AN ENDOTHELIAL CELL-DERIVED GROWTH FACTOR

CORINNE GAJDUSEK, PAUL DiCORLETO, RUSSELL ROSS, and STEPHEN M. SCHWARTZ.
From the Department of Pathology, University of Washington, School of Medicine, Seattle, Washington 98195

ABSTRACT

Cell-free plasma-derived serum (PDS) is deficient in the platelet-derived growth factor and will not support the growth of 3T3 cells, fibroblasts, or smooth muscle cells. However, when PDS-containing medium is preincubated with endothelial cells, the medium becomes modified so that it will support growth. The activity produced by the endothelial cells results from a polypeptide of 10,000 to 30,000 daltons which has several features that differ from those of the platelet-derived growth factor, including heat instability and lack of adsorption to CM Sephadex.

Growth of nontransformed cell strains in culture requires one or more exogenous growth factors. The availability of these polypeptides appears to determine the extent of growth, and account, at least in part, for the phenomenon of density-dependent inhibition of growth (12, 28, 31–33). In contrast, studies of endothelial cell strains have failed to demonstrate a requirement for platelet-derived growth factor, epidermal growth factor, fibroblast growth factor, or insulin (3, 4, 10, 14, 25, 26, 30, 34). These observations led us to examine the effects of endothelial cells on growth of other cell types in medium lacking the platelet-derived growth factor. We report here the presence of a potent growth factor produced by endothelial cells.

MATERIALS AND METHODS

Cells

Primary cultures of bovine aortic endothelial cells were isolated (27) in 15% cell-free plasma-derived serum (PDS). PDS was prepared from citrated bovine whole blood serum by the CM-Sephadex chromatography method of Vogel et al. (33) and heat inactivated for 30 min at 56°C. Bovine PDS contains 10% of the growth-promoting activity originally present in homologous whole blood serum. Under these conditions, it was possible to select for cultures with little or no evidence of smooth muscle overgrowth (3). Subcultures were grown in 75-cm^2 flasks (Corning Glass Works, Science Products Div., Corning, N. Y.) in 10 ml of Waymouth's medium containing 5% PDS. Duplicate experiments were performed with endothelial cells isolated by the thymidine selection technique (27). Results were identical using both isolation methods, including thymidine-selected cells grown in whole blood serum or PDS.

Cultures of Swiss and BALB/c-3T3 A_{31} cells, originally obtained from Dr. Stewart Aaronson, were maintained in 10% fetal calf serum (Grand Island Biological Co., Grand Island, N. Y.) in Dulbecco-Vogt modification of Eagle's medium. Human newborn skin diploid fibroblasts, in the 4th passage, were grown in Waymouth's medium containing 10% fetal calf serum. Bovine smooth muscle cells, isolated by the explant technique (20), were maintained as described for fibroblasts and used from passages 2–7.

Assay System

3T3 cell cultures, grown in 5% fetal calf serum, were used at saturation density, 5–7 d after plating, when no mitotic figures were evident and cultures appeared confluent. Thymidine incorporation ([^{3}H]thymidine, New England Nuclear, Boston, Mass., NET 027, 6.7 Ci/mmol) was determined by activity in acid-precipitable material (19).

Cocultivation Experiments

Incubation of confluent endothelial cells with latex beads (1 μm in diameter) for 24 h at 37°C leads to perinuclear labeling of the cells with beads. Beads are not released by vigorous washing or by suspension of the cells with trypsin and subsequent replating. Swiss 3T3 cells and bovine aortic smooth muscle cells were labeled by incubation for 24 h with [^{3}H]methionine (2 μCi/ml) in Dulbecco-Vogt modified Eagle's medium without methionine. After autoradiography, the methionine-labeled cells exhibited a homogeneous low grain count over the entire cell. After addition of endothelial cells to the 3T3 or smooth muscle cultures, nuclei were labeled by tritiated thymidine as described in the legend to Fig. 1. Under these conditions, nuclei were densely labeled and easily quantitated in spite of the methionine background.

Preparation of Conditioned Media

For serum-free conditioned medium, confluent endothelial cell cultures in 75-cm^2 flasks were rinsed with 20 ml of phosphate-buffered saline (PBS) and refed with 10 ml of fresh serum-free medium for 48 h. Medium was centrifuged at 1,000 rpm for 10 min and again at 48,000 *g* for 3 h at 4°C. The same procedure was followed for conditioned medium containing PDS. In biochemical assays, conditioned serum-free medium was lyophilized after exhaustive dialysis (Spectrapor No. 1, mol wt cutoff 6,000–8,000) against 1 mM NH_4HCO_3 and stored at −20°C.

Characterization

For heat stability, lyophilized material, representing 10 ml of serum-free conditioned medium, was resuspended in 10 ml of PBS, heated at 56°C for 30 min or at 100°C for 4 min, cooled rapidly, and tested for activity using the 3T3 cell assay. For trypsin sensitivity, lyophilized material was incubated in PBS containing Mg^{++} and Ca^{++} at 37°C for 1 or 2 h with 50 or 100 μg/ml trypsin TPCK (Worthington Biochemical Corp., Freehold, N. J.), followed by neutralization with 200 or 400 μg/ml, respectively, soybean trypsin inhibitor (Worthington Biochemical Corp.) for 1 h. pH stability was assayed after incubating conditioned serum-free medium for 24 h at 4°C, acidified or made basic with HCl or NaOH, respectively, and neutralized. Lyophilized serum-free conditioned medium was eluted through CM Sephadex under conditions established for purification of platelet-derived growth factor (33). Sample and gel were equilibrated with Tris-HCl (0.01 M, pH 7.4) containing 0.09 M NaCl. Elution was performed in the same buffer system.

RESULTS

We chose to examine the interaction of 3T3 cells and endothelial cells because 3T3 cells represent a standardized cell type that is quiescent in the absence of platelet-derived growth factor (17, 33). Swiss 3T3 and endothelial cells were each sparsely plated in medium containing cell-free PDS lacking platelet-derived growth factor. Under these conditions, endothelial cell growth in uninhibited, whereas 3T3 cells grow poorly (4, 26, 33). When endothelial cells were plated onto the 3T3 cell dish, the 3T3 cells showed a threefold increase in labeling index (Fig. 1). Endothelial cell replication, under these conditions, was slightly decreased. Similar data were obtained in cocultivation experiments using quiescent smooth muscle cells in place of 3T3 cells (not shown). An increase in smooth muscle cell labeling index upon cocultivation was also observed when the endothelial cells were not labeled with latex beads.

In parallel experiments, endothelial cell-conditioned medium was examined for stimulation of cell growth. Target cells included Swiss and BALB/c-3T3 cells, bovine aorta smooth muscle, and human dermal fibroblasts. Data for stimulation of [^{3}H]thymidine incorporation into Swiss 3T3 cells are illustrated in Fig. 2. Minimal incorporation was evident in nonconditioned serum-free medium. With the addition of PDS, there was a small increase in thymidine incorporation in contrast with a much greater increase in incorporation elicited by fetal calf serum. When medium containing PDS was pre-incubated with endothelial cells, this medium stimulated thymidine incorporation at a rate 6 to 10 times greater than that observed with nonconditioned, PDS-containing medium. A 15-fold increase in incorporation was also detected in serum-free medium that had been preincubated with endothelial cells. Prior incubation of medium in culture dishes without cells, or in cultures of smooth muscle cells, had no effect on mitogenic activity. Comparable results were obtained for stimulation of the other cell types.

The thymidine index was used as a more direct measure of stimulation of replication. Fig. 3 shows the effect of conditioned medium on quiescent smooth muscle cells. Between 16 and 20 h, there was a discrete wave of DNA synthesis after addi-

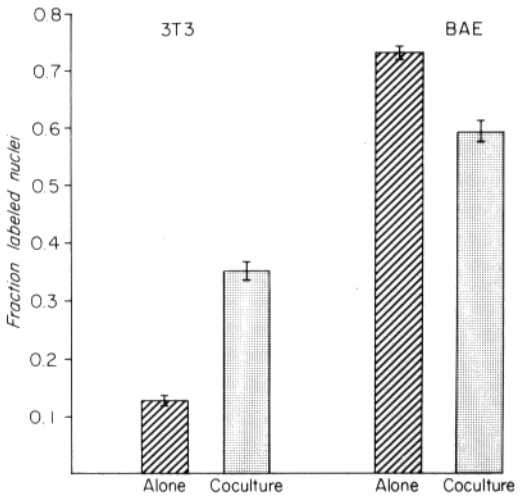

FIGURE 1 Cocultivation of endothelial cells and 3T3 cells. Endothelial cells were labeled by preincubation for 24 h with latex beads in a 75-cm^2 flask. Swiss 3T3 cells were plated in 5% PDS containing medium at 5 × 10^3 cells/cm^2 on round coverslips and labeled by incubation for 24 h with [^{3}H]methionine (2 μCi/ml). After labeling, endothelial cells in 5% PDS containing medium were plated alone at 5 × 10^3 cells/cm^2 and on the coverslip cultures of 3T3. Cultures were incubated with 2 μCi/ml [^{3}H]thymidine from 18 through 24 h after endothelial cell plating. Cultures were prepared for autoradiography. Error bars represent the standard deviation from the mean: Cross-hatched: 3T3 or endothelial cells alone; stippled: cocultures.

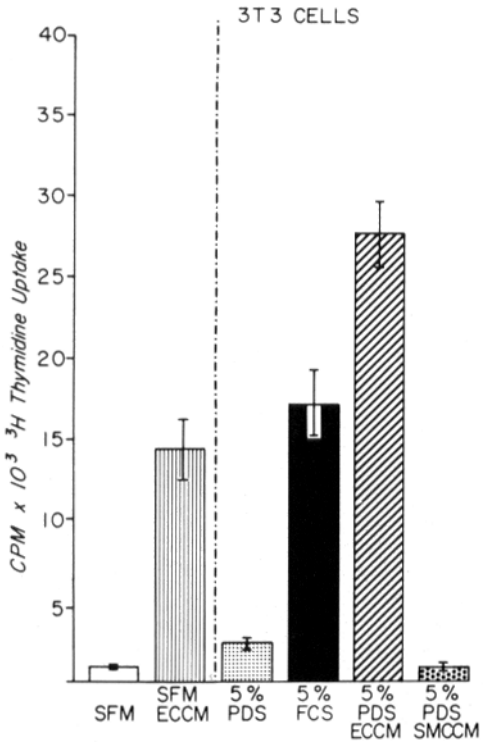

FIGURE 2 Stimulation of thymidine incorporation by endothelial cell conditioned media. 3T3 cells were plated at 5×10^3 cells/cm^2 in Falcon multiwells (Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.) and brought to saturation density. Medium was replaced with 200 μl test media diluted with 300 μl fresh serum-free medium. Thymidine incorporation was measured at 18–20 h with 1 μCi/ml [^{3}H]thymidine. Error bars represent the standard deviation from the mean: SFM: serum-free medium; SFM ECCM: serum-free medium that was preincubated with endothelial cells for 48 h; 5% PDS: medium containing 5% PDS; 5% FCS: medium containing 5% fetal calf serum; 5% PDS ECCM: 5% PDS containing medium that was preincubated with endothelial cells for 48 h; 5% PDS SMCCM: 5% PDS that was preincubated with smooth muscle cells for 48 h.

tion of either fetal calf serum or endothelial cell-conditioned serum-free medium. By 32 h, the labeling index in endothelial cell-conditioned serum-free medium had decreased to background levels, whereas the labeling index in cultures containing fetal calf serum remained elevated. When cell numbers were measured, both smooth muscle (Fig. 4*a*) and Swiss 3T3 cells (Fig. 4*b*) underwent minimal proliferation in nonconditioned PDS-containing medium. In contrast, after exposure to endothelial cells, the same medium supported proliferation at a rate comparable to that observed in medium containing whole blood serum. Serum-free medium, exposed to endothelial cells, also stimulated growth under these conditions. However, as expected from the thymidine index data, growth in serum-free, endothelial cell-conditioned medium was confined to one cell doubling.

A possible interpretation of these data is that endothelial cells store and release residual platelet-derived growth factor present in PDS. This was tested by examining the growth-promoting activity of serum-free medium as a function of the time during which the medium had been exposed to endothelial cells. Activity was first detected after 2 h and increased linearly over 72 with no evidence of diminution (Fig. 5). Growth-promoting activity released by endothelial cells into serum-free medium was also measured as a function of serum concentration in the media to which the cells had been previously exposed. These experiments were performed with cells that had been initially isolated and passaged only in PDS. After 48 h of incubation in medium containing concentrations of PDS ranging between 0.1 and 30%, cells were refed with serum-free medium. The level of activity detected in the serum-free medium was independent of the concentration of PDS to which the endothelial cells had been previously exposed.

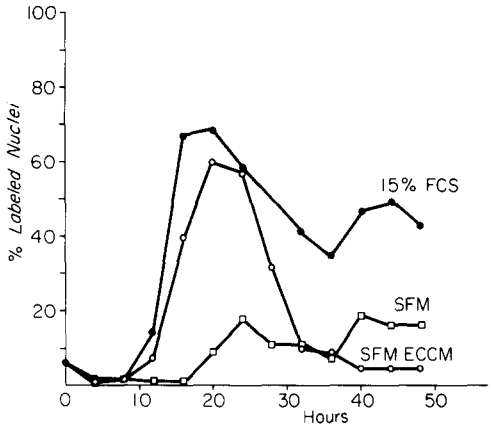

FIGURE 3 Stimulation of smooth muscle cell replication by endothelial cell conditioned medium. Bovine smooth muscle cells were plated at 5×10^3 cells/cm^2 in 15% fetal calf serum. This medium was removed, cultures washed with PBS, and cells brought to quiescence by 24-h exposure to fresh serum-free medium. Test media were added. Percent labeled nuclei represents a 4-h exposure to 1 μCi/ml [^{3}H]thymidine as determined by autoradiography: □ SFM: serum-free medium; ● 15% FCS: medium containing 15% fetal calf serum; ○ SFM ECCM: serum-free medium preincubated for 48 h with endothelial cells and diluted 1:1 with fresh serum-free medium.

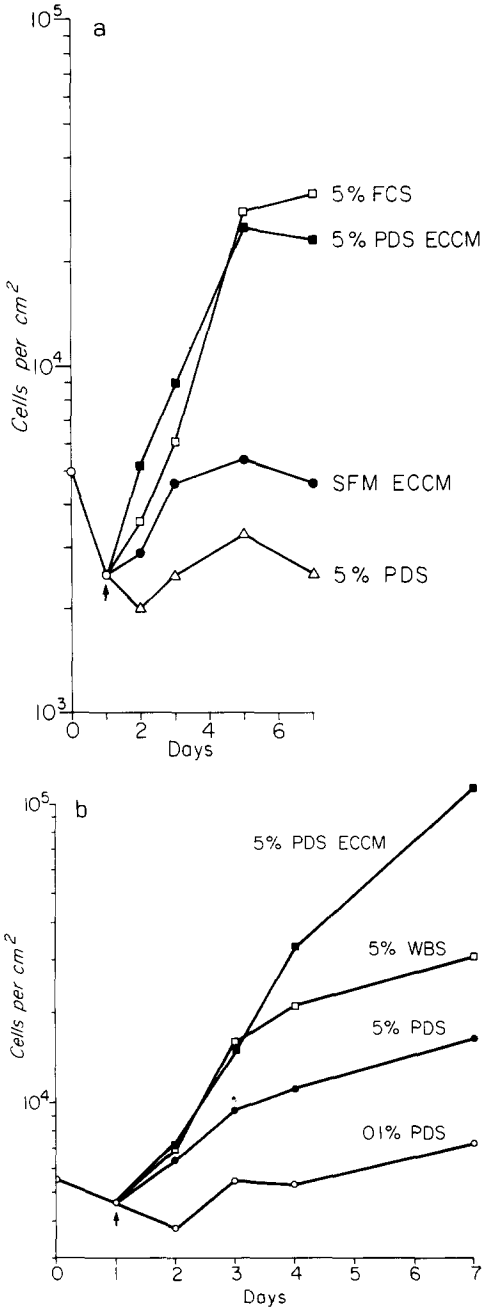

FIGURE 4 Stimulation of growth of smooth muscle cells (*a*) and 3T3 cells (*b*) by endothelial cell conditioned media. Cells were plated at 5×10^3 cells/cm^2 in medium containing 5% fetal calf serum. Test media were added at points indicated by arrows and media were replaced every other day. (*a*) □ 5% FCS: medium containing 5% fetal calf serum; ■ 5% PDS ECCM: endothelial cell conditioned medium containing 5% PDS; ● SFM ECCM: endothelial cell conditioned serum-free medium; △ 5% PDS: medium containing 5% PDS. (*b*) □ 5% WBS: medium containing 5% whole blood serum; ■ 5% PDS ECCM: endothelial cell conditioned medium containing 5% PDS; ● 5% PDS: medium containing 5% PDS; ○ 0.1% PDS: medium containing 0.1% PDS.

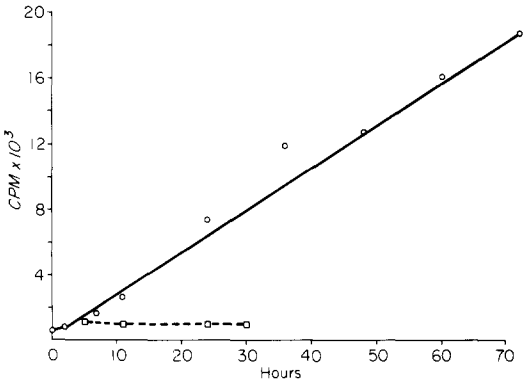

FIGURE 5 Growth-promoting activity in medium as function of time. Cultures of endothelial cells and smooth muscle cells were grown to saturation density in media containing 5% PDS and 15% fetal calf serum, respectively. Cells were rinsed in PBS and fed with serum-free medium. Medium was collected from replicate cultures at times indicated and activity was determined by use of the 3T3 assay as described in Materials and Methods. ○: serum-free endothelial cell conditioned medium; □: serum-free smooth muscle cell conditioned medium.

Similarly, endothelial cells grown in either 10% PDS or 10% whole blood serum released equivalent levels of activity when incubated for 48 h in serum-free medium.

Preliminary data indicate that this activity is caused by a polypeptide with characteristics different from those of the platelet-derived growth factor. In a 24-h dialysis of serum-free conditioned medium against an equal volume of nonconditioned, serum-free medium, all of the activity was recovered inside the dialysis tubing. No activity was found outside the tubing. Similarly, activity within the tubing was not lost when conditioned medium was dialyzed against three changes of medium at a ratio of 1:10. Growth-promoting activity, as determined by thymidine incorporation into 3T3 cells, was completely destroyed after incubation with 50 μg/ml trypsin for 1 h. Growth-promoting activity of lyophilized, conditioned serum-free medium, resuspended in PBS, was heat stable at 56°C for 30 min but lost 85% of its activity at 100°C for 4 min. Serum-free conditioned medium, incubated between pH 3 and 10 for 24 h, showed no loss of growth-promoting

activity. When concentrated serum-free conditioned medium was eluted from a Biogel P-150 column (Bio-Rad Laboratories, Richmond,Calif.) with 1 M acetic acid, activity was recovered in a mol wt range between 10,000 and 30,000. The activity was not retained by CM Sephadex under conditions in which the platelet-derived growth factor is absorbed to the column (11, 33).

DISCUSSION

This report provides evidence that aortic endothelial cells in culture produce a polypeptide growth factor or factors with mol wt of ~10,000–30,000. Although growth factors have been described in various biological fluids, have been isolated from tissues, and have been identified in the culture fluids of established cell lines, this observation represents production by a nonestablished cell strain of defined cell type (9). Activity has also been found in medium conditioned by monkey aorta endothelium (unpublished data) and human umbilical vein endothelium (unpublished data). Preliminary reports of the results presented here and similar data by Fass et al., in porcine endothelium, have been published in abstract form (5, 7).

Conditioning effects of other nontransformed cells in culture either can be attributed to low molecular weight substances (15, 16, 18) or are restricted to production of growth-promoting activity only in the presence of plasma (13). When plasma is present, it is difficult to determine whether the cells are acting to alter components in the medium rather than producing the factor themselves. The growth-promoting activity of endothelial cell-conditioned medium is detectable both in cell-free plasma-derived serum and in serum-free medium. Our observations show that the factor is produced by endothelial cells rather than stored and released from serum because activity increases linearly in serum-free medium over a period of 72 h.

This mitogenic factor has characteristics that are different from those of the platelet-derived growth factor (1, 11, 22, 33, 36). The endothelial cell-derived growth factor is unstable at 100°C for 4 min and is not absorbed by CM Sephadex. Also, the mitogenic activity is produced by endothelial cells even after multiple passages in cell-free PDS.

If this growth factor is produced by endothelial cells in vivo, it could serve a number of possible functions. The endothelium might act as an endocrine organ, either in the synthesis of circulating factors or in modulation of factors as they interact with the vessel wall. Functions of this sort have been identified for the synthesis of prostaglandins and for activation of angiotensin (8, 23, 29, 35). An endothelial cell-derived growth factor, if regulated by local events, could play an important role in the process of neovascular formation and in atherosclerosis. For example, endothelial cells are the first cell of the vessel wall to appear in new vessel formation (2, 6, 24). Smooth muscle cells appear later, suggesting a possible role for endothelial cell mediators in the induction of smooth muscle cell movement and differentiation. Smooth muscle cell proliferation plays a central role in the formation of the lesions of atherosclerosis (21). Recently, much emphasis has been given to the release of the platelet-derived growth factor at sites of endothelial cell injury and thrombosis in the pathogenesis of smooth muscle proliferation in atherosclerosis (22). The endothelial cell-derived growth factor may contribute to this process as well.

This work was supported in part by grants from U.S. Public Health SErvice HL 18645, HL 97312, RR 00166, and Am 13970, and from the R. J. Reynolds Industries, Inc. Dr. DiCorleto is supported by a fellowship from the American Heart Association of Washignton. Dr. Schwartz is an Established Investigator of the American Heart Association.

Received for publication 2 November 1979, and in revised form 1 February 1980.

REFERENCES

1. ANTONIADES, H. M., D. STATHKOS, and C. D. SCHER. 1975. Isolation of a cationic polypeptide from human serum that stimulates proliferation of 3T3 cells. *Proc. Natl. Acad. Sci. U. S. A.* **72**:2635–2639.
2. CLARK, E. R., and E. L. CLARK. 1935. Observations on changes in blood vascular endothelium in the living animal. *Am. J. Anat.* **57**:385–438.
3. COTTA-PERRIERA, G., H. SAGE, P. BORNSTEIN, R. ROSS, and S. M. SCHWARTZ. 1979. Studies of morphologically atypical "sprouting" cultures of bovine aortic endothelial cells. Growth characteristics and connective protein synthesis. *J. Cell. Physiol.* In press.
4. DAVIES, P. F., and R. ROSS. 1978. Mediation of pinocytosis in cultured arterial smooth muscle and endothelial cells by platelet-derived growth factor. *J. Cell Biol.* **79**:663–671.
5. FASS, D. N., M. R. DOWNING, P. MEYERS, E. J. W. BOWIE, and L. D. WITTE. 1978. A mitogenic factor from porcine arterial endothelial cells. 32nd Annual Meeting, Council on Atherosclerosis. American Heart Association. 11.
6. FOLKMAN, J., and R. COTRAN. 1976. Relation of vascular proliferation to tumor growth. Int. Rev. Exp. Pathol. **16**:207–248.
7. GAJDUSEK, C. M., S. M. SCHWARTZ, P. DICORLETO, and R. ROSS. 1979. Endothelial cell derived mitogenic activity. *Fed. Proc.* **38**:1075.
8. GIMBRONE, M. A., JR. 1974. Culture of vascular endothelium. *In* Progress in Hemostasis and Thrombosis. Vol. III T. H. Spaet, editor. Grune & Stratton, Inc., New York. 1–28.
9. GOSPODAROWICZ, D., and J. MORAN. 1976. Growth factors in mammalian cell culture. *Annu. Rev. Biochem.* **45**:531–558.
10. HAUDENSCHILD, C. C., D. ZAHNISER, J. FOLKMAN, and M. KLAGSBURN. 1976. Human vascular endothelial cells in culture: lack of response to serum growth factors. *Exp. Cell Res.* **98**:175–183.
11. HELDIN, C. H., A. WASTESON, and B. WESTERMARK. 1977. Partial

purification and characterization of platelet factors stimulating the multiplication of normal human glial cells. *Exp. Cell Res.* **109:**429–437.
12. HOLLEY, R. W. 1975. Control of growth of mammalian cells in culture *Nature (Lond.)* **258:**487–490.
13. LEIBOVICH, S. J., and R. ROSS. 1976. A macrophage-dependent factor that stimulates the proliferation of fibroblasts *in vitro*. *Am. J. Pathol.* **84:**501–514.
14. MCAUSLAN, B. R., W. REILLY, AND G. N. HANNAN. 1979. Stimulation of endothelial cell proliferation by precursors of thymidylate. *J. Cell Physiol.* **100:**87–94.
15. MELBYE, S. W., and M. A. KARASEK. 1973. Some characteristics of a factor stimulating skin epithelial cell growth *in vitro*. *Exp. Cell Res.* **79:** 279–286.
16. MILLIS, A. J. T., M. HOYLE, and B. Field. 1977. Human fibroblast conditioned media contain growth promoting activity for low density cells. *J. Cell Physiol.* **93:**17–24.
17. PLEDGER, W. J., C. D. STILES, H. M. ANTONIADES, and C. D. SCHER. 1977. Induction of DNA synthesis in BALB/c 3T3 cells by serum components: re-evaluation of the commitment process. *Proc. Natl. Acad. Sci U. S. A.* **74:**4481–4485.
18. POHJANPELTO, P., and A. RAINA. 1972. Identification of a growth factor produced by human fibroblasts *in vitro* as putrescine. *Nat. New Biol.* **235:**247–249.
19. POLLACK, R., and A. VOGEL. 1973. Isolation and characterization of revertant cell lines. II. Growth control of a polyploid revertant line derived from SV-40 transformed 3T3 mouse cells. *J. Cell. Physiol.* **82:** 93–100.
20. ROSS, R. 1971. The smooth muscle cell. II. Growth of smooth muscle in culture and formation of elastic fibers. *J. Cell Biol.* **50:**172–186.
21. ROSS, R., and J. GLOMSET. 1976. The pathogenesis of atherosclerosis. *N. Engl. J. Med.* **295:**369–377, 420–425.
22. ROSS, R., A. VOGEL, P. DAVIES, E. RAINES, B. KARIYA, M. J. RIVEST, C. GUSTAFSON, and J. GLOMSET. 1979. The platelet-derived growth factor and plasma control cell proliferation. *In* Hormones and Cell Culture. Cold Spring Habor 3rd Conference on Cell Proliferation, Vol. 6. R. Ross and G. Sato, editors. Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y. 3–16.
23. RYAN, J. W., and U. S. RYAN. 1977. Is the lung a para-endocrine organ? *Am. J. Med.* **63:**505–603.
24. SCHOEFL, G. A. 1963. Studies on inflammation. III. Growing capillaries: their structure and permeability. *Virchows Arch. Abt. A. Pathol. Anat.* **337:**97–141.
25. SCHWARTZ, S. M., C. M. GAJDUSEK, M. A. REIDY, S. C. SELDEN, and C.C. HAUDENSCHILD. 1979. Maintenance of integrity in aortic endothelium. *Fed. Proc.* In press.
26. SCHWARTZ, S. M., S. C. SELDEN, and P. BOWMAN. 1978. Growth control in aortic endothelium at wound edges. *In* Hormones and Cell Culture. Cold Spring Harbor 3rd Conference on Cell Proliferation, Vol. 6. R. Ross and G. Sato, editors. Cold Spring Harbor Laboratory., Cold Spring Harbor, N. Y. 593–611.
27. SCHWARTZ, S. M. 1978. Selection and characterization of bovine aortic endothelial cells. *In vitro. (Rockville).* **14:**966–980.
28. TEMIN, H. M. 1967. Control by factors in serum of multiplication of uninfected cells and cells infected and converted by avian sarcoma viruses. *In* Growth Regulating Substances for Animal Cells in Culture. Wistar Institute Symposium Monograph 7. V. Defendi and M. Stoker, editors. Wistar Institute Press, Philadelphia. 103–116.
29. THORGEIRSSON, G., and A. L. ROBERTSON, JR. 1978. The vascular endothelium-pathobiologic significance. *Am. J. Pathol.* **93:**803–848.
30. THORGEIRSSON, G., and A. L. ROBERTSON, JR. 1978. Platelet factors and the human vascular wall. Part 2. Such factors are not required for endothelial cell proliferation and migration. *Atherosclerosis.* **31:**231–238.
31. TODARO, G. J., G. K. LAZAR, and H. GREEN. 1965. The initiation of cell division in a contact-inhibited mammalian cell line. *J. Cell. Comp. Physiol.* **66:**325–334.
32. TODARO, G., Y. MATSUYA, S. BLOOM, A. ROBBINS, and H. GREEN. 1967. Stimulation of RNA synthesis and cell division in resting cells by a factor present in serum. *In* Growth Regulating Substances for Animal Cells in Culture, Wistar Institute Symposium Monograph 7. V. Defendi and M. Stoker, editors. Wistar Institute Press, Philadelphia. 87–101.
33. VOGEL, A., E. RAINES, B. KARIYA, M. RIVEST, and R. ROSS. 1978. Coordinate control of 3T3 cell proliferation by platelet derived growth factor and plasma components. *Proc. Natl. Acad. Sci. U. S. A.* **75:**2810 2814.
34. WALL, R. T., L. A. HARKER, L. J. QUADRACCI, and G. E. STRIKER. 1978. Factors influencing endothelial cell proliferation *in vitro*. *J. Cell. Physiol.* **96:**203–214.
35. WEKSLER, B. B., A. J. MARCUS, and E. A. JAFFE. 1977. Synthesis of prostaglandin I_2 (prostacyclin) by cultured human and bovine endothelial cells. *Proc. Natl. Acad. Sci. U. S. A.* **74:**3922–3926.
36. WESTERMARK, B., and A. WASTESON. 1976. A platelet factor stimulating human normal glial cells. *Exp. Cell Res.* **98:**170 174.