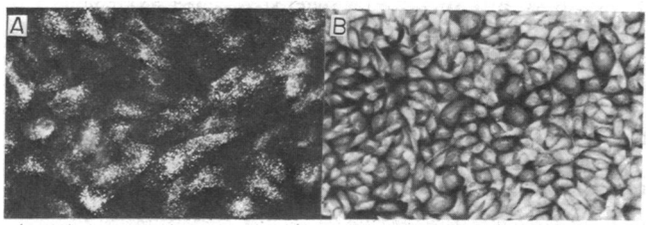


FIG. 5. Immunofluorescence of antihemophilic factor antigen in cultured bovine aortic endothelial cells. Cloned adult bovine aortic endothelial cells in passage number 7 (47 generations) were used. (A) Rabbit anti-AHF at 1:10,000 dilution. The cells are brightly stained. Vesicles that contain AHF antigen are apparent (×130). (B) Monolayer of endothelial cells stained with rabbit antiserum against AHF at 1:1000 dilution (×50). Similar fluorescence was observed with cloned endothelial cells from fetal bovine aorta. Cultures treated with normal rabbit antiserum at 1:100 dilution did not fluoresce.

best in 10% calf serum. Growth in 35% calf serum was similar to that observed with 10%. The addition of FGF greatly increased the rate of growth of cultures in either 10% or 35% calf serum (Fig. 6). Fetal calf serum (Gibco) was much less suitable for supporting growth.

## DISCUSSION

Our results indicate that bovine endothelial cells will not survive at low density unless FGF is present; thus, FGF is needed for survival and is also a mitogenic agent for these cells (19, 20). This is true for cells derived from vascular territories as different in age and origin as the bovine umbilical vein (5, 19) and the fetal or adult aortas. Similar results have been also obtained with human umbilical vein endothelium and endothelial cells derived from capillaries present in the bovine choroid plexus of the third ventricule (20).

Endothelial cells in tissue culture have a morphology so characteristic that they cannot be confused with smooth muscle cells. Furthermore, they exhibit characteristics of differentiated endothelial cells in that they contain AHF. In this context, we found that the presence of AHF antigen in cultured endothelial cells is a better cell marker than the presence of Weibel-Palade bodies. By using immunofluorescence, one can examine a much greater number of cells than is feasible with electron microscopy. In addition, while the endothelium of the umbilical vein contains a high concentration of Weibel-Palade bodies, it remains to be established that similar high concentrations exist in endothelial cells of other vascular territories. It has been our consistent experience that endothelial cells of the aorta contain very few Weibel-Palade bodies which makes these organelles an inadequate marker. In contrast, the concentration of AHF antigen in endothelial cells from bovine umbilical vein is similar to that found in endothelial cells from the aorta.

Most reports of successful culture of endothelial cells to date have concerned cells obtained from the human umbilical vein (14, 21–26) or from the rabbit (27) or bovine aorta (18). Gimbrone *et al.* (14) reported that one third of their primary, human umbilical vein cultures did not reach confluency and that inocula of less than $1.2 \times 10^4$ viable cells per $cm^2$ usually failed to become established. Thus this precluded cloning. Buonassisi and Venter developed clonal lines of rabbit vascular endothelial cells by using a feeder layer technique (27). They did not report on cell growth except to mention that the cells were transferred to new flasks at a 1:2 dilution, once each week.

Booyse *et al.* (18) have recently reported the culture of adult bovine aortic endothelial cells in RPMI-1640 medium supplemented with 35% fetal calf serum. Our results differ from theirs

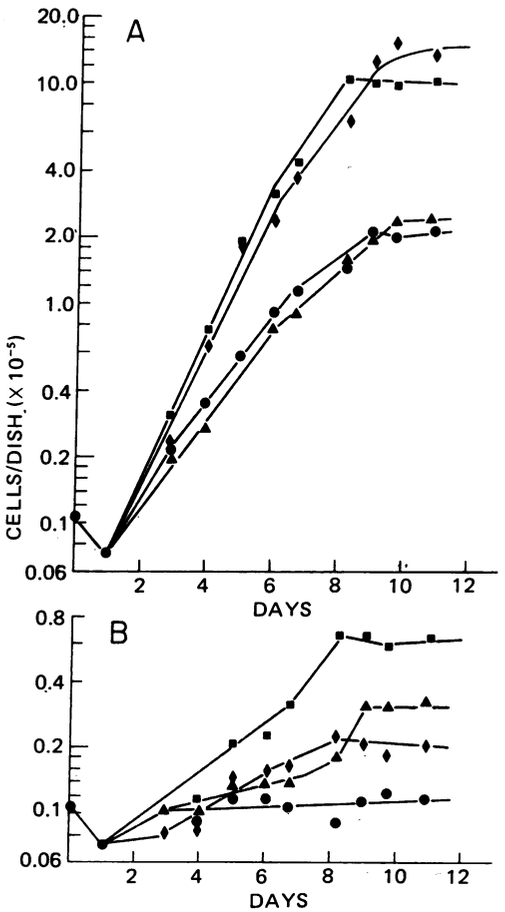


FIG. 6. Growth rate of cloned adult bovine endothelial cells maintained in the presence of serum with or without FGF. Cloned adult bovine endothelial cells in their 10th passage (62 generations) were seeded at 10,000 cells per 6 cm dish in DME with 10% calf serum. Twenty-four hours later, the medium was replaced with either: (A) 10% calf serum (▲—▲), 35% calf serum (●—●), 10% calf serum plus 100 ng/ml of FGF (■—■), or 35% calf serum plus 100 ng/ml of FGF (♦—♦). (B) 10% fetal calf serum (▲—▲), 35% fetal calf serum (●—●), 10% fetal calf serum plus 100 ng/ml of FGF (■—■) or 35% fetal calf serum plus 100 ng/ml of FGF (♦—♦). The addition of FGF was repeated every other day.

on the following points: (*i*) We found that fetal calf serum, at either 10% or 35%, was much inferior to calf serum for supporting cell growth. (*ii*) We found that with calf serum, the cells grew as well in 10% serum as in 35%. (*iii*) We found that cells did not grow at very low cell density (less than 1 cell per $cm^2$) unless FGF was added to the medium while Booyse *et al.* suggested that these cells do not require a minimum cell number in order to become established. (They did not, however, report on the growth of cells seeded at less than 400 cells per $cm^2$.) The differences between our results and those of Booyse *et al.* may be due to the fact that we maintain our cells in DME while they used RPMI medium.

Because FGF is a survival agent for endothelial cells in tissue culture, one wonders whether it has the same role *in vivo*. It has been shown by us (29) as well as by others (30–33) that FGF could be similar to factor(s) present in platelets and involved in the control of survival as well as proliferation of vascular (31) and other cell types (29, 30–33). Kohler and Lipton (30) showed that 3T3 cells maintained in platelet-poor plasma serum can proliferate as well as those maintained in blood serum only when a platelet lysate is added to the medium. Ross and Glomset have shown that FGF can substitute for a platelet ex-

tract to promote the growth of vascular smooth muscle cells maintained in platelet-poor plasma serum (31). We have shown that FGF can substitute for a platelet lysate to promote the growth of 3T3 cells in platelet-poor plasma serum (32). Westermark and Wasteson (33) have shown that either pure FGF or a partially-purified extract of platelets can be used to stimulate the proliferation of human glial cells. We have found that platelet extract contains 0.01–0.05% of material that crossreacts with FGF by radioimmunoassay (unpublished observation). Because platelets are known to be involved in maintaining the integrity of the vascular endothelium *in vivo* (34, 35), it will be of interest to know the relationship between platelets, FGF and endothelial cells.

**Note Added in Proof.** The mitogenic effect of FGF on bovine vascular endothelial cells and vascular smooth muscle has been compared to that of epidermal growth factor (EGF). While FGF is a strong mitogen for both endothelial cells and vascular smooth muscle, EGF is not mitogenic for endothelial cells and has a small mitogenic effect on smooth muscle (36). FGF also promotes the growth both of endothelial cells from human umbilical vein (when seeded at low density, 32 cells per $cm^2$) and of bovine vascular endothelial cells derived from capillaries present in the choroid plexus of the third ventricle. In contrast, EGF has no effect on either cell type. FGF is thus mitogenic *in vitro* for cells derived from aorta, vein, and capillary endothelium. This latter observation correlates well with our observation that acrylamide implants of FGF *in vivo* induced the proliferation of capillaries in the rabbit cornea (20, 37) at a concentration as low as 1 $\mu$g of FGF per implant.

We thank Drs. E. Davie and W. Brown for generous gifts of antiserum. This work was supported by grants from the National Institutes of Health and from the American Cancer Society. C.R.B. is a Fellow of the Anna Fuller Fund.

1. Aursnes, I. (1974) *Microvasc. Res.* **1**, 283–292.
2. Gore, I., Takada, M. & Austin, J. (1970) *Arch. Pathol.* **90**, 197–201.
3. Stemerman, M. B. & Spaet, T. H. (1972) *Bull. N.Y. Acad. Med.* **48**, 289–292.
4. Bell, E. T. (1960) *Diabetes Mellitus* (Charles C Thomas, Springfield, Ill.).
5. Gospodarowicz, D., Moran, J. & Bialecki, H. (1976) "Growth hormone and related peptides," *Excerpta Med. Int. Congr. Ser.*, 141–155.
6. Gospodarowicz, D. & Moran, J. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 4584–4588.
7. Boone, C. W., Takeichi, N., Paranjpe, M. & Gilden, R. (1976) *Cancer Res.* **36**, 1626–1633.
8. Gospodarowicz, D., Rudland, P., Lindstrom, J. & Benirschke, K. (1975) *Adv. Metab. Disord.* **8**, 301–335.
9. Smith, A. R. & Wolpert, L. (1975) *Nature* **257**, 224–226.
10. Gospodarowicz, D. (1975) *J. Biol. Chem.* **250**, 2515–2519.
11. Ross, R. (1971) *J. Cell Biol.* **50**, 172–186.
12. Schmer, G., Virby, E. P., Teller, D. C. & Davie, E. W. (1972) *J. Biol. Chem.* **247**, 2512–2521.
13. Jaffe, E. A., Hoyer, L. W. & Nachman, R. L. (1973) *J. Clin. Invest.* **52**, 2757–2764.
14. Gimbrone, M. A., Cotran, R. S. & Folkman, J. (1974) *J. Cell Biol.* **60**, 673–684.
15. Weibel, E. R. & Palade, G. E. (1964) *J. Cell Biol.* **23**, 101–113.
16. Haudenschild, C. C., Cotran, R. S., Gimbrone, M. A. & Folkman, J. (1975) *J. Ultrastruct. Res.* **50**, 22–32.
17. Hoyer, L. W., de los Santos, R. P. & Hoyer, J. R. (1973) *J. Clin. Invest.* **52**, 2737–2744.
18. Booyse, F. M., Sedlak, B. J. & Rafelson, M. E. (1975) *Thrombos. Diath. Haemorrh.* **34**, 825–839.
19. Gospodarowicz, D. & Moran, J. (1976) in *Membranes in Neoplasia, New Approaches and Strategies*, ed. Marchesi, V. T. (A. R. Liss, Inc., New York), pp. 1–20.
20. Gospodarowicz, D., Mescher, A. & Binder, P. (1976) "Gene expression and regulation," *Natl. Cancer Inst. Monogr.*, in press.
21. Maruyama, Y. (1963) *Z. Zellforsch. Mikrosk. Anat.* **60**, 69–82.
22. Fryer, D. G., Birnbaum, G. & Lutrell, C. N. (1966) *J. Atheroscler. Res.* **6**, 151, 155.
23. Fryer, D. G., Birnbaum, G. & Lutrell, C. N. (1966) *J. Atheroscler. Res.* **6**, 155.
24. Pomerat, C. M. & Slick, W. C. (1963) *Nature* **198**, 859–861.
25. Jaffe, E. A., Nachman, R. L., Becker, C. G. & Minick, C. R. (1973) *J. Clin. Invest.* **52**, 2745–2756.
26. Suddith, R. L., Keely, P. J., Hutchison, H. T., Murray, E. A. & Huber, B. (1975) *Science* **190**, 682–684.
27. Buonassisi, V. (1973) *Exp. Cell Res.* **76**, 363–368.
28. Buonassisi, V. & Venter, J. C. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 1612–1616.
29. Gospodarowicz, D., Greene, G. & Moran, J. (1975) *Biochem. Biophys. Res. Commun.* **65**, 770–787.
30. Kohler, N. & Lipton, A. (1974) *Exp. Cell Res.* **87**, 297–301.
31. Ross, R. & Glomset, J. (1976) *J. Cell Biol.*, in press.
32. Westermark, B. & Wasteson, A. (1975) *Adv. Metab. Disord.* **8**, 85–100.
33. Westermark, B. & Wasteson, A. (1976) *Exp. Cell Res.* **98**, 170–174.
34. Kitchens, C. S. & Weiss, L. (1975) *Blood* **46**, 567–578.
35. Gimbrone, M. A., Aster, R. H., Cotran, R. S., Corkery, J., Jandl, J. H. & Folkman, J. (1969) *Nature* **222**, 33–36.
36. Gospodarowicz, D., Moran, J. & Braun, D. (1976) *J. Cell. Physiol.*, in press.
37. Gospodarowicz, D., Moran, J., Mescher, A. & Binder, P. (1976) "Molecular Control of Proliferation and Cytodifferentiation," *35th Symposium of the Society for Developmental Biology*, in press.