Proc. Natl. Acad. Sci. USA
Vol. 78, No. 9, pp. 5656–5660, September 1981
Cell Biology

Mitogenic factors present in serum but not in plasma

(chicken heart mesenchymal cells/Rous sarcoma virus/3T3 cells/platelet-derived growth factor/thrombin)

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Communicated by Armin C. Braun, May 26, 1981

ABSTRACT **In culture medium containing heparinized, heat-inactivated, chicken plasma, normal chicken heart mesenchymal cells do not proliferate but their Rous sarcoma virus-infected counterparts proliferate maximally. In medium containing serum derived from chicken whole blood or plasma, on the other hand, normal chicken heart mesenchymal cells proliferate actively, at similar overall rates and to similar extents. The rate and extent of normal cell proliferation are decreased by a factor of approximately ½ with whole blood-derived serum that is heparinized and inactivated; proliferation ceases in plasma-derived serum that is heparinized and inactivated. Heparinization and inactivation of serum does not affect the proliferation of Rous sarcoma virus-infected cells, indicating that this combined treatment eliminates a mitogenic (regulatory) rather than a supportive (nutrient) factor(s) for cell replication. We hypothesize that mitogen(s) is released from plasma protein precursors when plasma clots in the presence of formed elements of the blood or when plasma-derived serum is exposed to cultured cells; heparinization and inactivation, within the framework of this hypothesis, would render nonfunctional the plasma protein precursor(s) from which the mitogen(s) is generated. Alternatively, our data are consistent with the release of two mitogens during blood clotting, one from plasma protein precursors and the other from formed elements of the blood. We also have studied the proliferative behavior of Swiss and BALB/c 3T3 cells in whole blood-derived and plasma-derived human serum. Our studies suggest that the platelet-derived growth factor has an artifactual supportive (nutrient) role, rather than an authentic mitogenic role, in cell replication.**

Between 1971 and 1973, Balk and coworkers (1–3) demonstrated that normal chicken pectoral muscle fibroblasts did not proliferate in low-calcium culture medium containing chicken plasma but proliferated actively when this medium contained chicken serum. On the basis of that observation, it was postulated that a mitogen might be released from plasma protein precursors or from formed elements of the blood during the clotting process and that this mitogen, found in serum but not in plasma, might represent a "wound hormone" capable of initiating cell proliferation at sites of tissue injury. Because of uncertainties inherent in the interpretation of data derived from studies using a culture medium with a low calcium concentration (4), however, further studies of the serum–plasma difference were deferred until a culture system was available in which normal cells were quiescent in a plasma-containing medium with physiological ion concentrations.

Other workers subsequently have claimed that the mitogenic property present in serum, but not plasma, resided in a specific polypeptide "platelet-derived growth factor" (for general reviews, see refs. 5 and 6; for critical reviews, see refs. 7 and 8). Ross and Vogel (5) demonstrated that arterial smooth muscle cells and Swiss 3T3 cells proliferate in medium containing dialyzed serum derived from whole blood but are inactive in medium containing dialyzed and chromatographed (CM-Sephadex) serum derived from plasma (9). The addition of platelet-derived growth factor to the dialyzed and chromatographed serum derived from plasma causes proliferative activity similar to that seen in dialyzed serum derived from whole blood. Scher *et al.* (6) demonstrated that BALB/c 3T3 cells tend not to proliferate under the artificially inhibitory conditions of density-dependent inhibition (8) or culture in low concentrations (3%) of dialyzed, heat-defibrinogenated plasma. Again, replacement of the dialyzed plasma with dialyzed serum or addition of platelet-derived growth factor to the dialyzed-plasma medium caused proliferative activity of the cells. Paradoxically, Westermark and his colleagues (10) found that platelet-derived growth factor alone causes cultured cells to proliferate in the complete absence of any plasma preparation. Similarly, Scher *et al.* (6) stated that "continuous incubation of cells with partially purified platelet growth factor alone or with [dialyzed] plasma alone could induce a significant fraction of the cells to synthesize DNA."

Because the activity of the platelet-derived growth factor has been defined in media containing serum derived from plasma or plasma that have been depleted by dialysis, chromatography, and growth of high-density cells and because this factor is capable of sustaining proliferative activity in the absence of plasma, Balk (7) has argued that the platelet-derived growth factor is not an authentic mitogen but functions merely by compensating for deficiency or absence of the plasma component of the culture medium.

Balk recently described (7) a culture system in which normal chicken heart mesenchymal cells proliferate only sluggishly but in which their rigorously comparable Rous sarcoma virus (RSV)-infected counterparts proliferate maximally. The medium is nutritionally replete and contains ions at physiological concentrations; it also contains heparinized or heat-inactivated (defibrinogenated) chicken plasma. This chicken heart mesenchymal system is free of starvation artifacts like density-dependent inhibition (8) or the use of dialyzed or chromatographed plasma.

We have now used the chicken heart mesenchymal system to study differences in mitogenic properties of chicken serum and plasma. In addition to providing phenomenological data essential to an understanding of this critical serum–plasma difference, these studies have allowed us to perfect the chicken heart mesenchymal system to the point such that normal chicken heart mesenchymal cells show absolute proliferative quiescence but their neoplastic counterparts continue to proliferate maximally. We have also studied the proliferative behavior of Swiss and BALB/c 3T3 cells in media containing whole blood-derived and plasma-derived human serum—i.e., in the

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Abbreviations: RSV, Rous sarcoma virus; PF4, platelet factor 4; EGTA, ethylene glycol bis(β-aminoethyl ether)*N,N,N',N'*-tetraacetic acid.

presence and absence of platelet factors. These latter studies provide confirmatory evidence for the argument (7) that the platelet-derived growth factor is not an authentic mitogen.

MATERIALS AND METHODS

Our basic materials and methods have been described (7). The synthetic medium was modified by the inclusion of uridine and hypoxanthine, each at 1 μM. In order to ensure an adequate number of cells for experiments, each set of primary chicken heart mesenchymal cell cultures was established from the hearts of two 8- to 12-week-old cockerels. Primary cultures were prepared and infected with Schmidt–Ruppin RSV and secondary experimental cultures were seeded by using heparinized (4 units/ml), heat-inactivated (57°C, 35 min) rooster plasma at 5%. Cultures of avian cells were incubated at 41.9°C.

Swiss 3T3 cells were generously provided by H. Green. BALB/c 3T3 cells (clone A31) were generously provided by Bruce Zetter. 3T3 cells were incubated at 37°C and were propagated in Dulbecco's modified Eagle's medium with 10% calf serum. For experiments, 20,000 Swiss or BALB/c 3T3 cells were seeded per 35-mm dish in Dulbecco's modified Eagle's medium containing 3% plasma-derived human serum.

Blood was collected (1) from roosters or from mature men; the specific calcium chelator ethylene glycol bis(β-aminoethyl ether)*N,N,N',N'*-tetraacetic acid (EGTA) was the anticoagulant (10 ml of blood was collected into chilled, sterile, silicone-treated tubes containing 0.6 ml of 25 mM EGTA). The quantity of EGTA used would chelate calcium in slight excess of that present in the plasma component (55–60%) of the blood and yielded absolute anticoagulation.

The following protocol was used to prepare, from single lots of rooster blood, samples of whole blood-derived serum, plasma-derived serum, and plasma; some lots were heparinized or heat-inactivated or both. Approximately one-third of a lot of EGTA-anticoagulated rooster blood was stoichiometrically recalcified (0.15 ml of 100 mM $CaCl_2$ per 10 ml of blood) and allowed to clot overnight at 41.9°C; this yielded *whole blood-derived rooster serum*. The remaining two-thirds of the lot of rooster blood was centrifuged by a standard method (1) to yield EGTA-anticoagulated, *formed element-free plasma*. One half of this EGTA-plasma was recalcified (0.15 ml of 100 mM $CaCl_2$ per 6 ml of plasma) and allowed to clot overnight in polycarbonate centrifuge tubes at 41.9°C; the clot that formed was removed by centrifugation at 60,000 × *g* for 5 hr to yield *plasma-derived rooster serum*. The remaining EGTA-plasma was heparinized or heat-inactivated and recalcified. For heparinization, 0.08 ml of heparin concentrate (10,000 units/ml) was added per 10 ml of serum or plasma (final heparin concentration, 4 units/ml). Heat-inactivation was carried out at 57°C for 35 min. The heat-inactivation treatment denatures and precipitates the fibrinogen in plasma. Three cycles of freezing and thawing are necessary to ensure quantitative precipitation of this denatured fibrinogen. For experimental rigor, all samples of rooster serum were frozen and thawed three times.

Lots of EGTA-anticoagulated human blood were divided into halves. One-half was recalcified and allowed to clot overnight to yield *whole blood-derived human serum*. The other half was distributed into polypropylene tubes and centrifuged at 12,000 × *g* for 10 min at 4°C. The plasma supernatant was aspirated off with a plastic pipette and again centrifuged. This EGTA-anticoagulated, formed element-free plasma was then recalcified in polycarbonate tubes and allowed to clot overnight at 37°C. The clot was removed by centrifugation to yield *plasma-derived human serum*. Whole blood-derived and plasma-derived human sera were assayed for platelet factor 4 (PF4) by the method of Levine and Krentz (11).

Experimental cell proliferation curves were determined over 6- or 8-day periods. Two milliliters of experimental culture medium was used per 35-mm dish; medium was changed on day 2 and on each day thereafter. Cell counts were done with a Coulter electronic cell counter. Standard errors are not presented because they would compromise the clarity of the figures; however, no standard error exceeded half the height of the symbol used to represent a point.

RESULTS AND DISCUSSION

The use of heparinized, heat-inactivated chicken plasma reduced the normal chicken heart mesenchymal cells to absolute proliferative quiescence without affecting proliferation of the RSV-infected cells (Fig. 1). Virtually identical results (data not shown) were obtained with the same plasma preparations at concentrations of 5%. (Untreated plasma cannot be used for experiments because it causes culture media to clot.) The use of heparinized, inactivated plasma thus yields a striking, qualitative difference in the proliferative behaviors of normal and neoplastic chicken heart mesenchymal cells. The fact that this qualitative difference is independent of plasma concentration and is obtained without affecting the rate or extent of proliferation of the RSV-infected cells indicates that the combined treatment of heparinization and inactivation does not affect the growth-supporting or nutrient properties of the plasma. Rather, heparinization plus inactivation appears to eliminate a potential mitogen-generating system.

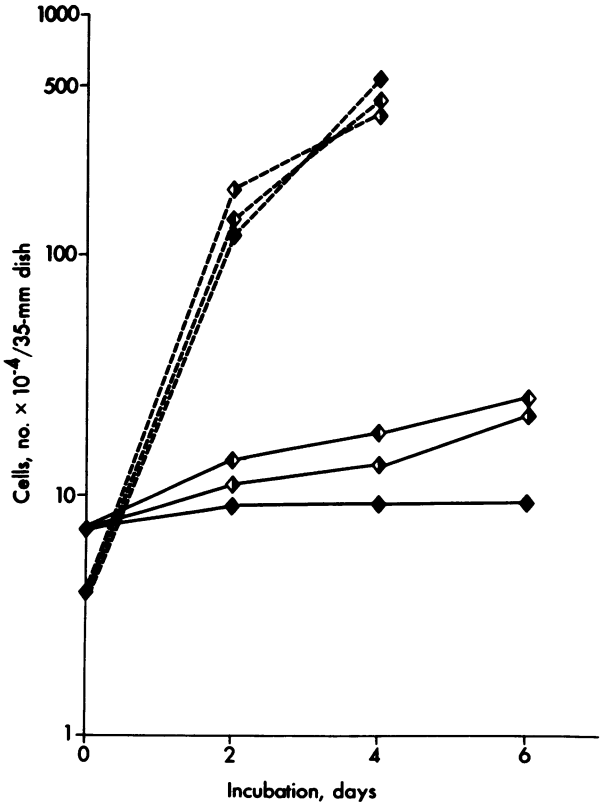

FIG. 1. Proliferative behavior of normal (—) and RSV-infected (---) chicken heart mesenchymal cells in media containing heparinized chicken plasma (◆), heat-inactivated plasma (◆), or heparinized, inactivated plasma (◆), each at 10%. The plasma preparations were made from the same lot of pooled rooster blood. Culture media were changed on day 2 and on each day thereafter. Each point represents the mean of four culture dishes; no standard errors exceeded half the height of the symbols used.

Fig. 2 presents the results of an experiment comparing the proliferative behavior of normal chicken heart mesenchymal cells in culture media containing whole blood-derived chicken serum, plasma-derived serum, and heparinized, inactivated plasma at concentrations of 5% and 10%. The cells proliferated at a somewhat greater rate in the presence of whole blood-derived serum than plasma-derived serum during the initial 2 days of exponential growth, but the rates became equal during the subsequent 4 days of exponential growth. Overall, the normal cells proliferated actively, at similar rates and to similar extents, in the presence of whole blood-derived serum or plasma-derived serum but they were quiescent in the presence of heparinized, inactivated plasma. (Plasma that has been defibrinogenated with the enzyme reptilase, which directly converts fibrinogen to fibrin, has the same properties as plasma-derived serum.) Doubling the concentration of whole blood-derived or plasma-derived serum (from 5% to 10%) caused only a small increase in rate and extent of proliferation of normal chicken heart mesenchymal cells, suggesting that the serum mitogen(s) in question is not overwhelmingly potent. Such modest potency would be expected of an authentic serum mitogen because cells *in vivo* are exposed to serum, rather than plasma, only in wounds, where serum is found in essentially undiluted ("100%") form.

Fig. 3 presents the results of an experiment comparing the effects of heparinization, heat inactivation, and heparinization plus heat inactivation on the mitogenic effects of whole blood-derived chicken serum and plasma-derived serum. First, as noted in regard to plasma, the fact that heparinization and inactivation of the sera reduce or abolish the proliferation of normal chicken heart mesenchymal cells without affecting the proliferation of their RSV-infected counterparts indicates that these treatments affect mitogenic (regulatory) rather than supportive ("nutrient") factor(s) in the sera. Indeed, the neoplastic state is defined in terms of autonomy—i.e., initiation of cell replication that is independent of mitogens (e.g., serum mitogen) (1–4, 7, 8). Second, the observation that heparinization and inactivation of the sera are required to reduce or abolish the proliferation of normal cells indicates that serum contains mitogen(s) rather than lacks inhibitors. Third, the observation that heparinization of plasma-derived serum causes far greater inhibition of cell proliferation than does heparinization of whole blood-derived serum indicates that heparin acts directly on the sera (or plasma) rather than on the cultured cells themselves, just as heat inactivation obviously acts directly on sera or plasma. If heparin were acting on the cultured cells themselves, heparinized whole blood-derived serum and heparinized plasma-derived serum would have identical properties.

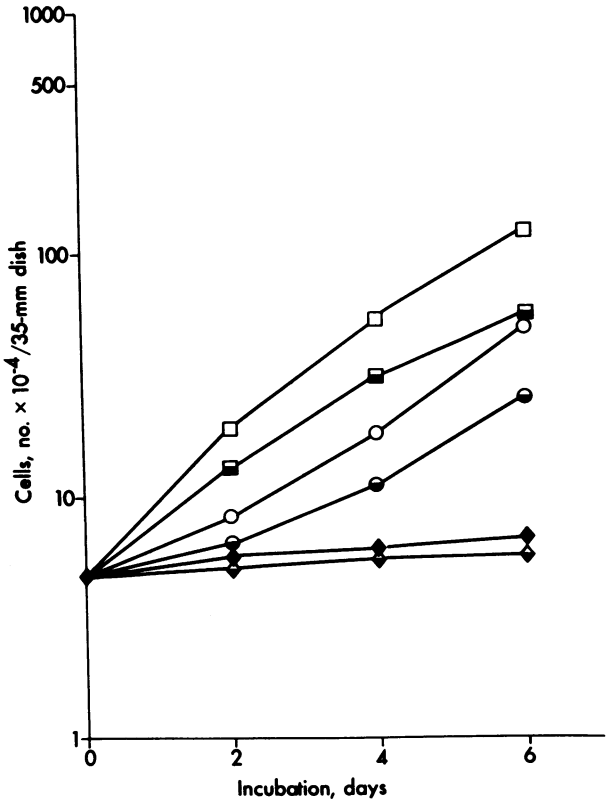

FIG. 2. Proliferative behavior of normal chicken heart mesenchymal cells in media containing whole blood-derived chicken serum at 10% (□) and at 5% (■), plasma-derived serum at 10% (○) and at 5% (●), and heparinized, inactivated plasma at 10% (◆) and at 5% (◆). The serum and plasma preparations were made from the same lot of pooled rooster blood. Each point represents the mean of two culture dishes.

We observed (Fig. 1) that limited mitogenic potential is present in chicken plasma that has been treated solely by heparinization or solely by heat inactivation (i.e., in plasma preparations that have not been subjected to any clotting). This mitogenic potential is eliminated by combining heparinization and heat inactivation. We also observed that the rate and extent of proliferation of normal chicken heart mesenchymal cells are similar in whole blood-derived and in plasma-derived serum (Figs. 2 and 3). The rate and extent of normal cell proliferation are reduced by a factor of approximately ½ when whole blood-derived serum is heparinized and inactivated, whereas proliferation ceases when plasma-derived serum is heparinized and inactivated (Fig. 3). On the basis of these observations, we hypothesize that mitogen(s) is released from plasma protein precursors when plasma clots in the presence of formed elements of the blood or when plasma-derived serum (or heparinized or inactivated plasma) is exposed to cultured cells; heparinization and inactivation, within the framework of this hypothesis, would render nonfunctional the plasma protein precursor(s) from which the mitogen is generated. Candidate plasma precursors of the serum mitogen would include the proteins of the clotting cascade itself, the kinin system, or the complement system. Alternatively, our data are consistent with the release of two mitogens during blood clotting, one from plasma protein precursors and the other from formed elements of the blood.

Other workers claim that the mitogenic activity that is present in serum, but not in plasma, is represented by a specific platelet-derived growth factor, a protein secreted from platelet α granules during platelet release. Swiss and BALB/c 3T3 cells have been used in the bulk of the published studies on this platelet-derived growth factor. We found (Fig. 4) that the exponential-phase proliferation rate of Swiss 3T3 cells in the presence of plasma-derived human serum that has not been depleted by dialysis and chromatography is equal to their exponential proliferation rate in the presence of whole blood-derived serum—i.e., an 8-fold increase in number in 2 days. P4, another platelet α granule protein, is released simultaneously and in equal proportions to platelet-derived growth factor when platelet secretion occurs during clotting (12). Because the whole blood-derived human serum used in this experiment contained 8700 ng of PF4 per ml whereas the plasma-derived serum contained only 6.4 ng/ml, the latter can be considered to be virtually free of platelet factors, including the platelet-derived growth factor. Therefore, the initiation of replication of Swiss 3T3 cells appears to be independent of any so-called platelet-derived growth factor. As reported by Ross and his coworkers (13), we found differences in the saturation densities of Swiss 3T3 cells in culture media containing whole blood-de-

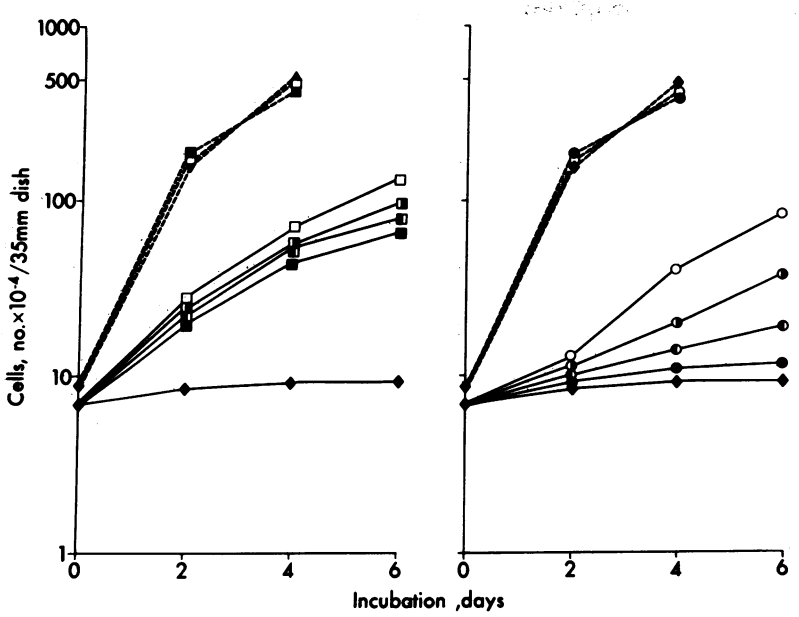

FIG. 3. Proliferative behavior of normal (—) and RSV-infected (---) chicken heart mesenchymal cells in media containing the following chicken serum or plasma preparations, each at a concentration of 10%: whole blood-derived serum (□), heparinized whole blood-derived serum (◧), heat-inactivated whole blood-derived serum (◨), heparinized, inactivated whole blood-derived serum (■), plasma-derived serum (○), heparinized plasma-derived serum (◐), inactivated plasma-derived serum (◑), heparinized, inactivated plasma-derived serum (●), and heparinized, inactivated plasma (◆). The serum and plasma preparations were made from the same lot of pooled rooster blood. Each point represents the mean of two culture dishes.

rived and plasma-derived sera—i.e., in the presence and absence of platelet-derived growth factor (Fig. 4). Such "density-dependent inhibition of proliferation" and its determinants, however, appear on careful analysis to represent starvation or artifacts of cell culture systems (8).

Although BALB/c 3T3 cells proliferate in culture medium containing whole blood-derived human serum (PF4, 5400 ng/ml) but not in medium containing plasma-derived serum (PF4, 6.6 ng/ml), BALB/c 3T3 cells that have been kept for 6 days in the presence of plasma-derived serum die, rather than proliferate, when exposed to whole blood-derived serum (Fig. 5). This cell death suggests that the proliferative inactivity of

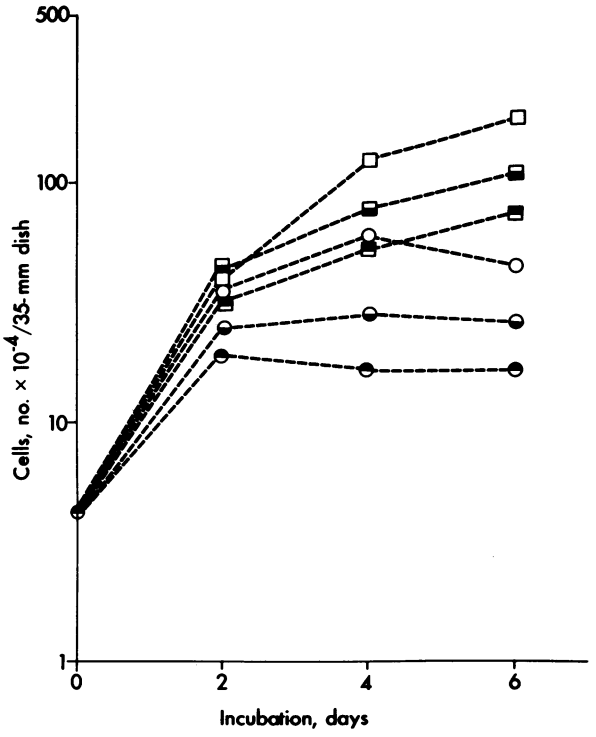

FIG. 4. Proliferative behavior of Swiss 3T3 cells in media containing whole blood-derived human serum at 10% (□), 5% (◨), or 3% (■) or plasma-derived human serum at 10% (○), 5% (◒), or 3% (◓). Both sera were prepared from the same lot of blood. The whole blood-derived serum contained 8700 ng of PF4 per ml; the plasma-derived serum contained 6.4 ng/ml. The data shown were obtained by using our own physiological-composition synthetic culture medium; essentially identical data were obtained with Dulbecco's modified Eagle's medium. Each point represents the mean of two culture dishes.

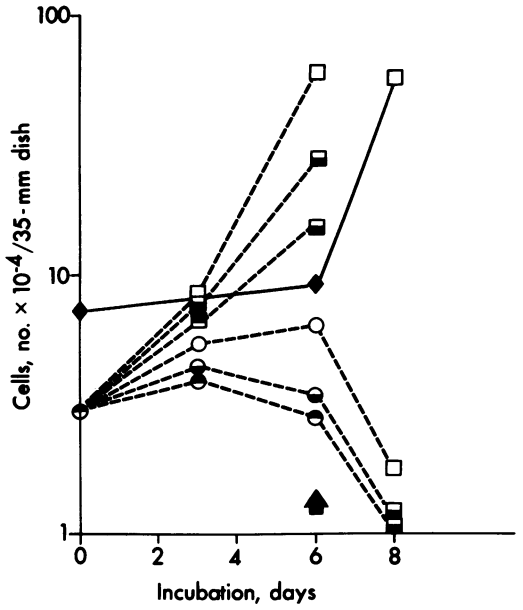

FIG. 5. Proliferative behavior of BALB/c 3T3 cells (---) in media containing whole blood-derived human serum at 10% (□), 5% (◨), or 3% (■) or plasma-derived human serum at 10% (○), 5% (◒), or 3% (◓). On day 6, cultures of BALB/c 3T3 cells that had been kept in media containing plasma-derived human serum were changed to media containing whole blood-derived human serum (both sera were prepared from the same lot of blood). The whole blood-derived serum contained PF4 at 5400 ng/ml; the plasma-derived serum contained 6.7 ng/ml. The data shown were obtained with our own synthetic culture medium, but essentially identical data were obtained with Dulbecco's modified Eagle's medium. For comparison to the proliferative behavior of BALB/c 3T3 cells, a trial was included in which cultures of normal chicken heart mesenchymal cells (—) were maintained for 6 days in medium containing heparinized, inactivated chicken plasma at 10% (◆) and then changed to medium containing whole blood-derived chicken plasma at 10% (□). Each point represents the mean of two culture dishes.

BALB/c 3T3 cells in the absence of the so-called platelet-derived growth factor is not physiological but is based on a state of unbalanced growth (14)—i.e., starvation. The death of BALB/c 3T3 cells following change from medium containing plasma-derived human serum to medium containing whole blood-derived human serum contrasts sharply with the active proliferation of chicken heart mesenchymal cells that follows change from medium containing heparinized, inactivated chicken plasma to medium containing whole blood-derived chicken serum (Fig. 5). Because vascular endothelial cells are bathed in undiluted (100%) plasma *in vivo*, our evidence that BALB/c 3T3 cells starve in medium containing an interstitial concentration (10%) of plasma-derived human serum is consistent with the published evidence suggesting that BALB/c 3T3 cells are endothelial in nature (15, 16). Alternatively, it is possible that cells of the 3T3 lines have become habituated to metabolic support by nonspecific serum factors during their long years of propagation in serum-containing media. Indeed, Scher *et al.* (17) have demonstrated that, unlike aneuploid, highly culture-selected 3T3 cells, diploid human fibroblasts proliferate actively in plasma-containing media.

We argue that the metabolic support afforded by the platelet-derived growth factor is nonspecific because of the multitude of agents that have been demonstrated to be capable of relieving 3T3 cells from starvation imposed by serum (plasma) withdrawal or density-dependent inhibition. These include precipitates and particulates (8), "fibroblast-growth factor" (18), vasopressin (19), bovine colostrum (20), "platelet basic protein" (21), "Sertoli cell mitogenic peptide" (22), a chondrocyte-derived "growth factor" (23), insulin (24), and a "growth factor" derived from spinal cord (25). The possession of neoplastic properties (16, 26), moreover, makes BALB/c 3T3 cells unsuitable for mitogen assays because neoplastic cells are by definition independent of mitogens; factors that allow neoplastic cells to proliferate must therefore have a supportive (nutrient), rather than a regulatory, role in cell replication. On the basis of our own observations and all of the above considerations we argue, in sum, that the platelet-derived growth factor is not an authentic mitogen but instead plays an artifactual, nonspecific, supportive role in cell replication. Braunstein *et al.* (27) have found that it was not possible to demonstrate a direct, fibroblast-stimulating effect of platelet components *in vivo*.

Since the original publication by Chen and Buchanan (28), a number of workers have claimed that thrombin is a mitogen for fibroblast-like cells (29). All of these claims are based on observations that thrombin, like other proteases, causes some degree of proliferative activity of cells rendered inactive by the use of a serum-free medium. Again because the normal extracellular fluids have a protein concentration at least 10% of that of the plasma (30, 31), proliferative inactivity imposed by culture in a serum (plasma)-free medium cannot be considered to represent physiological proliferative quiescence. Likewise, the very limited proliferative activity that follows treatment of serum-starved cells with thrombin cannot be considered to represent mitogenesis. Rather, thrombin and other proteases appear to be capable of affording to cells some degree of transient relief from serum (plasma) starvation.

Using the chicken heart mesenchymal cell system, with its nutritionally replete, physiological ion concentration baseline, we have now confirmed the original observation (1–3) that serum contains mitogen(s) not present in plasma. Heparinization and heat inactivation of plasma appear to eliminate the system from which this mitogen is generated. The serum mitogen, as originally postulated by Balk, may function as a wound hormone. Ross and Vogel (5) have suggested that the release of mitogens from thrombi formed at sites of damage to the linings of arteries may induce the formation of the intimal smooth muscle lesions of atherosclerosis. Because we question the authenticity of the platelet-derived growth factor, we think that the mitogenic activity that we have demonstrated to be present in serum, but not in plasma, is a more legitimate candidate for such a role in atherogenesis.

These investigations were supported by grants from the National Cancer Institute of Canada, the Manitoba Health Services Foundation, and the Medical Research Service of the Veterans Administration. S.P.L. is a Veterans Administration Clinical Investigator.

1. Balk, S. D. (1971) *Proc. Natl. Acad. Sci. USA* **68**, 271–275.
2. Balk, S. D. (1971) *Proc. Natl. Acad. Sci. USA* **68**, 1689–1692.
3. Balk, S. D., Whitfield, J. F., Youdale, T. & Braun, A. C. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 675–679.
4. Balk, S. D., Polimeni, P. I., Hoon, B. S., LeStourgeon, D. N. & Mitchell, R. S. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 3913–3916.
5. Ross, R. & Vogel, A. (1978) *Cell* **14**, 203–210.
6. Scher, C. D., Shepard, R. C., Antonaides, H. N. & Stiles, C. D. (1979) *Biochim. Biophys. Acta* **560**, 217–241.
7. Balk, S. D. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 6606–6610.
8. Balk, S. D. (1980) *Life Sci.* **27**, 1917–1920.
9. Ross, R., Nist, C., Kariya, B., Rivest, M. J., Raines, E. & Callis, J. (1978) *J. Cell. Physiol.* **97**, 497–508.
10. Heldin, C. H., Wasteson, A. & Westermark, B. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 6611–6615.
11. Levine, S. P. & Krentz, L. S. (1977) *Thromb. Res.* **11**, 673–686.
12. Kaplan, K. L., Broekman, M. J., Chernoff, A., Lasznick, G. R. & Drillings, M. (1979) *Blood* **53**, 604–618.
13. Vogel, A., Ross, R. & Raines, E. (1980) *J. Cell Biol.* **85**, 377–385.
14. Giese, A. C. (1979) *Cell Physiology* (Saunders, Philadelphia), pp. 561–562.
15. Porter, K. R., Todaro, G. J. & Fonte, V. (1973) *J. Cell Biol.* **59**, 633–642.
16. Boone, C. W. (1975) *Science* **188**, 68–70.
17. Scher, C. D., Pledger, W. J., Martin, P., Antoniades, H. & Stiles, C. D. (1978) *J. Cell. Physiol.* **97**, 371–380.
18. Thomas, K. A., Riley, M. C., Lemmon, S. K., Baglan, N. C. & Bradshaw, R. A. (1980) *J. Biol. Chem.* **255**, 5517–5520.
19. Rozengurt, E. & Mendoza, S. (1980) in *Growth Regulation by Ion Fluxes*, ed. Leffert, H. (N.Y. Acad. Sci., New York), pp. 175–190.
20. Klagsbrun, M. & Neumann, J. (1979) *J. Supramol. Struct.* **11**, 349–359.
21. Paul, D., Niewiarowski, S., Varma, K. G., Rucinski, B., Rucker, S. & Lange, E. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5914–5918.
22. Feig, L. A., Bellve, A. R., Erickson, N. H. & Klagsbrun, M. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 4774–4778.
23. Azizkhan, J. C. & Klagsburn, M. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 2762–2766.
24. Petrides, P. E. & Bohlen, P. (1980) *Biochem. Biophys. Res. Commun.* **95**, 1138–1144.
25. Jennings, T., Jones, R. D. & Lipton, A. (1979) *J. Cell. Physiol.* **100**, 273–278.
26. Boone, C. W. & Scott, R. E. (1981) *J. Supramol. Struct.*, in press.
27. Braunstein, P. W., Jr., Cuenod, H. F., Joris, I. & Magno, G. (1980) *Am. J. Pathol.* **99**, 53–62.
28. Chen, L. B. & Buchanan, J. M. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 131–135.
29. Glenn, K. C., Carney, D. H., Fenton, J. W. & Cunningham, D. D. (1980) *J. Biol. Chem.* **255**, 6609–6616.
30. Renkin, E. M. (1979) in *Pulmonary Edema*, eds. Fishman, A. F. & Renkin, E. M. (Am. Physiol. Soc., Bethesda, MD), pp. 145–159.
31. Altman, P. & Katz, D. D., Eds. (1974) *Biology Data Book* (Fed. Am. Soc. Exp. Biol., Bethesda, MD), 2nd Ed., pp. 1942–1975.