Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 1641–1645, March 1983
Cell Biology

Dialyzable factor in human serum of platelet origin stimulates endothelial cell replication and growth

(atherosclerosis/mitogen/growth factor/repair)

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Communicated by K. M. Brinkhous, December 13, 1982

ABSTRACT Porcine aortic endothelial cells were isolated and maintained in Dulbecco's modified Eagle's medium (DME medium)/10% citrate-treated human plasma. They were stimulated by DME medium/10% human serum to grow from a density of 10,100 ± 500 per well to a final density of 83,000 ± 1,800 per well over a 9-day period. On the other hand these cells grew poorly (11% increase) in DME medium/10% human platelet-poor plasma prepared without chelating agents and containing platelet factor 4 at 18 ng/ml by radioimmunoassay. Dialysis of the human serum (M_r cutoff, 3,500) eliminated all the stimulatory activity. The activity recovered from the dialysate stimulated growth when added to endothelial cultures in conjunction with either dialyzed serum or platelet-poor plasma alone. The dialyzable factor could be obtained directly from platelets; both acetic acid extracts and boiled NaCl extracts stimulated porcine aortic endothelial cell replication. Gel filtration chromatography on Sephadex G-15 showed that the endothelial growth factor had a molecular weight of 700. Partially purified material induced a concentration-dependent stimulation of porcine aortic endothelial cell replication in the presence of DME medium alone; however, simultaneous incubation with platelet-poor plasma resulted in a much greater response. Fibroblast growth factor isolated from bovine brain was found to be mitogenic only in the presence of nondialyzed serum or of the dialyzable factor together with plasma. In the absence of this serum factor, fibroblast growth factor had no effect. We conclude that human serum contains a potent endothelial cell mitogen of platelet origin. Human plasma that is devoid of platelet content does not stimulate endothelial cell growth. This growth factor may be an important stimulant of the endothelial cell response to vascular wall injury.

Vascular endothelial cells form a selective barrier to the transport of nutrients and macromolecules to subendothelial tissues (1). Disruption of this single layer of endothelial cells results in loss of the barrier function and exposure of both the damaged endothelium and the subendothelium to plasma molecules (2). Platelets immediately adhere to subendothelial substances such as collagen, and this may result in subendothelial exposure to released platelet contents (3). Platelets have been shown to release platelet-derived growth factor, a potent mitogen for smooth muscle cells (4), and fibroblasts (5). Because damaged endothelial cells are directly exposed to platelet contents, one would expect that growth factors released after platelet adhesion might have potent stimulatory effects for this cell type. Repair of endothelium is not believed to be dependent on platelet-derived growth factors, however, since dialyzed serum supplements do not have greater mitogenic activity than citrated plasma for cultured vascular endothelial cells (6, 7). However, like serum, citrated plasma contains greater concentrations of platelet factor 4 (PF4) (8) than EDTA-treated plasma, indicating that platelet release of α granules has occurred and that intracellular factors have been released. The presence of one or more of these factors might obscure presumed differences between citrated plasma and serum. Furthermore, dialysis of citrated plasma or serum reduces their mitogenic activity for endothelial cells when compared with nondialyzed serum (9). It is possible, therefore, that results obtained by comparing dialyzed citrated plasma and serum are not strictly applicable as an *in vivo* model of vascular injury. We, therefore, undertook the present study to make a direct comparison between nondialyzed serum and plasma that contained minimal or no platelet content and to determine whether serum contains an endothelial cell mitogen that can be removed by dialysis. Since platelet adhesion to vessel walls may be an important determinant of the response to vascular injury, we also investigated whether platelets were the source of this endothelial mitogen.

MATERIALS AND METHODS

Preparation of Plasma and Serum. Citrated human plasma (16 mM Na citrate/7 mM citric acid) was purchased from the American Red Cross. One liter of plasma was centrifuged at 1,800 × *g* for 15 min, and the supernatant was centrifuged at 22,000 × *g* for 30 min to remove residual platelet contents. The supernatant was recalcified by adding 20 ml of 1.0 M $CaCl_2$ and incubating the solution at 37°C for 2 hr. After clot formation, the solution was centrifuged at 22,000 × *g* for 30 min and the supernatant was dialyzed against 13 liters of Ringer's bicarbonate buffer (pH 7.4) for 24 hr at 4°C. Extensively dialyzed citrated plasma was prepared by heating this material to 37°C for 24 hr and dialyzing against Ringer's bicarbonate buffer (pH 7.4) for 5 days at 4°C. Citrated platelet-rich plasma was obtained from outdated platelet packs containing 10 units of platelets and 40 ml of residual plasma. The plasma was separated from the platelets by centrifugation and prepared by a method identical to that used to prepare citrated plasma. EDTA- and oxalate-treated plasma specimens were prepared by placing freshly drawn blood obtained from normal adult donors in glass tubes containing 150 mM EDTA or 10 mM Na oxalate and centrifuging at 1,800 × *g* for 15 min. The plasma was recalcified by adding 1.0 M $CaCl_2$ to a final Ca^{2+} concentration of 20 mM. It was then centrifuged at 1,800 × *g* for 15 min and the supernatant was subsequently heated to 56°C for 30 min. Heparin-treated plasma was obtained by placing freshly drawn blood in a 10-ml prechilled (4°C) glass tube and then adding 143 units of heparin and centrifuging at 1,800 × *g* for 15 min. Human serum was prepared by placing freshly drawn blood in glass tubes, allowing it to clot, and centrifuging it for 15 min at 1,800 × *g*. The supernatant was stored at −20°C until use. Dialyzed serum was prepared by using

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Abbreviations: PF4, platelet factor 4; DME medium, Dulbecco's modified Eagle's medium; FGF, fibroblast growth factor; PDECM, platelet-derived endothelial cell mitogen.

Spectropor 3 membranes (Spectrum Medical Industries) (M_r cutoff, 3,500) and dialyzing for 24 hr at 4°C with three exchanges of Ringer's bicarbonate buffer (pH 7.4) [ratio, 1:100 (vol/vol)]. Human platelet-poor plasma was obtained by placing freshly drawn blood in prechilled (4°C) plastic tubes, immediately centrifuging at 1,800 × *g* for 15 min, and then centrifuging the supernatant at 22,000 × *g* for 30 min. The fibrinogen was precipitated with broken glass and the solution was clarified by centrifugation. The supernatant was heat inactivated at 56°C for 30 min. The PF4 contents of the plasma preparations were determined by radioimmunoassay (10) (carried out by G. White). The limit of detectability in this assay is <1 ng/ml and the PF4 concentration in EDTA plasma is 0–15 ng/ml.

Purification of Endothelial Cell Growth Factor. Platelet-rich plasma was obtained from outdated (10-unit) platelet packs (American Red Cross), each containing 40 ml of residual plasma. The platelet/plasma mixture (400 ml) derived from 100 units of platelets was incubated at 37°C for 4 hr and then filtered through a YM-5 filter (Amicon). Fifty milliliters of the filtrate was acidified with 1 M acetic acid (1:1) and lyophilized to dryness. The lyophilized material was reconstituted in 2.0 ml of 0.5 M acetic acid; 1.0 ml was applied to a Sephadex G-15 (Sigma) column (90 × 1.6 cm) and eluted with 0.5 M acetic acid (pH 2.9) at a flow rate of 1.0 ml/min. The column was calibrated using bovine serum albumin, thyrotropin-releasing hormone (TRH), and $Na^{125}I$. The 2.0-ml fractions that were collected were also lyophilized to dryness. These fractions were reconstituted in 2.0 ml of Dulbecco's modified Eagle's (DME) medium and their activity was determined by their capacity to stimulate [^{3}H]thymidine incorporation into quiescent porcine endothelial cells.

Preparation of Platelet Extracts for Biological Studies. NaCl platelet extracts were prepared by centrifuging 6 units of outdated platelet plasma mixture at 10,000 × *g* for 30 min to remove residual plasma. The platelet pellet was suspended in 2 vol of 0.01 M Na_2HPO_4/0.9 M NaCl, pH 7.4, and freeze-thawed three times. The suspension was centrifuged at 5,000 × *g* for 30 min and the supernatant was tested for mitogenic activity. Boiled extracts were prepared by heating the NaCl extract to 100°C for 15 min, allowing the extract to cool to 24°C, and centrifuging it at 5,000 × *g* for 10 min. Acetic acid extracts of platelets were obtained by suspending the pellet from 6 units of outdated platelets in 2 vol of 0.5 M acetic acid, pH 2.9, and stirring for 14 hr at 4°C. The suspension was centrifuged at 5,000 × *g* for 30 min and the supernatant was neutralized to pH 7.4 with 10 M NaOH.

Preparation of Fibroblast Growth Factor (FGF). FGF was purified from bovine brain extracts by a published method (11). The purification procedure was monitored by testing the capacity of column fractions to stimulate DNA synthesis in BALB/c 3T3 cells (12). At 55 ng/ml, the partially purified material stimulated [^{3}H]thymidine incorporation equal to 10% bovine serum.

Isolation of Endothelial Cells. Porcine aortic endothelial cells were isolated as described (13). Aortas from young piglets were incubated for 30 min at 37°C in DME medium (Flow Laboratories)/0.1% type II collagenase (Worthington, lot 42C074; 126 units/mg). The endothelial cells that detached were plated in two 10-cm dishes (Falcon) in DME medium/20% bovine serum (Colorado Serum, Denver, CO). After attachment (6–8 hr), the medium was removed and replaced with DME medium/10% citrated human plasma. The cells were removed from the plates with 0.5% trypsin/0.2% EDTA (GIBCO) and subcultured every 10–15 days at a split ratio of 1:3. Cells processed in this manner formed a homogenous monolayer, had double nucleoli, had no myofilaments by electron microscopy, and stained for factor VIII-related antigen (14). There was no evidence of transformation or loss of the endothelial monolayer (15, 16) under these conditions for periods of up to 6 wk.

Assessment of Growth of Cultured Endothelial Cells. For growth experiments, porcine endothelial cells were subcultured at a plating density of 10,000 cells per well in 1.5-cm 24-well plates (Falcon) using DME medium/10% citrated plasma. The cells were incubated for 4 hr to allow attachment, and then the medium was removed and the test media were added. After a 72-hr incubation, the media were aspirated from duplicate wells for determination of cell number. The cells were removed by incubating for 10 min in 0.5% trypsin/0.2% EDTA. The cell suspension was withdrawn and added to 9.5 ml of 0.15 M NaCl and cell number was determined in a particle counter (Coulter model ZBI). The remaining wells received fresh test media and this procedure was repeated every 3 days.

[^{3}H]Thymidine Incorporation. To assess DNA synthesis, porcine endothelial cells were plated in microtest wells (Falcon) at 5,000 cells per well in DME medium/3% platelet-poor plasma (total vol, 200 μl). After 3 days, test materials were added directly with 0.5 μCi of [*methyl*-^{3}H]thymidine (6 Ci/mmol; 1 Ci = 37 GBq) (Schwartz-Mann). Test mixtures were incubated for 36 hr, and wells were washed twice with Ringer's bicarbonate (pH 7.4) at 4°C and twice with 5% trichloroacetic acid. The DNA was extracted with two 0.2-ml portions of 0.1 M NaOH/1% $NaDodSO_4$, and the radioactivity was determined by liquid scintillation counting.

RESULTS

Porcine Endothelial Cell Growth Requires a Dialyzable Serum Factor. Porcine aortic endothelial cells that had been maintained in medium containing 10% citrated human plasma for three passages were plated at 10,000 cells per well and their growth rate was determined. Cultures exposed to 10% human serum grew from a density of 10,100 cells per well to a density of 83,000 cells per well over the 9-day test period. On the other hand, cells exposed to 10% platelet-poor plasma remained nearly stationary over this interval (11% increase) (Fig. 1). Dialysis of serum removed the growth-stimulating activity (Fig. 2). The serum dialysate was concentrated 1,000-fold by evaporation to the original serum volume (1.0 ml), and addition of 0.05 ml of this concentrate to 0.5 ml of DME medium/10% dialyzed platelet-poor plasma gave medium that stimulated endothelial cell growth to a final cell density comparable with that of normal serum (Fig. 2). These results are consistent with the hypothesis that the mitogenic factor is removed by dialysis rather than inactivated.

The Dialyzable Factor Is of Platelet Origin. Because serum supported growth much more effectively than plasma, it appeared that the dialyzable mitogen might be derived from platelets. Therefore, outdated human platelet concentrates were extracted under a variety of conditions. Extracts prepared by using 0.5 M acetic acid stimulated [^{3}H]thymidine incorporation 122% in the presence of 3% platelet-poor plasma (Table 1). An extract prepared with 0.15 M NaCl was inhibitory; however, after it was boiled, there was a 73% increase in DNA synthesis. To distinguish between the possibility of inhibitor inactivation by boiling versus generation of some other stimulant from an inactive form, nonboiled 0.15 M NaCl extracts were dialyzed or sequentially dialyzed and boiled. Neither of these maneuvers resulted in a significant increase in mitogenic activity. A mixing experiment was carried out in which the nonboiled 0.15 M NaCl extract was mixed (1:1) with human serum and assayed at a final concentration of 10% serum. This concentration of platelet extract was found to cause 86% inhibition of serum-stimulated replication. These results indicated that the endothelial mitogen was not

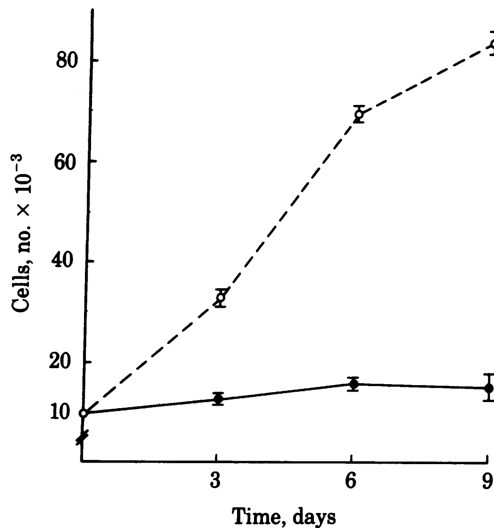

FIG. 1. Growth of porcine endothelial cells in human serum and platelet-poor plasma. Cells were plated at a density of 10,000 cells per well in DME medium/10% citrated human plasma, allowing 4 hr for attachment. Then, the medium was removed and fresh DME medium containing either 10% serum (○) or 10% platelet-poor plasma (●) (PF4 content, <6 ng/ml) was added. At 72-hr intervals, trypsinized cells in triplicate wells were counted and the remaining wells received fresh medium containing serum or plasma. Results are mean ± 1 SD of three determinations.

generated by heating and that it was detectable only after destruction of inhibitory substances present in the crude platelet extract.

Because citrated plasma is rich in platelet contents, several other plasma preparations were compared for growth-stimulating activity. Recalcified oxalate-, EDTA-, and heparin-treated plasmas (PF4 values, <18 ng/ml) and platelet-poor plasma were tested for their PF4 content and their capacity to stimulate endothelial cell replication (Table 2). Each was found to have stimulatory activity that correlated directly with the PF4 content of

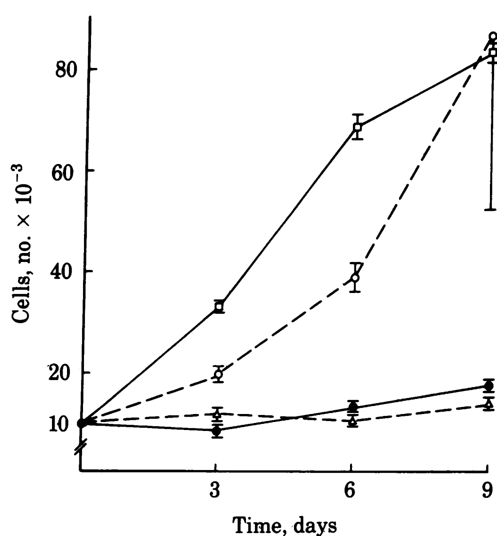

FIG. 2. Growth of porcine endothelial cells in dialyzed human serum. Cells were plated as in Fig. 1. After attachment, the cultures were exposed to DME medium containing 10% nondialyzed human serum (□), 10% dialyzed human serum (△), 10% platelet-poor plasma (●), or 10% concentrated dialysate/10% platelet-poor plasma (○) (final vol, 0.5 ml). At 72-hr intervals, cells in triplicate wells were counted and the remaining wells received fresh DME medium and test serum or plasma.

Table 1. Stimulation of [^{3}H]thymidine incorporation into porcine endothelial cells by platelet extracts

Factor	% increase over control
Human serum (10%)	255
Platelet-poor plasma (5%)	11
0.5 M acetic acid platelet extract	122
0.15 M NaCl platelet extract	−31
Boiled 0.15 M NaCl platelet extract	73
Dialyzed 0.15 M NaCl platelet extract	−8
Dialyzed then boiled 0.15 M NaCl platelet extract	2
0.15 M NaCl platelet extract/10% human serum*	37

Quiescent porcine endothelial cells were plated at 5,000 cells per well. After cell attachment, each well received 200 μl of DME medium/3% platelet-poor plasma. After a 3-day incubation, 0.5 μCi of [^{3}H]thymidine and 5 μl of test material were added directly to each well. After 48 hr, [^{3}H]thymidine incorporation was determined. Control cultures were exposed to 3% platelet-poor plasma alone.

* The 0.15 M NaCl extract was added to an equal volume of human serum and the mixture was incubated at 24°C for 30 min and then added to the endothelial cultures to give a final human serum concentration of 10%.

that specific plasma preparation. Citrated platelet-rich plasma caused the greatest increase in [^{3}H]thymidine incorporation (290%). Both incubation at 37°C for 24 hr and exhaustive dialysis [i.e., a ratio of 1:100 (vol/vol) with six exchanges] were required to remove the stimulatory factor(s) from citrated plasma.

Properties of the Endothelial Mitogen. A molecular weight estimate of the platelet-derived endothelial mitogen (PDECM) was obtained by gel filtration chromatography. One milliliter of concentrated platelet-rich plasma filtrate was applied to a Sephadex G-15 column (90 × 1.6 cm). The growth-promoting activity eluted at a distribution coefficient (Kd) of 0.5 corresponding to a molecular weight of approximately 700 (Fig. 3). It was estimated that approximately 62% of the biological activity was recovered in the active fractions and that the material had been purified 15-fold over filtered platelet-rich plasma. The active fractions (Kd, 0.46–0.54) were pooled and further tested for stimulatory activity. This partially purified extract from platelets stimulated a concentration-dependent increase in porcine endothelial cell replication when tested in the presence of DME medium/3% platelet-poor plasma (Fig. 4). In the presence of DME medium alone (no platelet-poor plasma), PDECM induced much lower increases in thymidine incorporation at each concentration tested. Therefore, incubation with platelet-poor plasma enhanced the activity of PDECM. PDECM-induced

Table 2. Effect of plasma preparations on endothelial cell replication

Plasma type	PF4 content, ng/ml	% increase over control
Citrated	125	101
Platelet rich (citrated)	850	290
Oxalate treated	8	5
Heparin treated	18	11
EDTA treated	5	−2
Platelet poor	21	16
Extensively dialyzed citrated*	106	12

Plasmas were used at 10%. Control cultures were incubated with 3% platelet-poor plasma for 3 days and received only [^{3}H]thymidine at the initiation of the experiment.

* Citrated plasma was incubated at 37°C for 24 hr and then dialyzed against Ringer's bicarbonate [ratio, 1:100 (vol/vol)] for 5 days at 4°C. PF4 has a molecular weight of 8,000 and is nondialyzable when using Spectrapor 3 tubing.

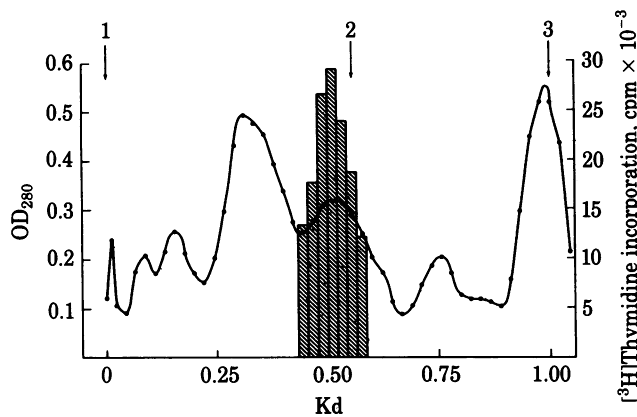

FIG. 3. Gel filtration chromatography of the endothelial cell growth factor. One-milliliter of a concentrated filtrate of platelet-rich plasma was applied to a Sephadex G-15 column that had been equilibrated with 0.5 M acetic acid (pH 2.9). The column (1.6 × 90 cm) was eluted at a flow rate of 1.0 ml/min, and 2.0 ml fractions were collected. The fractions were lyophilized to dryness and reconstituted in 2.0 ml of DME medium. The activity in each fraction was determined by adding 5 μl of the fraction directly to quiescent porcine endothelial cells in DME medium/3% platelet-poor plasma and measuring [^{3}H]thymidine incorporation after 36 hr. Markers: 1, bovine serum albumin; 2, thyrotropin releasing hormone; 3, ^{125}I. Kd, coefficient of distribution.

stimulation of replication was relatively specific for endothelial cells. When identical concentrations of PDECM were added to cultures of BALB/c 3T3 fibroblasts and human fibroblasts and the results were compared using an identical serum standard, these cell lines were stimulated only 22% and 7% compared with endothelial cell stimulation. No activity was obtained when PDECM was added to smooth muscle cell cultures.

Growth-Promoting Effects of the Endothelial Mitogen. When stimulation of growth was analyzed, PDECM was found to be active in the presence of platelet-poor plasma and dialyzed human serum (Fig. 3). Therefore, partially purified material was capable of stimulating growth as well as replication. In contrast, when FGF at 50 ng/ml was added in the presence of platelet-poor plasma or dialyzed human serum, no growth-promoting activity could be demonstrated (Fig. 5). FGF was active in the presence of undialyzed human serum but required the simultaneous presence of PDECM to be active when incubated with either platelet-poor plasma or dialyzed human serum. In the presence of PDECM, FGF induced a further increase in growth that was greater than that induced by PDECM/platelet-poor plasma or PDECM/dialyzed human serum.

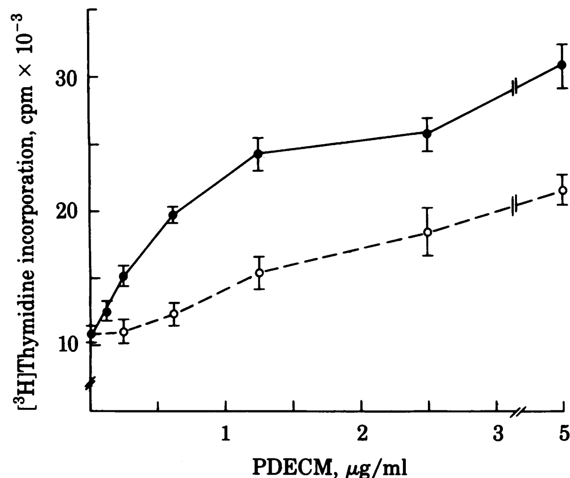

FIG. 4. Concentration-dependent increase in DNA synthesis by PDECM. Partially purified PDECM (see Fig. 3) was added at the concentrations indicated to quiescent endothelial cell monolayers in DME medium/3% platelet-poor plasma (●) or DME medium without platelet-poor plasma (○). Each well contained 0.5 μCi of [^{3}H]thymidine. After a 36-hr incubation, the cells were washed twice with 5% trichloroacetic acid, and the DNA was extracted with 0.1 M NaOH/1% $NaDodSO_4$. [^{3}H]Thymidine incorporation was determined by liquid scintillation counting.

DISCUSSION

The results of this study show that human serum contains a potent stimulant of porcine endothelial cell replication and growth. In contrast, human platelet-poor plasma, prepared to contain minimal concentrations of factors released during platelet aggregation, has virtually no stimulatory effect (Fig. 1). These findings emphasize that the method of plasma preparation is crucial to accurate interpretation of results because our data suggest that the capacity of plasma to stimulate endothelial cell growth is directly proportional to its PF4 content (Table 2). Platelet-rich plasma and citrated plasma that contain high concentrations of PF4 (>100 ng/ml) are much more potent than EDTA-treated or oxalate-treated plasma (PF4, <10 ng/ml). The finding of such a correlation between PF4 content and plasma mitogenicity suggested that platelets were the source of this material. Further evidence to support this hypothesis was obtained when it was noted that both platelet extracts and platelet contents released after degranulation contained a similar mitogenic activity (Table 1). Crude extracts also contained inhibitors of porcine endothelial cell replication that were removed by boiling or acid treatment. Platelets, therefore, release a low molecular weight mitogen that is a potent stimulant of endothelial cell growth.

Dialysis removed the growth-promoting activity from serum and addition of concentrated dialysate to either dialyzed serum or platelet-poor plasma restored endothelial cell growth. These results suggested that the PDECM was of low molecular weight

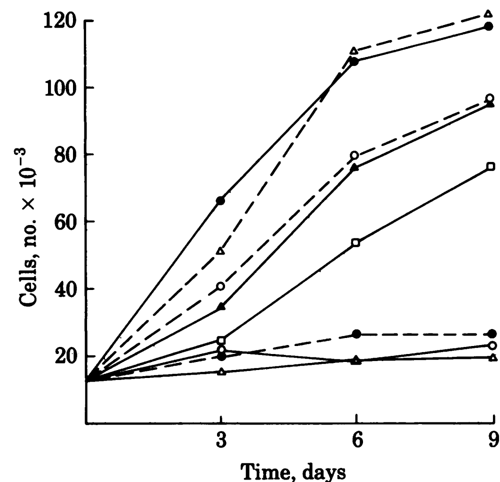

FIG. 5. Effect of FGF on endothelial cell growth. Cells were plated as in Fig. 1 and then exposed to DME medium containing FGF/5% dialyzed human serum/partially purified PDECM (●——●), FGF/5% platelet-poor plasma/PDECM (△– –△), PDECM/platelet-poor plasma (○– –○), PDECM/dialyzed human serum (▲——▲), FGF/human serum (□——□), FGF/platelet-poor plasma (●– –●), platelet-poor plasma (○——○), or FGF/dialyzed human serum (△——△). After 72 hr of incubation, the cells were detached and counted. As in the previous experiments, the remainder of the wells received fresh DME medium together with the test reagent(s) for an additional 72 hr and this was repeated. Concentrations of reagents used: FGF, 50 ng/ml; PDECM, 2.5 μg/ml; sera and platelet-poor plasma, 5%.

and this was confirmed by chromatography on Sephadex G-15, which gave a molecular weight estimate of approximately 700 (Fig. 3). PDECM was also found to be heat and acid stable and, in the presence of platelet-poor plasma, caused a concentration-dependent increase in endothelial cell replication (Fig. 4). The mitogen showed some specificity for endothelial cells since comparable concentrations were much less effective in stimulating DNA synthesis in both BALB/c 3T3 fibroblasts and human fibroblasts. PDECM had no effect on replication of cultured porcine aortic smooth muscle cells.

Partially purified PDECM was approximately 40% as effective in the absence of platelet-poor plasma. Pledger *et al.* (17) and Ross and Vogel (18) have shown that exposure of fibroblasts or smooth muscle cells to both platelet-derived growth factor and platelet-poor plasma results in a synergistic increase in replication. Like these cell types, endothelial cells require factors in platelet-poor plasma for the optimal effects of PDECM to occur (Fig. 5). The availability of PDECM will make it possible to identify the specific plasma components that are required for optimal stimulation of endothelial cell growth.

FGF (9), macrophage growth factor (19), and a recently described endothelial cell growth factor (20) isolated from bovine hypothalamus have all been shown to stimulate endothelial cell growth. Our results show that FGF is inactive in the presence of platelet-poor plasma or dialyzed serum and that stimulation of porcine endothelial cells is dependent on the presence of PDECM. In the presence of PDECM, however, FGF did stimulate endothelial cell growth, confirming that each factor stimulates growth by a different mechanism. Because each of these three factors has been tested in the presence of serum or citrated plasma, it is possible that each requires simultaneous exposure to PDECM.

Citrated plasma that had been dialyzed for 24 hr had mitogenic activity, but extensive dialysis of citrated plasma after incubation at 37°C removed the endothelial cell growth-stimulating activity (Table 2). Several groups have failed to detect an endothelial cell growth-promoting effect of partially purified platelet extracts or serum when compared with citrated plasma (6, 7, 21, 22). In their studies, both platelet extracts and serum were extensively dialyzed or chromatographed prior to testing; therefore, low molecular weight substances would have been at least partially removed. In contrast, inclusion of whole platelets in the incubation medium has been reported to stimulate endothelial cell growth (23, 24). Likewise, nondialyzed bovine serum had significantly greater stimulatory activity for bovine aortic endothelial cells than did extensively dialyzed citrated bovine plasma (9). In summary, these studies support the concept that serum contains an endothelial cell mitogen that is at least partially removed by dialysis. Our observation that crude NaCl platelet extracts inhibit endothelial cell replication is consistent with the findings of Wall *et al.* (6) and does not exclude the possibility that platelets are the source of a mitogen. This conclusion is supported by the observation that boiling or acid extraction removes the inhibitory effects of these extracts.

The results of this study may have important implications for understanding the mechanism of repair of vascular endothelium after injury. Disruption of the integrity of the endothelial cell layer *in vivo* results in platelet deposition and release of various platelet factors. This would result in a high local concentration of platelet mitogens that could initiate replication and growth after wounding. The presence of PDECM together with plasma growth factors would provide an optimal environment for rapid endothelial cell division. Since endothelial cells with histologic characteristics similar to "transformed" appearing cells described *in vitro* are seen after injury *in vivo* (20), this factor might also play a role in initiating the development of these cells after injury.

We gratefully acknowledge the technical assistance of Ms. Debra Shaw. We thank Mrs. Sheila Majors for her help in preparing the manuscript and Drs. Louis Underwood and Herbert Cooper for reviewing it. We thank Dr. Gilbert White for carrying out the PF4 assays and Dr. C. J. Edgell for carrying out the factor VIII antigen immunofluorescence studies. D.R.C. is supported in part by General Clinical Research Center Grant RR00046. This work was supported by U.S. Public Health Service Research Grant HL26309.

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