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# Serum contains a platelet-derived transforming growth factor

(growth in soft agar/cell transformation/bovine serum/human serum)

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**ABSTRACT** **High concentrations of fetal bovine serum induced colony formation in soft agar by anchorage-dependent, nontransformed mouse AKR-2B and rat NRK cells. The colony-stimulating activity in fetal bovine serum was precipitated by 45% saturated ammonium sulfate and migrated in molecular sieve chromatography as a single peak of activity in the 10,000–15,000 molecular weight range. The colony-stimulating activity was heat and acid stable and was destroyed by trypsin and dithiothreitol, indicating the activity is due to a polypeptide that requires disulfide bonds for biological activity. No competition for binding to the epidermal growth factor receptor was associated with the colony-stimulating activity. Isoelectric focusing revealed activity in the pI 4–5 range. The colony-stimulating activity in serum appeared to be of platelet origin because platelet-poor plasma and platelet-poor plasma-derived serum contained little activity, whereas acid/ethanol extracts of bovine and human platelets had potent colony-stimulating activity. Chromatography of platelet extracts on Bio-Gel P-60 revealed peaks of AKR-2B colony-stimulating activity in the 12,000 and 20,000 molecular weight ranges. The other biological and chemical properties of the platelet colony-stimulating activity were the same as those for the serum activity. The data indicate the presence in serum of a platelet-derived growth factor(s) with properties similar to those of the transforming growth factors.**

Cells that are normally anchorage dependent can be stimulated to form colonies when suspended in semisolid medium in the presence of higher than usual concentrations of serum. O'Neill *et al.* (1) reported that anchorage-dependent NIL-8 hamster fibroblasts exhibited a serum concentration-dependent growth in soft agarose and that the rate of growth of attached and suspended cells was approximately the same when the concentration of fetal bovine serum was raised to 66%. Peehl and Stanbridge (2) found that normal human fibroblasts would grow suspended in methylcellulose in the presence of 20% fetal bovine serum but not in medium with 10% fetal bovine serum; this anchorage-independent growth was enhanced by the addition of hydrocortisone.

The capability of stimulating growth in soft agar of normally anchorage-dependent cells is a property of the transforming growth factors (TGFs) (3, 4) that is not shared by the well-known growth factors such as epidermal growth factor (EGF), fibroblast growth factor (FGF), cationic platelet-derived growth factor (PDGF), insulin, or the insulin-like growth factors (3, 5). TGFs are a family of acid- and heat-stable, relatively low molecular weight (5,000–25,000) polypeptides with disulfide bonds (4–6). Some of the TGFs have been shown to compete with EGF for binding to available membrane receptors but do not crossreact with EGF antibodies (7). Other TGFs separable from the EGF-competing TGF activity have been shown to have their activity markedly enhanced by the addition of exogenous EGF (5, 8). A variety of neoplastic and non-neoplastic cells have been found to contain TGF, to release TGF into serum-free medium, or both (3–6, 8–10).

The present study was undertaken in an attempt to determine whether the capability of high levels of serum to stimulate growth in soft agar was due to the presence of TGF-like compounds within serum. The results indicate that serum contains a platelet-derived TGF-like compound(s) that is apparently separate and distinct from the well-known serum growth factors such as the cationic PDGF, EGF, insulin, and insulin-like growth factors.

## MATERIALS AND METHODS

**Soft Agar Assay.** Soft agar assays using nontransformed anchorage-dependent mouse AKR-2B and rat NRK cells were performed as described (6, 10). Colonies were quantitated after 5–14 days either by using an inverted microscope with a calibrated grid or by computerized image analysis systems. The two systems used were an Omnicon Feature Analysis Stem model II (Bausch & Lomb, Rochester, NY) and a Quantimet 800 image analyzer (Cambridge Instruments, Monsey, NY). In all circumstances, colonies greater than 60 $\mu$m in diameter (containing at least 50 cells) were scored as positive, and colony sizes ranged up to several hundred cells per colony. Control dishes generally contained no colonies greater than four cells.

To test for stimulation of colony formation by known growth factors, the following factors were added to the soft agar assay in the concentration ranges indicated: EGF, 1–800 ng/ml; multiplication-stimulating activity (Collaborative Research, Waltham, MA), 1–100 ng/ml; bovine insulin (Sigma), 1 ng/ml to 1 mg/ml; somatomedin C (obtained from Judson Van Wyk, University of North Carolina at Chapel Hill), 1–100 ng/ml; and PDGF (obtained from W. Jackson Pledger, University of North Carolina at Chapel Hill), 1–100 units/ml.

**Serum and Plasma.** Fetal bovine serum for analysis was obtained from Reheis Chemical (Phoenix, AZ) and GIBCO. Fetal bovine plasma was obtained from two near-term calf fetuses delivered by caesarean section. Blood was obtained in citrated form (10% of a solution of 3.8 g/100 ml of sodium citrate) and without anticoagulants to allow comparisons of serum and plasma from the same animal. Citrated and clotted blood were centrifuged at 600 × *g* for 30 min at room temperature, followed by removal of plasma and serum and storage at −20°C. Human blood was obtained from laboratory volunteers and processed as described above. Platelet-poor plasma-derived serum was prepared as described (11). Serum, plasma, and plasma-derived serum from both human and bovine sources was dialyzed against 3 changes of 50 volumes of serum-free McCoy's 5a medium at 4°C, followed by centrifugation at 1,500 × *g* before use directly in soft agar assays.

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Abbreviations: EGF, epidermal growth factor; FGF, fibroblast growth factor; PDGF, platelet-derived growth factor; TGF, transforming growth factor.

The ammonium sulfate precipitation procedure used for processing serum or plasma was the same as that described by Pedersen (12) for preparation of fetuin. The precipitate was then dissolved in 1% acetic acid, dialyzed against 1% acetic acid (two changes of 50 vol) in Spectropor 3 dialysis tubing (molecular weight cutoff of approximately 3,500; Spectra Medical Industries no. 132720) at 4°C, lyophilized, and stored at −20°C.

**Platelet Extraction.** Outdated human platelets were obtained from the Mayo Clinic Blood Bank. Bovine platelets were separated from blood obtained by jugular vein puncture of Holstein dairy cows and platelet separation was performed by the Mayo Clinic Blood Bank (13). Human and bovine platelet preparations were washed as described (14). Platelet concentrates were then extracted by a modification (10) of the acid/ethanol extraction procedure described by Roberts *et al.* (9). The extract, after extensive dialysis against 1% acetic acid, was lyophilized and stored at −20°C.

**Gel Exclusion Chromatography.** Aliquots of lyophilized material were dissolved in 1 M acetic acid and clarified by centrifugation, and the supernatant was applied to one of three Bio-Gel P-60 (Bio-Rad) columns (5 × 90, 2.5 × 87, and 1.6 × 87 cm) as described (6). Protein determination was by the dye-binding technique (15).

**Isoelectric Focusing.** Pooled fractions from the Bio-Gel P-60 chromatography containing the peak of colony-stimulating activity were dissolved in 1 ml of distilled water. The samples were applied in the region corresponding to fractions 4–6 of the grid plate. The gel consisted of 4 g of Ultradex (LKB) in 10% (vol/vol) glycerol/distilled water plus Ampholine (3.5 to 10 pH). The sample was focused on a flat bed isoelectric focusing apparatus (LKB Multiphor no. 2117-301) for 8 hr at 0°C at 8 W constant power. The gel bed was then fractionated into 31 zones and the pH of each zone was determined. The fractions were then eluted with 1 M acetic acid and the samples were dialyzed at 4°C against 1% acetic acid and lyophilized.

**DNA Synthesis and Morphologic Transformation.** Methods for the stimulation and quantitation of DNA synthesis and for induction of morphologic transformation have been described (6, 10).

**Competition for $^{125}$I-Labeled EGF Binding.** The competition assay using the A431 human carcinoma cell line has been described (6, 10).

## RESULTS

The use of medium supplemented with 10% fetal bovine serum in the soft agar assay with either the AKR-2B or NRK cells did not result in the formation of colonies. However, at higher levels of serum there was a serum concentration-dependent increase in the number and size of colonies formed by both AKR-2B and NRK cells (Fig. 1*A*). Increasing concentrations of fetal bovine plasma in the soft agar assay caused some stimulation of the NRK cells, but no detectable colony formation was observed with the AKR cells (Fig. 1*B*). As a first step in determining the factor or factors present in serum that cause colony formation, serum was precipitated with 45% saturated ammonium sulfate and the supernatant and precipitate were examined for colony-stimulating activity. The supernatant exhibited no colony stimulation (data not shown), whereas the precipitate exhibited a concentration-dependent stimulation of both NRK and AKR-2B cells (Fig. 2*A*). Ammonium sulfate precipitates from plasma caused only minimal stimulation of colony formation (Fig. 2*B*). Newborn calf and calf sera and ammonium sulfate precipitates of these sera were approximately one-half as active as fetal bovine serum and fetal bovine serum precipitates (data not shown).

Bio-Gel P-60 chromatography of the acid-soluble part of the ammonium sulfate precipitate from fetal bovine serum gave a single peak of colony-stimulating activity in the 10,000–15,000 molecular weight range (Fig. 3). The AKR-2B and NRK-stimulating activity cochromatographed, but the NRK activity was considerably less than that obtained with the AKR-2B cells. The addition of EGF at 2.0 ng/ml to the soft agar assay caused no alteration in the activity obtained with the AKR-2B cells. Added EGF did enhance the stimulation obtained with the NRK cells about 2-fold, but no new peaks of NRK colony-stimulating activity appeared in the presence of EGF.

Pooled active fractions from the Bio-Gel P-60 chromatography did not compete with $^{125}$I-labeled EGF for binding to A431 cells. The pooled material gave 50% maximal colony-stimulating activity at 33 μg, indicating a 100-fold purification over that present in the crude precipitate (Fig. 2*A*). The colony-stimulating activity was sensitive to trypsin and dithiothreitol but

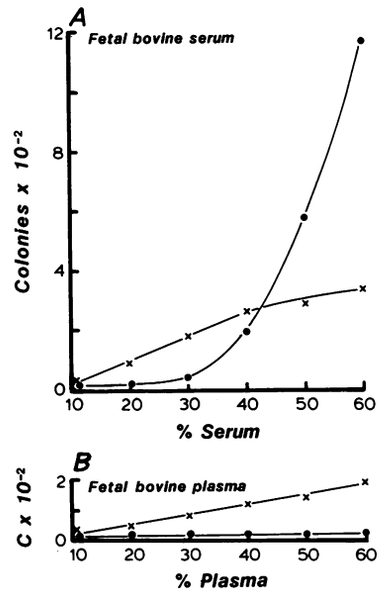


FIG. 1. Effect of serum (*A*) and plasma (*B*) concentration on growth in soft agar of AKR-2B (●) and NRK (×) cells. Fetal bovine serum and plasma obtained from the same fetus were dialyzed against serum-free medium at 4°C and centrifuged before use directly in the soft agar assay in the concentrations indicated.

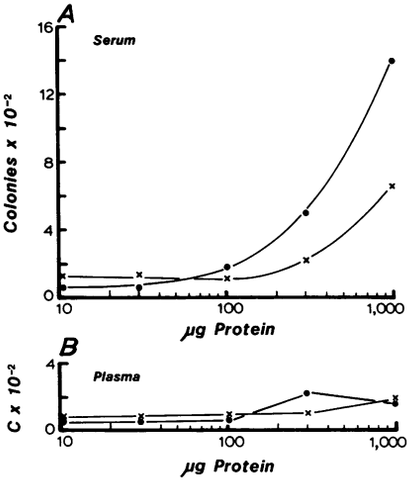


FIG. 2. Effects of ammonium sulfate precipitates from serum (*A*) and plasma (*B*) on stimulation of growth in soft agar of AKR-2B (●) and NRK (×) cells. Serum and plasma obtained from the same bovine fetus were precipitated by 45% saturated ammonium sulfate, dialyzed against dilute acetic acid, and lyophilized.

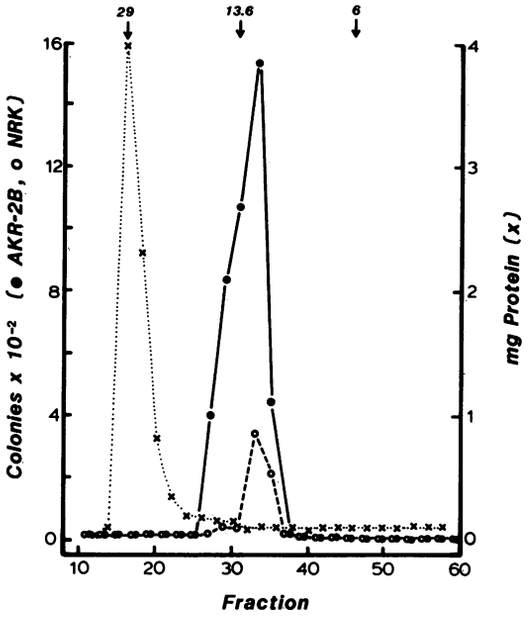


FIG. 3. Bio-Gel P-60 chromatography of acetic acid extracts of fetal bovine serum precipitate. Twenty milligrams of protein was dissolved in 1 M acetic acid and centrifuged at 100,000 × *g*, applied to a 1.6 × 87 cm Bio-Gel P-60 column, and eluted with 1 M acetic acid, with 3-ml fractions being collected. Soft agar assays of every other fraction was performed, using 1/25th fraction on both AKR-2B (●) and NRK (○) cells. Protein content per tube (×) was quantitated by the dye-binding assay (15). The standard markers used were carbonic anhydrase (29,000), RNase (13,800), and insulin (6,000) (molecular weight × $10^{-3}$ is shown).

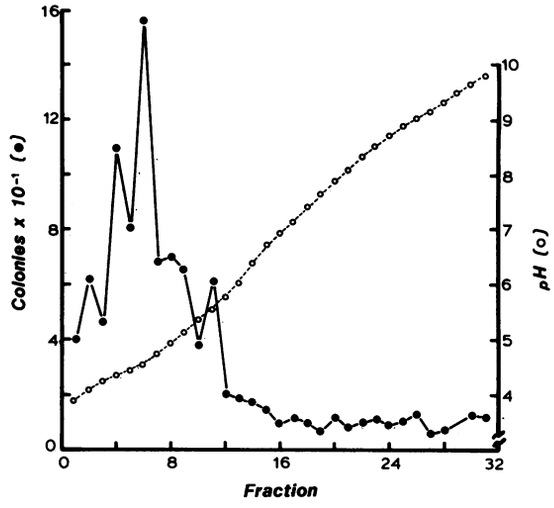


FIG. 4. Isoelectric focusing of colony-stimulating activity from fetal bovine serum. Fractions showing maximal colony-stimulating activity from the Bio-Gel P-60 chromatography of fetal bovine serum precipitates were pooled, and 6 mg of protein was focused on a flat-bed device. The gel was then fractionated into 31 zones and the pH (○) was determined. The colony-stimulating activity of each fraction with AKR-2B cells (●) was determined.

stable to heating to 56°C for 30 min or to 100°C for 3 min (Table 1), indicating that the activity is due to a polypeptide that must have intact disulfide bonds for biological activity.

Isoelectric focusing of pooled colony-stimulating activity from the Bio-Gel P-60 column revealed activity in the 4.0–5.5 pH range (Fig. 4). Because of the impure nature of the starting material and the propensity for compounds under isoelectric focusing conditions to bind to other compounds, the apparent pI may be inaccurate. However, the data do provide evidence that the activity is not due to a highly basic molecule such as the cationic PDGF, which has a pI of 9.8 (16).

Pooled colony-stimulating activity from the Bio-Gel P-60 column was shown to stimulate DNA synthesis in growth factor deficient arrested AKR-2B cells, with a concentration of 100 μg/ml giving a 5-fold increase in [$^3$H]thymidine incorporation into DNA (data not shown). In addition, the AKR-2B cells when grown in the presence of the TGF-like material from fetal bovine serum showed morphologic transformation identical to that induced by TGF extracted from mouse embryos and produced by the chemically transformed AKR-MCA cells as illustrated previously (6, 10).

The observation that serum contains much more colony-stimulating activity than does plasma suggests that the compound or compounds responsible for the activity is produced or released in the clotting reaction. To define the source of this material, human serum, platelet-poor plasma, and platelet-poor plasma-derived serum were examined for colony-stimulating activity. As shown in Table 2, human serum contained more colony-stimulating activity than either plasma or plasma-derived serum. Acid/ethanol extracts of both human and bovine platelets were tested for activity and found to contain potent colony-stimulating activity for both the AKR-2B and NRK cells (Table 2).

Bio-Gel P-60 chromatography of the bovine platelet extract revealed a major peak of NRK colony-stimulating activity in the 14,000 molecular weight range and a smaller higher molecular weight peak (Fig. 5). The AKR-2B cells responded to compounds present in the 14,000 and 20,000 molecular weight range (Fig. 5). The major peaks of NRK and AKR-2B stimulation were in the same molecular weight range as similar activity from

Table 1. Effect of chemical and physical treatments on AKR-2B colony-stimulating activity from fetal bovine serum*

| Treatment | Soft agar colonies† |
|---|---|
| Control | 175 |
| Heat (56°C for 30 min) | 125 |
| Trypsin (50 μg/ml, 2 hr at 37°C) | 0 |
| Trypsin (50 μg/ml) plus soybean trypsin inhibitor (100 μg/ml) (2 hr at 37°C) | 115 |
| Dithiothreitol (0.065 M in 0.1 M $NH_4HCO_3$, 1 hr at 20°C) | 0 |

* Fractions from the Bio-Gel P-60 chromatography showing maximal colony-stimulating activity were pooled and aliquots were treated as indicated above and described previously (6, 10).

† The results are expressed as the mean number of colonies per 1,000 cells.

Table 2. Stimulation of colony formation in soft agar by serum, plasma, plasma-derived serum, and platelet extracts

| Treatment | Soft agar colonies* AKR-2B | NRK |
|---|---|---|
| Human serum† | 95 | 170 |
| Human platelet-poor plasma† | 17 | 7 |
| Human platelet-poor plasma-derived serum† | 7 | 1 |
| Human platelet extract‡ | 1,820 | 840 |
| Bovine platelet extract‡ | 1,830 | 470 |

* The results are expressed as colonies per dish, mean of two determinations.

† Human serum, plasma, or plasma-derived serum, 20% concentration for each, were used instead of fetal bovine serum in the soft agar assay.

‡ Acid/ethanol extracts of human and bovine platelets (1 mg of protein per ml) were added to the standard soft agar assay.

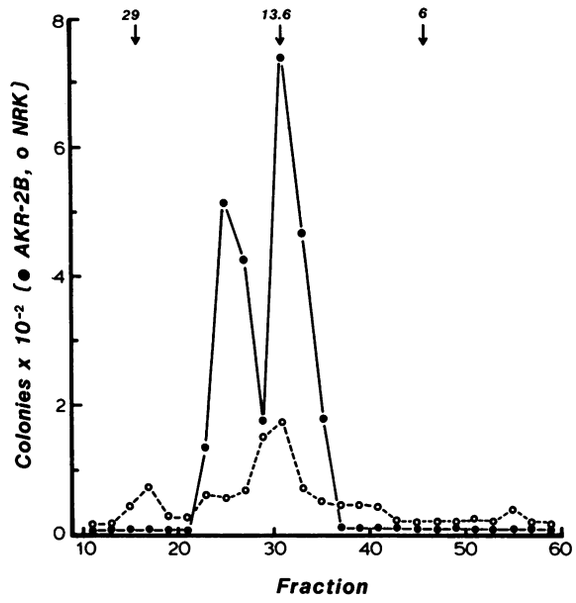


FIG. 5. Bio-Gel P-60 chromatography of acid/ethanol extracts of bovine platelets. Adult bovine platelets were extracted by the acid/ethanol procedure and the precipitate was extracted with 1 M acetic acid. The acetic acid-soluble material (10 mg of protein) was applied to a 1.7 × 87 cm Bio-Gel P-60 column and eluted with 1 M acetic acid in 3-ml fractions. One-half of every other fraction was used for soft agar assay with the AKR-2B (●) or NRK (○) cells. The standard markers were the same as those described in the legend for Fig. 3.

fetal bovine serum (Fig. 3). Bio-Gel P-60 chromatography of human platelet extracts gave a somewhat different profile. A single peak of AKR-2B stimulating activity was present in the 14,000–20,000 molecular weight range (Fig. 6), accompanied by a small amount of NRK-stimulating activity (data not shown). However, when EGF (2 ng/ml) was added to the soft agar assay, two prominent peaks of EGF-enhanced NRK-stimulating activity were observed, one in the 12,000–16,000 molecular weight range and one eluting after the insulin marker (molecular weight less than 6,000) (Fig. 6). The addition of EGF to the AKR-2B colony-stimulating assay did not cause significant enhancement of colony formation (data not shown).

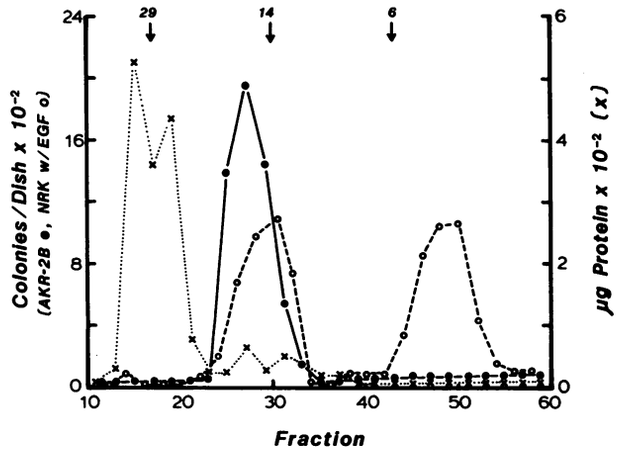


FIG. 6. Bio-Gel P-60 chromatography of acid/ethanol extracts of human platelets. The methods were the same as those described in the legend for Fig. 5 except that 5 mg of protein was applied to the Bio-Gel P-60 column. One-fifth of every other fraction was used for soft agar assays on AKR-2B cells (●) and one-fifth fractions were supplemented with EGF at 2 ng/ml and assayed on NRK cells (○). Protein content per tube (×) was determined by the dye-binding assay (12).

Pooled fractions containing major colony-stimulating activity from the Bio-Gel P-60 chromatography of human platelet extracts were also shown to be trypsin and dithiothreitol sensitive and relatively heat stable (data not shown). There was no associated competition for binding to the EGF receptor by this activity.

In an attempt to determine whether the colony-stimulating activity from serum and platelets was due to a previously described growth factor known to be present in serum or platelets, various growth factors were examined for their ability to stimulate colony formation by AKR-2B and NRK cells. EGF, somatomedin C, insulin, and multiplication-stimulating activity did not cause stimulation of colony formation by either cell type even at concentrations 100-fold greater than the concentration necessary for obtaining stimulation of DNA synthesis. More pure preparations of cationic PDGF [purified through the Bio-Gel P-150 step in the scheme reported by Antoniades *et al.* (16)] did not stimulate colony formation by either of the indicator cell types with concentrations up to 100-fold greater than the concentration required for stimulation of DNA synthesis. These results are in agreement with previous reports by other investigators (3, 5).

## DISCUSSION

The results of this study indicate that the capability of high levels of serum to cause anchorage-independent growth of certain cells is due to a specific polypeptide factor(s). The serum factor(s) appears to be derived from platelets because it is not present in platelet-poor plasma and platelet-poor plasma-derived serum or is present in very low concentrations and because it is present in very high concentrations in platelets.

These serum and platelet polypeptides stimulate cell growth and may be termed growth factors. They have properties very similar to those of many of the previously described transforming growth factors (3–6); therefore, they may be additional members of this group of biologically active compounds. Previous studies with TGFs have used as indicator cells the nontransformed rat NRK cells (3–5, 8, 9) or the mouse AKR-2B cells (6, 10). Both cell lines were used in the present study, and the serum and platelet factors are potent stimulators of soft agar growth of both cell types.

Several different types of TGFs have been described. One is the sarcoma growth factor isolated from medium conditioned by murine sarcoma virus-transformed cells (3). This TGF has a molecular weight of approximately 10,000, stimulates growth of NRK cells, and competes for binding to the EGF receptor but does not crossreact with EGF antibodies (7). Very similar factors having NRK colony-stimulating activity and the ability to compete for binding to the EGF receptor have been isolated from medium conditioned by a variety of human cancer cell lines (4). The serum and platelet TGF described in the present study appears to be different from these TGFs because it does not compete for binding to the EGF receptor. Chemically transformed cells have been shown to release into serum-free medium (6), and mouse embryos have been shown to contain (10), a TGF-like compound that is a potent stimulator of colony formation with AKR-2B cells and that does not compete for binding to the EGF receptor. Roberts *et al.* (5, 8) have recently reported the presence in a wide variety of tissues of intracellular TGF activity that does not compete for binding to the EGF receptor and that is a potent stimulator of colony formation by NRK cells, but only in the presence of added EGF (2 ng/ml). The TGF activities described in this report from serum and platelets may be the same as the activity reported by Roberts *et al.* (5, 8), but the bovine serum and platelet AKR-2B-stimulating activities are not enhanced by EGF and the NRK-stimulating activity shows

only about 2- to 3-fold EGF enhancement of colony stimulation by NRK cells. The NRK-stimulating activity in human platelets showed greater enhancement by addition of EGF to the soft agar assay. The specific relationship of these different TGFs remains to be established.

The possibility that the TGF-like compounds described in this study are the same as well-known serum factors for which another action has been discovered must be considered. Previous work showing that EGF, FGF, cationic PDGF, insulin, multiplication-stimulating activity, and insulin-like growth factors do not stimulate growth in soft agar of NRK cells largely excludes this possibility (3, 5). We have shown that the AKR-2B and NRK cells are not stimulated to grow in soft agar by EGF, highly purified cationic PDGF, multiplication-stimulating activity, stomatomedin C, and insulin. Other evidence that the compound is not cationic PDGF is also provided by the elution from the Bio-Gel P-60 column with an apparent molecular weight of 14,000–20,000, which is much smaller than the reported molecular weight of PDGF, 36,000 (17). In addition, isoelectric focusing of the serum factor gave activity only in the anionic range, whereas PDGF is a highly cationic polypeptide with a pI of 9.8.

Platelets have been shown to contain growth factors other than the cationic PDGF. Heldin *et al.* (14) reported that platelets contain two anionic growth factors, one with a molecular weight of approximately 40,000 and another with an apparent molecular weight of slightly less than 10,000. These apparent growth factors have not been well characterized. Castor *et al.* (18) have shown that platelets release a polypeptide with a calculated molecular weight of 11,633 that is a potent mitogen for a variety of connective tissue cells. This factor, called connective tissue-activating peptide, has been purified to homogeneity and is not the same as the cationic PDGF. Eastment and Sirbasku (19) have found in platelets a factor distinct from cationic PDGF that stimulates growth of rat mammary adenocarcinoma cells. The relationship of the TGF described in the present report to these less well-known platelet-derived growth factors remains to be established.

The presence of TGF-like growth factor(s) in serum and platelets has possible significance in several respects. The capability of the TGFs to stimulate growth in soft agar of normally anchorage-dependent indicator cells has caused considerable interest in these compounds because this property is the best *in vitro* correlate of tumorigenicity (20). The TGFs have, therefore, been proposed as the proximal effectors of the transformed phenotype (21). Their presence in serum and platelets would suggest that these compounds may also play a role in normal cell proliferation. The serum and platelet growth factors could play a role in any of the various normal and pathologic processes for which the cationic PDGF has been implicated (22, 23). These include induction of the early proliferative atherosclerotic plaque, wound healing, and implantation and growth of metastatic neoplasms. Another possible significance is that the serum TGF-like compound(s) could be a cofactor in the soft agar growth induced by other compounds. All of the soft agar assays used to detect TGFs are carried out in the presence of 10% calf serum or fetal bovine serum. They may also contribute to TGFs identified in serum-free conditioned medium or extracted directly from cells cultured in the presence of serum if the serum TGF adheres tightly to the cells and is not readily removed by rinsing with serum-free medium.

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1. O'Neill, C. H., Riddle, P. N. & Jordan, P. W. (1979) *Cell* **16**, 909–918.
2. Peehl, D. M. & Stanbridge, E. J. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 3053–3057.
3. DeLarco, J. E. & Todaro, G. J. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 4001–4005.
4. Todaro, G. J., Fryling, C. & DeLarco, J. E. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5258–5262.
5. Roberts, A. B., Anzano, M. A., Lamb, L. C., Smith, J. M., Frolik, C. A., Marquardt, H., Todaro, G. J. & Sporn, M. B. (1982) *Nature (London)* **295**, 417–419.
6. Moses, H. L., Branum, E. L., Proper, J. A. & Robinson, R. A. (1981) *Cancer Res.* **41**, 2842–2848.
7. DeLarco, J. E. & Todaro, G. J. (1980) *J. Cell. Physiol.* **102**, 267–277.
8. Roberts, A. B., Anzano, M. A., Lamb, L. C., Smith, J. M. & Sporn, M. B. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 5339–5343.
9. Roberts, A. B., Lamb, L. C., Newton, D. L., Sporn, M. D., DeLarco, J. E. & Todaro, G. J. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 3494–3498.
10. Proper, J. A., Bjornson, C. L. & Moses, H. L. (1982) *J. Cell. Physiol.* **110**, 169–174.
11. Rutherford, R. B. & Ross, R. (1976) *J. Cell Biol.* **69**, 196–203.
12. Pedersen, K. O. (1947) *J. Phys. Colloid Chem.* **51**, 164–171.
13. Slichter, S. J. & Harker, L. A. (1976) *Br. J. Haematol.* **34**, 395–402.
14. Heldin, C. H., Wasteson, A. & Westermark, B. (1977) *Exp. Cell Res.* **109**, 429–437.
15. Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254.
16. Antoniades, H. N., Scher, C. D. & Stiles, C. D. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1809–1813.
17. Antoniades, H. N. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 7314–7317.
18. Castor, C. W., Ritchie, J. C., Williams, C. H., Jr., Scott, M. E., Whitney, S. L., Myers, S. L., Sloan, T. B. & Anderson, B. E. (1979) *Arthritis Rheum.* **3**, 260–272.
19. Eastment, C. T. & Sirbasku, D. A. (1980) *In Vitro* **16**, 694–705.
20. Cifone, M. A. & Fidler, I. J. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 1039–1043.
21. Sporn, M. B. & Todaro, G. J. (1980) *N. Engl. J. Med.* **303**, 878–880.
22. Ross, R. (1981) *Handb. Exp. Pharmacol.* **57**, 133–159.
23. Sporn, M. B. & Harris, E. D. (1981) *Am. J. Med.* **70**, 1231–1236.