FIBROBLAST GROWTH FACTOR CAN SUBSTITUTE FOR PLATELET FACTOR TO SUSTAIN THE GROWTH OF BALB/3T3 CELLS IN THE PRESENCE OF PLASMA

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SUMMARY: The Fibroblast Growth Factor isolated from either brain or pituitary, when added with plasma and dexamethasone, promotes the growth of 3T3 cells seeded at low density (7 cells/mm^2) to the same extent as does serum. This suggests that the Fibroblast Growth Factor could be similar or related to the growth-promoting activity present in platelets.

Serum contains one or more factors that are essential for the growth and survival of most mammalian cell lines (1-4). Plasma, in contrast, has been found to be less effective than serum for promoting the growth of fibroblasts (5), smooth muscle (6) or established cell lines such as 3T3 (7). The addition of platelet lysate with plasma promotes cell growth at a level comparable to that of serum (6, 7). On the basis of this evidence, it has been postulated that serum mitogens may be released from platelets during the coagulation process.

Fibroblast Growth Factor (FGF) is a polypeptide of 13,000 MW purified from mammalian brain or pituitary (8, 9). It has been shown to be mitogenic at nanomolar concentrations for 3T3 cells maintained in low serum (8-12) or in depleted high (10%) serum (10,11). It provokes pleiotypic effects similar to those observed in the presence of optimal serum concentrations (13). The effect of FGF is enhanced by a glucocorticoid but the combination does not, in the absence of serum, support growth indefinitely, indicating that some factor in serum is needed for the long term survival and proliferation of 3T3 cells (12).

In this communication we report that FGF, when added with dexamethasone and plasma, promotes the growth of 3T3 cells to the same extent as that observed with the optimal concentration of serum.

We suggest, therefore, that plasma contains the factor or factors necessary for long-term survival of 3T3 cells in the absence of serum although it contains very little mitogenic activity, and that FGF is similar to the mitogenic factor that has been found in platelets.

MATERIALS AND METHODS

FGF was purified from bovine pituitary glands or brains as described previously (8, 9). Sera and plasma were from Gibco. Citrated bovine saline was defibrinated before use by dialysis against phosphate-buffered saline followed by Millipore filtration. Both plasma and serum were heat-activated at 56 C for 30 minutes. A platelet solution was prepared as described by Kohler and Lipton (7). A Balb/c 3T3 cell line maintained in our laboratory was used; it has been described elsewhere (9-11). Cell culture and cell counting techniques have been described (9-11).

RESULTS

A. Growth-promoting effect of plasma compared to serum

The growth of sparse populations of 3T3 cells maintained in the presence of various concentrations of plasma was compared to population growth in serum. Plasma was 5 to 10 times less potent than serum for supporting the growth of 3T3 cells (figs. 1 A and B). When a platelet lysate was added to cells maintained quiescent in the presence of 1% plasma, the rate of growth became similar to that observed with 10% serum (fig. 1 C), demonstrating that mitogenic factors present in serum may come, in fact, from the lysis of platelets.

B. Growth-promoting effect of FGF and dexamethasone for 3T3 cells maintained in plasma as compared to serum

Pituitary FGF stimulates the rate of growth of sparse populations of

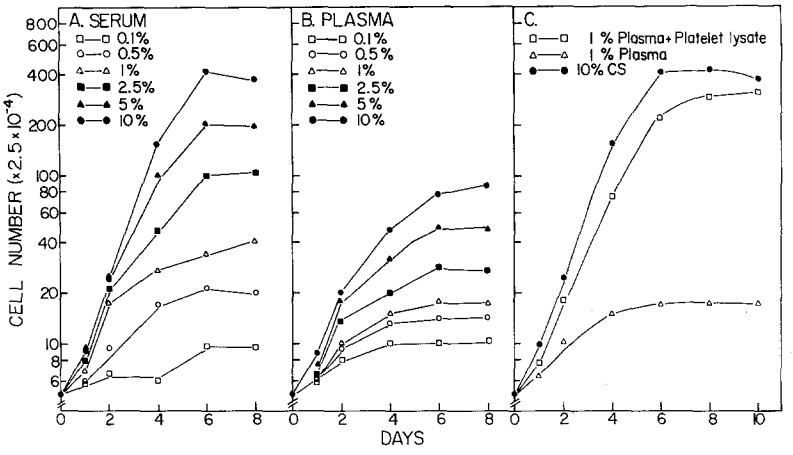
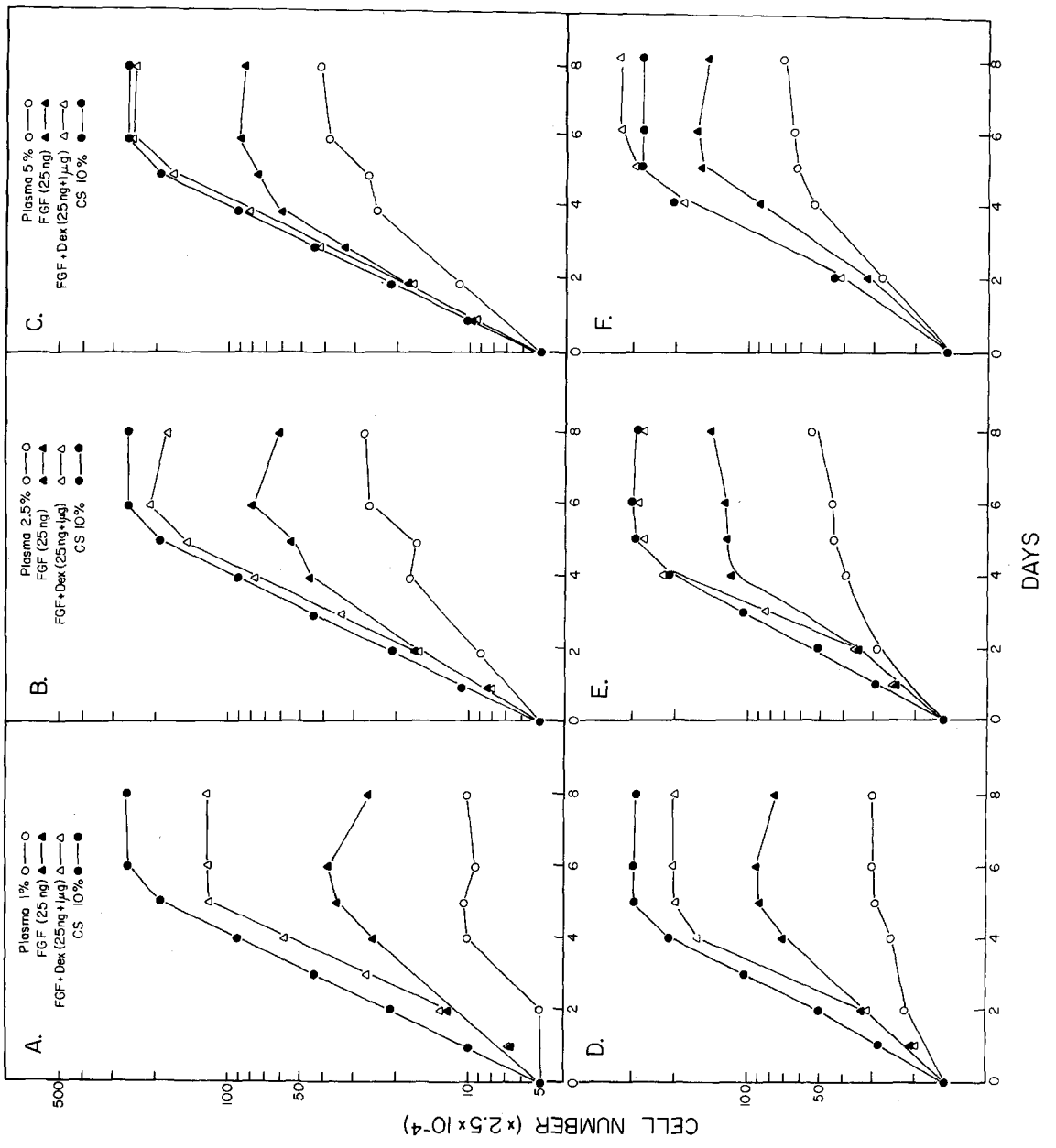


Figure 1. Growth curve of 3T3 cells maintained in the presence of plasma, serum, or plasma plus platelet lysate.
A-B: 20,000 cells per 6 cm dish were plated in 5 ml of DME, 10% calf serum on day 0. Eight hours later the medium was changed to the different serum or plasma concentrations.
C: 20,000 cells were plated as already described. Eight hours later the media was changed to either 10% calf serum, 1% plasma or 1% plasma plus 2 units/ml A_{280} of platelet lysate and 1 μg/ml of dexamethasone.

3T3 cells maintained in the presence of various concentrations of plasma (fig. 2). With an initial density of 7 cells/mm^2, in the presence of 1% plasma, the cell number doubled over 8 days (fig. 2 A). When FGF was added, the cell number increased eight-fold, corresponding to three division cycles. With FGF plus dexamethasone, five cycles of division were observed; the final density was half that observed with 10% serum. With 2.5% plasma, even better stimulation of division was observed, the final density in the presence of FGF plus dexamethasone was only 20% lower than that observed in 10% serum (fig. 2B). With 5% plasma, the final density was similar to that of 10% serum (fig. 2 C). It should, however, be noted that with neither 2.5 nor 5% plasma were the cells fully quiescent before day 5, thus giving a baseline that increased with time.


A.
B.
C.
D.
E.
F.
Plasma 1% o—o
FGF (25 ng) ▲—▲
FGF + Dex (25ng + 1μg) Δ—Δ
CS 10% ●—●
Plasma 2.5% o—o
FGF (25 ng) ▲—▲
FGF + Dex (25 ng + 1μg) Δ—Δ
CS 10% ●—●
Plasma 5 % o—o
FGF (25 ng) ▲—▲
FGF + Dex (25 ng + 1μg) Δ—Δ
CS 10% ●—●
500
100
50
10
5
0
2
4
6
8
CELL NUMBER (× 2.5 × 10⁻⁴)
DAYS

When cells were seeded at three times the initial density (21 cells/mm^2) (fig. 2 D, E and F), essentially similar results were obtained with the difference that it was with 2.5% (rather than 5%) plasma and FGF plus dexamethasone that the cells reached the same density as with 10% serum. With 5% plasma (fig. 2F), the final cell density with FGF plus dexamethasone was 50% higher than that observed with 10% serum. Similar results were obtained when the effect of brain FGF (fig. 3) on division in sparse populations of 3T3 cells was investigated.

These results in the presence of 1, 2.5 or 5% plasma can be compared with the stimulation by pituitary FGF or pituitary FGF plus dexamethasone of cells maintained in serum-starved conditions. When cells were maintained at a density of 7 cells/mm^2 in the presence of 0.1% serum, the addition of FGF and dexamethasone provoked three division cycles. The final density was 14% of that observed with 10% serum alone (fig. 4).

DISCUSSION

Serum is an artificial medium, developed when tissue culture was originated (14) because it induced animal cells to proliferate in vitro.

Since serum is such a potent promoter of growth, it is necessary, in order to impede cell division in cultures maintained in the presence of serum, to lower the serum concentration to very low levels (serum starvation). In doing so, one limits not only the concentration of growth factors present in

Figure 2. Growth curves of 3T3 cells maintained in different plasma concentrations with pituitary FGF or FGF plus dexamethasone added to them. Cells were plated at a concentration of 20,000 cells (A, B, C) or 60,000 (D, E, F) per 6 cm dish as described in fig. 1. The medium was changed 6 hours later to 1% plasma (A, D), 2.5% plasma (B, E), 5% plasma (C, F) or 10% calf serum. FGF was added in 50 µl volume of a 0.5% solution of crystalline bovine serum albumin to a final concentration of 25 ng/ml. Dexamethasone was added in 10 µl of absolute ethanol to a final concentration of 1 µg/ml. FGF and dexamethasone were added every other day.

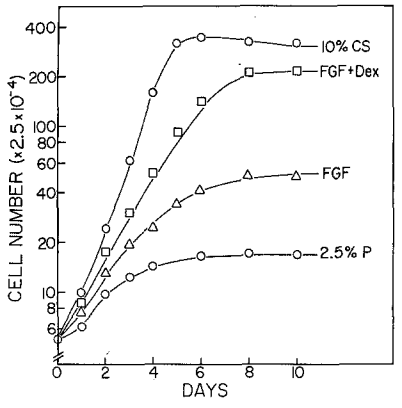


Figure 3. Growth of 3T3 cells maintained in the presence of 1% plasma with brain FGF or brain FGF plus dexamethasone added to them. 20,000 cells were plated in 5 ml of DME, 10% calf serum on day 0. Eight hours later the medium was changed to 2.5% plasma or 10% calf serum. 50 ng/ml of brain FGF or 50 ng/ml of brain FGF plus 1 μg/ml of dexamethasone were added every other day to the cells maintained in 1% plasma as described in fig. 2. Cells were counted in triplicate and their growth compared to that of cells maintained in 10% calf serum.

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the medium, but also the concentration of other factors necessary for the survival and good health of the cells. While certain cell types can survive quite well in low serum or in the absence of serum, others will die or will become unresponsive to further growth stimuli.

3T3 cells are particularly susceptible to serum starvation and their survival and response to growth stimuli in low serum is mainly dependent on their density. When 3T3 cells are plated at an initial density of 35 cells/mm^2, and maintained in the presence of 0.4% serum, the final density reached when FGF plus dexamethasone is added was half that seen in 10% serum (10-11). A greater effect on cell proliferation is seen in subconfluent and confluent cultures (10-11). When lower cell densities are used (7 cells/mm^2), or when lower serum concentrations are used (0.1%), considerably less response to FGF and dexamethasone is observed (12). We have attributed the low sensi-

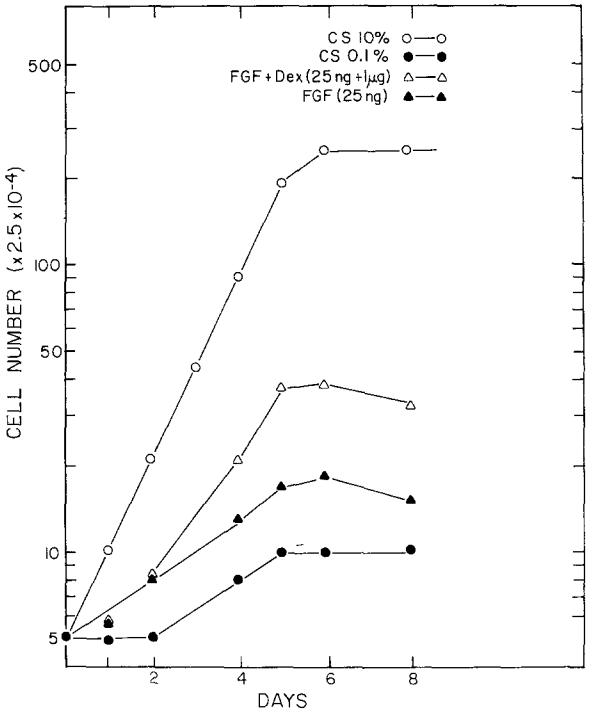

Figure 4. Growth of 3T3 cells maintained in the presence of low serum concentrations with pituitary FGF or FGF plus dexamethasone added to them.
20,000 cells were plated in 5 ml of DME, 5% calf serum on day 0. Eight hours later the medium was changed to 0.1% calf serum or 10% calf serum. 25 ng/ml of pituitary FGF or 25 ng/ml of pituitary FGF plus 1 μg/ml of dexamethasone were added every other day as described in fig. 2.

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tivity to FGF of serum-starved 3T3 cells to irreversible deterioration that takes place when cells are maintained under too drastic conditions, such as in very low serum concentrations (12).

Since plasma has been reported to have little growth-promoting activity compared to serum (5-7), it may be possible to maintain the cells in a quiescent state in the presence of higher concentrations of plasma than of serum. With plasma, the concentration of growth factors in the medium may still be limiting at concentrations at which the plasma provides the cell with

adequate concentrations of factors needed for survival and for the maintenance of homeostatic mechanisms. Since these "survival" factors are normally present in the blood, they can be expected to occur at similar concentrations in both serum and plasma. It should be kept in mind that cells in vivo are normally exposed to plasma (that fraction of whole blood that passes through capillary walls) and that only in wounds are cells exposed to serum (the liquid fraction of clotted blood). Therefore, cells maintained in a quiescent state at low density in plasma should survive better and be more fully responsive to further stimulation by growth factors than cells maintained in a quiescent state in low serum.

This postulate agrees with what we have observed when we have maintained the cells in the presence of plasma. Cell densities as low as 7 cells/mm^2 can be used and the final density with FGF and dexamethasone reached 257 to 500 cells/mm^2, depending on the initial cell density. These values are comparable to the values observed with 10% serum (430 cells/mm^2).

Since the effect of FGF is additive over plasma, FGF is unlikely to be one of the "growth factors" normally present in equal concentration in plasma and serum, such as insulin, NSILA, growth hormone, or the somatomedins, even though it may contribute to plasma mitogenic activity by being present in low concentration. Furthermore, since FGF can replace the platelet lysate, it can be concluded that FGF may be similar to the mitogenic factor(s) contained in platelets. This does not prove, however, that FGF is identical to the platelet factor that has been held responsible for the control of division of diverse cell types (5-7). Proof should be obtained when antibodies against FGF are obtained.

The use of plasma, rather than serum, should also be useful to distinguish between survival factors (in plasma and serum) and mitogenic factors

(in serum) and should provide an ideal medium to make cells quiescent without killing them.

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