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# New class of transforming growth factors potentiated by epidermal growth factor: Isolation from non-neoplastic tissues

(soft agar growth/cell transformation)

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**ABSTRACT** Proteins potentiated by epidermal growth factor (EGF) to induce a transformed phenotype in non-neoplastic rat kidney fibroblasts in cell culture have been isolated from many non-neoplastic tissues of the adult mouse, including submaxillary gland, kidney, liver, muscle, heart, and brain. They resemble previously described transforming growth factors (TGFs) isolated from neoplastic cells as follows: they are extractable by acid/ethanol and are acid-stable, low molecular weight (6000–10,000) polypeptides requiring disulfide bonds for activity, and they cause anchorage-independent growth of non-neoplastic indicator cells that will not grow in soft agar in their absence. Partial purification of these TGFs from submaxillary glands of male mice shows that they are distinct from EGF. Unlike previously described extracellular TGFs, but like certain cellular TGFs from neoplastic cells, they are potentiated by EGF in their ability to promote anchorage-independent growth. The isoelectric point of the submaxillary gland TGF protein is near neutrality. Chromatography on Bio-Gel P-30 followed by high-pressure liquid chromatography has resulted in a 22,000-fold overall purification. The most purified protein is active in inducing growth in soft agar at 1 ng/ml when assayed in the presence of EGF. The data add further evidence to the concept that neoplasia may result from a quantitative, rather than qualitative, alteration in non-neoplastic biochemical processes.

We have recently described (1) the isolation and characterization of a set of low molecular weight, acid-stable polypeptides, called transforming growth factors (TGFs), from several neoplastic mouse tissues including fibroblasts transformed by Moloney sarcoma virus (MSV) and a transplantable bladder carcinoma originally induced by a chemical carcinogen. These polypeptides are intracellular proteins that are extractable by acid/ethanol. Similar extracellular transforming polypeptides, called sarcoma growth factors (SGFs), were first isolated by De Larco and Todaro (2) from the conditioned medium of MSV-transformed mouse fibroblasts grown in culture. Several other extracellular transforming polypeptides derived from neoplastic cells of both human (3) and animal (4) origin have been reported recently.

All of these polypeptides cause the following set of changes when applied to untransformed, non-neoplastic indicator cells growing in culture, and these changes provide an operational definition of TGFs: (*i*) loss of density-dependent inhibition of growth in monolayer; (*ii*) overgrowth of cells in monolayer; (*iii*) change in cellular shape, with the result that the indicator cells assume the neoplastic phenotype; and (*iv*) acquisition of anchorage independence, with the resultant ability to grow in soft agar. Untransformed, non-neoplastic cells will not form progressively growing colonies in soft agar, and this property of anchorage-independent growth of cells in culture has a particularly high correlation with neoplastic growth *in vivo* (5–7).

Using methods essentially identical to those previously used for the isolation and characterization of TGFs from neoplastic tissues (1), we have found a related set of intracellular TGFs from many non-neoplastic tissues of the adult mouse, including skeletal muscle, heart, liver, kidney, brain, and submaxillary gland. Particular attention was given to the submaxillary gland because a number of biologically active peptides have been isolated from this tissue (8, 9). The TGFs isolated from non-neoplastic cells resemble the intracellular TGFs from neoplastic cells; when assayed in the presence of epidermal growth factor (EGF) they are equally potent inducers of anchorage-independent growth of untransformed, non-neoplastic, indicator cells. This property clearly distinguishes the TGFs from many other known mitogenic growth factors such as insulin, the insulin-like growth factors, platelet-derived growth factor, nerve growth factor, fibroblast growth factor, and EGF, none of which have this property (unpublished data).

## MATERIALS AND METHODS

**Extraction and Gel Exclusion Chromatography of Mouse Tissues.** Mature male Swiss Webster mice were given an intraperitoneal injection of 10 mg of testosterone propionate in sesame oil (10). One week later, the mice were killed by $CO_2$ narcosis; the excised tissues were immediately frozen and stored over liquid nitrogen. The tissues were powdered at liquid nitrogen temperature (Tekmar laboratory mill with cryostatic attachment) before extraction with acid/ethanol, precipitation with ethanol and ether, dialysis, and lyophilization of the extract, as described for the extraction of TGFs from neoplastic cells (1). Chromatography on Bio-Gel P-60 or P-30 (100–200 mesh) was done with upward flow of 1 M acetic acid on 2.5 × 70 cm or 5.0 × 90 cm columns at flow rates of 15 or 35 ml/hr (1). Protein concentrations in all assays were determined by dye binding (11) with bovine serum albumin as standard.

**Soft Agar Assay.** Assay for growth of anchorage-dependent rat kidney fibroblasts, clone 49 F, in soft agar medium was modified from that described (1). Cells were stained after 7 days (12), and colonies were counted and measured by using a Bausch and Lomb Omnicon image analysis system consisting of a light microscope with attached television camera, image analyzer, and data terminal. The field was displayed two-dimensionally, and all values were derived from the area of the colony cross section. The image analyzer system was programmed to size colonies on a logarithmic scale from 500 $\mu m^2$ to a maximum of 353,000 $\mu m^2$ in 25 categories and to determine the mean colony size from the data obtained from each plate. A total area of 0.5 $cm^2$ was read for each plate in 15 separate fields; the data represent a compilation of the averages of three counts of each field.

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Abbreviations: MSV, Moloney sarcoma virus; SGF, sarcoma growth factor; TGF, transforming growth factor; EGF, epidermal growth factor; HPLC, high-pressure liquid chromatography; RSV, Rous sarcoma virus.

In the absence of any added growth factors, few cells undergo even a single cell division. The addition of TGFs to the assay produces a dose-dependent increase in both mean colony size and absolute number of colonies. Colonies larger than 9000 $\mu m^2$ (0.1 mm in diameter) were determined to have particular significance for the assay of EGF-potentiated TGF activity because the background level of EGF-induced colonies of this size is low. Within a particular assay, the number of colonies or the mean colony size determined on duplicate plates typically agreed within 5–10%. Although the maximal cellular response to TGFs showed some variation from assay to assay, both the median and relative positions of the dose–response curves remained essentially unchanged.

**Epidermal Growth Factor (EGF).** EGF was extracted from the submaxillary gland of male mice together with TGFs by using the same acid/ethanol extraction procedure and chromatography on polyacrylamide gels in 1 M acetic acid as described for TGFs (1). EGF was retained on the Bio-Gel P-60 column and eluted just after the salt peak (see Fig. 2A), as described by Savage and Cohen (13). The pooled EGF from the Bio-Gel column was lyophilized, redissolved, dialyzed against 0.02 M ammonium acetate (pH 5.6), and further purified by chromatography on Whatman DE-52 DEAE-cellulose (13). This EGF was shown to be free of lysine, alanine, and phenylalanine after acid hydrolysis, to migrate as a single band on basic polyacrylamide gels (pH 8.4), and to migrate as a single band on isoelectric focusing. The yield of DEAE-cellulose-purified EGF was 0.25–0.35 mg/g (wet weight) of tissue. Iodination was by the chloramine-T method (14). In a standard radioimmunoassay, rabbit antiserum raised against this EGF responded identically to antiserum kindly provided for comparison by Stanley Cohen.

**Isoelectric Focusing.** Lyophilized samples pooled after chromatography on Bio-Gel P-60 columns were dissolved in 100 $\mu l$ of 4 mM HCl and diluted to 6 ml in 2% Pharmalyte, pH 3–10 (Pharmacia). The sample was applied as a zone to a prefocused bed (217 × 217 mm) consisting of 7 g of Ultradex (LKB-Produkter, Bromma, Sweden) in Pharmalyte solution. The sample was focused on a flat-bed isoelectric focusing apparatus (Pharmacia) for 15 hr at 10°C with 8 W constant power. The gel bed was then fractionated into 26 zones and the pH was measured by using a surface electrode. Samples were eluted from each zone with 1 M acetic acid, dialyzed extensively at 4°C against 0.17 M acetic acid to remove the carrier Pharmalytes (Spectrapor tubing, $M_r$ cut-off, 3500), lyophilized, and reconstituted in 1 M acetic acid for determination of activity in the soft-agar assay, EGF by radioimmunoassay, and protein.

**High-Pressure Liquid Chromatography (HPLC).** Lyophilized samples pooled after chromatography on Bio-Gel P-30 columns were dissolved in 24% $n$-propanol in aqueous 0.1% trifluoroacetic acid (pH 2) and chromatographed on an analytical $\mu$Bondapak CN column (3.9 × 300 mm; Waters Associates) with a flow rate of 1.0 ml/min and a gradient of 24–48% $n$-propanol in 0.1% trifluoroacetic acid over 40 min. Aliquots of the the 1-ml fractions were removed for determination of protein, soft-agar activity, and EGF by radioimmunoassay. Remaining fractions were taken to dryness on a Vortex evaporator (Buchler) and stored at −20°C. Recovery of protein ranged from 85% to 110%.

## RESULTS

Acid/ethanol extracts of all non-neoplastic mouse tissues stimulated anchorage-independent growth of rat kidney fibroblasts in soft agar. EGF, which potentiates the ability of certain cellular TGFs from neoplastic tissues to induce the formation of large colonies in soft agar (unpublished data), was found here to be a strong determinant of the activities of the TGFs from non-neoplastic mouse tissues. When assayed in the absence of added EGF, extracts of kidney, liver, muscle, and submaxillary gland all had a higher apparent specific activity in the soft-agar assay than did cellular SGF extracted from neoplastic mouse 3T3 cells (1) (Fig. 1A). However, a parallel soft-agar assay carried out in the presence of EGF at 2 ng/ml [a concentration shown to be optimal for the potentiation of the TGF-induced response (unpublished data)] showed that the specific activity of the extracts from all the non-neoplastic tissues was approximately equal to that of the cellular SGF (Fig. 1B). EGF itself stimulated only minimal formation of large colonies (Fig. 1B). The disparity between the results obtained in the presence and absence of EGF can be explained, in part, by the EGF levels of the crude acid/ethanol extracts of each of these tissues (Table 1). There was sufficient intrinsic EGF in extracts of the submaxillary gland or kidney for full potentiation of the TGFs from these tissues in the soft-agar assay. Extracts of tissues such as liver, muscle, brain, and heart contained only very low amounts of EGF, and the crude cellular SGF contained essentially none. Comparison of Fig. 1 A and B, as well as the data in Table 1, shows that the specific activity of those tissue extracts low in EGF is markedly increased in the presence of EGF. In the presence of optimal concentrations of EGF, the specific activities of acid/ethanol extracts of liver, muscle, heart, brain, submaxillary gland, and kidney all were similar to the specific activity of extracts of transformed cells, represented by cellular SGF. Also, like the cellular SGF (1) and other TGFs (1–3) isolated from neoplastic tissues, the activities of the TGFs from non-neoplastic tissues were <5% of the controls after treatment with either trypsin or dithiothreitol (data not shown).

Chromatography of the acid/ethanol extracts of mouse submaxillary gland (Fig. 2A) and kidney (Fig. 2B) on Bio-Gel P-60 columns in 1 M acetic acid showed that, like the cellular SGF (1), the soft-agar colony-forming activity eluted from the column in a broad profile after the RNase marker ($M_r$, 13, 000) and just

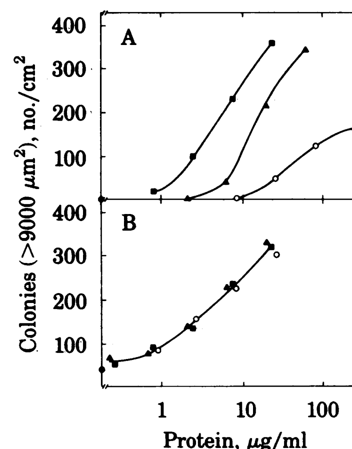


FIG. 1. Dilution curves of soft-agar colony-forming activity of acid/ethanol extracts of representative tissues of the male mouse. Frozen tissues (25–100 g) were extracted with acid/ethanol, solvent-precipitated, dialyzed, and lyophilized (1). The average yield was 6–10 mg/g (wet weight) of tissue. These crude extracts were dissolved in 4 mM HCl at 10 times their final concentration in the soft-agar assay. Assays were carried out as described, stained at 7 days, and read on the image analyzer. (A) Assayed in the absence of added EGF; (B) assayed in the presence of added EGF at 2 ng/ml. The background levels of colonies in the assay with no TGF added, with and without EGF, are shown on the ordinates. ■, Kidney; ▲, liver; ○, equivalent acid/ethanol extract of MSV-transformed mouse 3T3 fibroblasts (cellular SGF) used in each assay as a marker.

Table 1. Effect of variations in intrinsic tissue EGF levels on assayable TGF activity in different mouse tissues

| Tissue | Specific activity of TGF* | | Intrinsic EGF,† ng/mg protein |
|---|---|---|---|
| | Without added EGF | With added EGF (2 ng/ml) | |
| Submaxillary gland | 21 | 21 | 178,000 |
| Kidney | 37 | 41 | 163 |
| Liver | 10 | 52 | 45 |
| Muscle | 18 | 18 | 42 |
| Brain | 4.3 | 24 | 21 |
| Heart | 2.5 | 48 | 19 |
| Transformed fibroblasts (cellular SGF) | 1.3 | 50 | <1 |

* Specific activity (expressed as colonies per $\mu$g per ml) was calculated from the amount of protein required to cause the formation of 160 colonies (>9000$\mu m^2/cm^2$), using acid/ethanol extracts of the various tissues.

† As determined by radioimmunoassay of acid/ethanol extracts.

before the insulin marker ($M_r$ 6,000), having an apparent $M_r$ of 7000–10,000. EGF, determined by radioimmunoassay, eluted from the Bio-Gel P-60 column after the salt peak, as reported by Savage and Cohen, who used Bio-Gel P-10 in a different solvent system (13). Although Fig. 2*A* shows that EGF and the TGF activity of submaxillary gland extracts were generally separated on a Bio-Gel column, nanogram amounts of EGF could be detected by radioimmunoassay in the region of TGF activity (less than 1% of the EGF on the column). This minute amount of EGF was sufficient for optimal potentiation of the submaxillary gland TGF activity in the soft-agar assay, thus making it insensitive to the addition of EGF (2 ng/ml) (see Fig. 5). A similar effect was seen with the pooled TGFs from Bio-Gel chromatography of kidney extracts, even though the kidney extracts contained less than 0.1% of the EGF of the submaxillary gland (Table 1).

Isoelectric focusing of the pooled TGFs from Bio-Gel P-30 chromatography of the submaxillary gland extract showed that the major TGF activity potentiated by EGF focused at an isoelectric point of approximately 6.8–7.4 (Fig. 3*A*, fractions 7–10), similar to the isoelectric point of 6.8 determined by De Larco *et al.* (15) for the $M_r$ 7000 SGF of conditioned medium. EGF (determined by radioimmunoassay) in the submaxillary gland TGF (purified on Bio-Gel P-30) focused at an isoelectric point of 4.2–4.4 (Fig. 3*B*), in good agreement with published values (16). As reported elsewhere (unpublished data), in soft agar, EGF itself is able to induce the formation only of small colonies (mean size 2000–3000 $\mu m^2$), and the response is independent of EGF concentration in the range 2–100 ng/ml. However, in the presence of optimal TGF levels or suboptimal concentrations of TGFs in combination with EGF, a dose-dependent response of large colonies (>9000 $\mu m^2$) can be demonstrated. A similar phenomenon can be seen in Fig. 3*A*, where it is shown that pooled submaxillary gland fractions 7–10 (neutral isoelectric point), assayed in the presence of EGF at 2 ng/ml, caused a dose-dependent formation of large colonies in the soft-agar assay, whereas pooled fractions 15–20 (EGF region) behaved similarly to known EGF in the assay, causing dose-independent formation of small colonies (unpublished data).

HPLC also gave an excellent separation of TGFs from contaminating EGF and other unwanted proteins still remaining in submaxillary gland extracts after purification by gel exclusion chromatography (Fig. 4). The submaxillary gland TGF purified by HPLC was markedly potentiated (18-fold) by EGF in the soft agar assay (Fig. 5), as reported for partially purified preparations of cellular SGF and a TGF derived from bladder carcinomas (unpublished data). It had measurable activity in the soft-agar assay at a concentration of 18 ng/ml in the absence of EGF and at approximately 1 ng/ml in the presence of EGF at 2 ng/ml. The overall purification of the TGF from the submaxillary gland was approximately 2200-fold from the crude acid/ethanol extract or approximately 22,000-fold from the original tissue (Fig. 5*B*). It is expected that one or two additional purification steps will result in a homogeneous preparation. Preliminary results with $\mu$ Bondapak $C_{18}$ columns and acetonitrile gradients in 0.1% trifluoroacetic acid show that excellent separation and recovery of TGFs from other proteins can be obtained (17). The data in Fig. 5 emphasize the need for assessing the degree of purification of this class of TGFs by assay in the presence of the synergistic growth factor EGF, whose ubiquitous presence in the initial steps of purification results in an amplification of the TGF activity over the intrinsic activity of these factors once they have been freed of contaminating EGF.

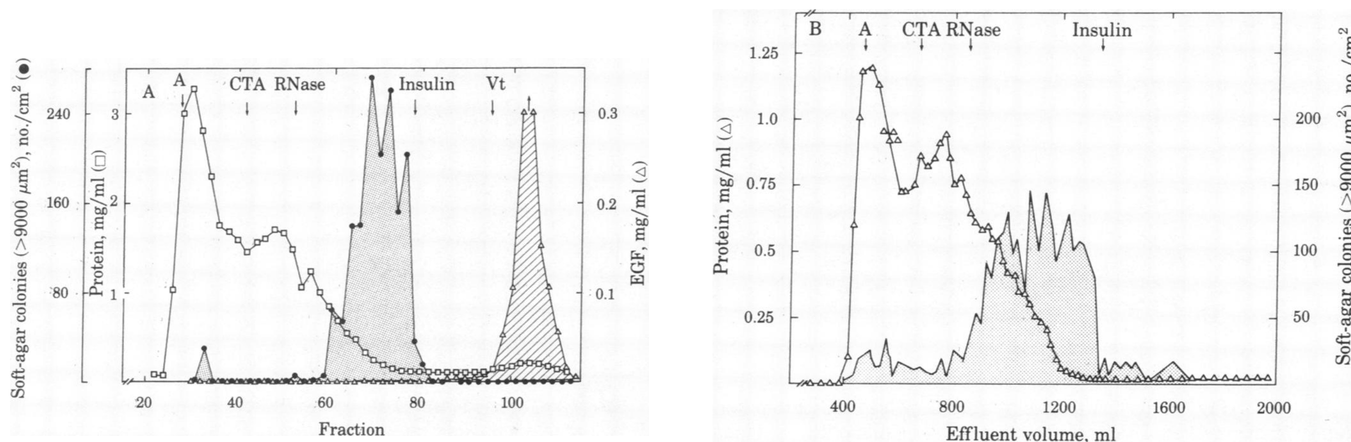


FIG. 2. Gel exclusion chromatography of acid/ethanol extracts. (*A*) Chromatography of 250 mg of acid/ethanol extract of male mouse submaxillary gland on a 2.5 × 70 cm column of Bio-Gel P-60. The sample was applied in 6 ml of 1 M acetic acid and eluted in 3-ml fractions. Protein analyses and soft-agar assays were performed on lyophilized 50-$\mu$l aliquots. EGF was determined by radioimmunoassay. (*B*) Chromatography of 1 g of acid/ethanol extract of male mouse kidney. The sample was dissolved in 17 ml of 1 M acetic acid, applied to a 5 × 100 cm Bio-Gel P-30 column, and eluted in 10-ml fractions. Aliquots (100 $\mu$l and 200 $\mu$l) were taken for determination of protein and soft-agar activity (stippled area), respectively. Markers were: A, bovine serum albumin (68,000); CTA, Chymotrypsinogen A(25,000); RNase (13,800): and insulin (6000).

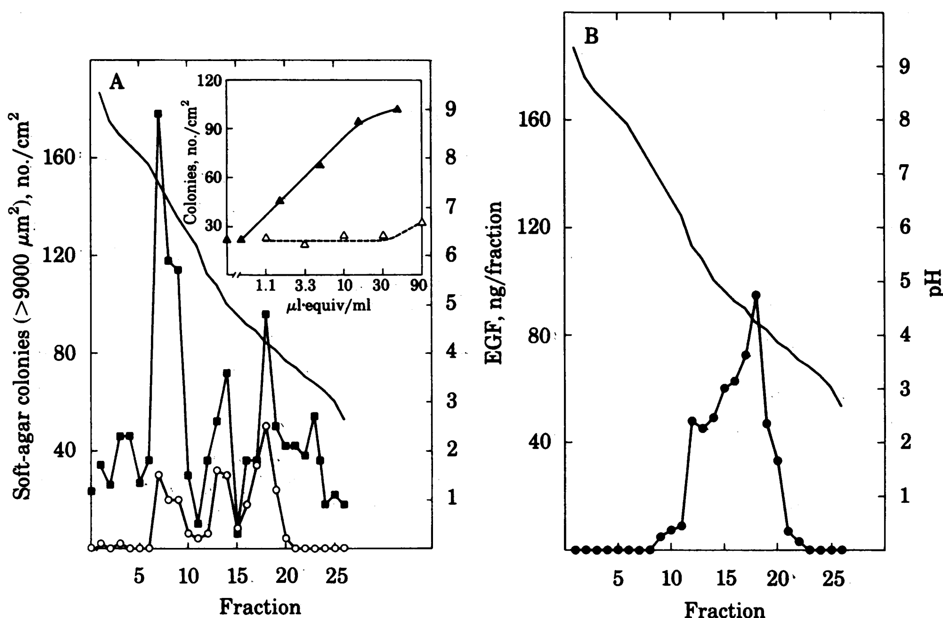


FIG. 3. Isoelectric focusing of pooled fractions from Bio-Gel P-30 chromatography of acid/ethanol extracts of male mouse submaxillary glands. Focusing was carried out in carrier ampholytes, pH range 3–10, on a flat-bed device. A sample load of 1.6 mg from the Bio-Gel column was applied to the focusing gel bed. The lyophilized residue from each zone was reconstituted to 1 ml with 1 M acetic acid. Aliquots (70 μl) were then used for determination of soft-agar activity (final concentration in assay, 35 μl-equiv/ml) and EGF by radioimmunoassay. (*A*) Soft-agar assay of the focused fractions, assayed in the absence (○) or presence (■) of EGF at 2 ng/ml. The background levels of colonies in the assay with no TGF added, with and without EGF, are shown on the ordinates. (*Inset*) Soft-agar assay of serial 1:3 dilutions of pooled fractions 7–10 (▲) or fractions 15–20 (△). Both pools were assayed in the presence of added EGF at 2 ng/ml. (*B*) Determination of EGF (●) by radioimmunoassay after isoelectric focusing as in *A*; the pH (right ordinate) was determined by using a glass surface electrode and pertains to both *A* and *B*.

## DISCUSSION

The results presented indicate that transforming polypeptides, which are potentiated by EGF to stimulate anchorage-independent growth of untransformed indicator cells, can be extracted from many non-neoplastic tissues of the adult mouse. Although the assayable level of such transforming activity in crude extracts appears to differ from tissue to tissue, when EGF is included at optimal levels in the soft-agar assay a similar level of transforming activity is found in the different tissues. Characterization thus far of the transforming polypeptides extracted from non-neoplastic mouse tissues, such as the submaxillary gland and kidney, indicates that they are similar to peptides found in neoplastic mouse tissues, such as the cellular SGFs derived from virus-transformed fibroblasts (1). Transforming polypeptides extracted from both neoplastic and non-neoplastic mouse tissues have similar molecular weights (as determined by gel exclusion chromatography) and are dependent on integrity of disulfide bonds for activity. As reported here, one class of TGFs is markedly potentiated in activity by nanogram levels of EGF, which in itself is not a potent TGF. Although the amino acid sequence of any of the TGFs remains to be determined, the ability to purify these peptides on HPLC which we have demonstrated in the present study indicates that this should be an attainable goal once the preparations can be scaled up to larger amounts.

The finding of TGFs in many non-neoplastic tissues of an adult animal at first may seem surprising, but it is not unexpected in light of new insights regarding the molecular biology of the transforming (*src*) genes of RNA tumor viruses. Indeed, recent studies on the *src* gene products in both avian (18, 19) and rat (20, 21) sarcoma systems indicate that a "mere change in the level of expression of a normal cellular gene and gene product may be sufficient to induce the transformed pheno-

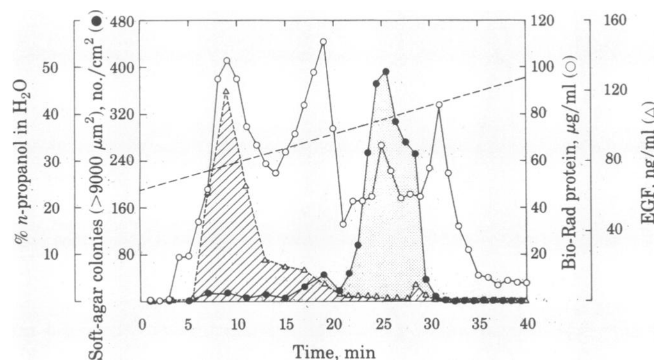


FIG. 4. HPLC of pooled fractions from Bio-Gel P-30 chromatography of acid/ethanol extracts of male mouse submaxillary glands. The sample (1.2 mg) was dissolved in 250 μl of 24% *n*-propanol in 0.1% aqueous trifluoroacetic acid and injected onto an analytical μBondpak CN column, 3.9 × 300 mm. The column was developed with a gradient of 24–48% *n*-propanol (dashed line) in aqueous 0.1% trifluoroacetic acid over 40 min. Aliquots (50 μl) of the 1-ml fractions were used for determination of soft agar activity (●), EGF by radioimmunoassay (△), and protein (○).

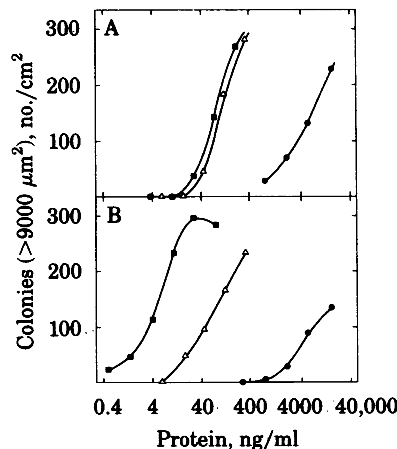


FIG. 5. Dilution curves showing sequential purification of TGFs from the male mouse submaxillary gland. Samples were: crude acid/ethanol extract (●); pooled fractions of soft-agar colony-forming activity from Bio-Gel P-30 chromatography, as shown in Fig. 2*A* (△); and peak fractions from HPLC purification, as shown in Fig. 4 (■). (*A*) Assayed in the absence of EGF. (*B*) Assayed in the presence of EGF at 2 ng/ml. In *B* the background of 35 colonies per cm² caused by EGF alone has been subtracted from each point. Note the marked potentiation of activity by EGF after HPLC.

type" (21). In the Rous sarcoma virus (RSV) transformation system it has been suggested that the virus may simply introduce a normal cellular gene into an untransformed cell, and subsequent transformation might then be a matter of dosage —i.e., the extent of expression of that gene (18). The RSV *src* gene appears to be derived from a cellular gene that, in uninfected, non-neoplastic cells, expresses a phosphoprotein phosphokinase structurally and functionally closely related to the RSV transforming gene product, the protein kinase $pp60^{src}$ (19).

Moreover, in several other non-neoplastic cells, regulatory polypeptides have been found that bear close relationship to gene products derived from transforming (*src*) genes. EGF recently has been shown to activate an endogenous cell membrane kinase which then phosphorylates tyrosine residues on a target protein molecule (22, 23), in a manner analogous to avian RSV $pp60^{src}$ (18, 19). Furthermore, a well-characterized rat brain membrane protein has been shown to have close functional resemblance to the *src* gene product of the Kirsten and Harvey rat sarcoma viruses in that all three bind guanine nucleotides in a similar manner (24).

The physiological role of the TGFs that we have found in many non-neoplastic tissues in the present study is not clear. Their lower molecular weight indicates that they are not identical to *src* gene products of either the avian (Rous) or rat (Harvey, Kirsten, Rasheed) sarcoma system. The isolation of these polypeptides in a form in which they can be assayed exogenously for enhancement of the ability of untransformed indicator cells to grow in soft agar does not imply that these same TGFs are phenotypically expressed in a continuous manner in the cells from which they came. Endogenously, the TGFs may be in an inactive form, as a polypeptide precursor (a pro form) or in a physically sequestered state (within a membranous vesicle or other compartment or complexed with a binding protein). The dissociative properties of the acid/ethanol which has been used for their isolation would be likely to unmask some of these latent activities.

Although it has been demonstrated that the secretion by transformed cells of TGFs which compete with EGF for membrane receptors is correlated with the expression of the transformed phenotype (2–4), the finding of EGF-potentiated TGFs in a wide variety of non-neoplastic tissues, some of which ordinarily have little DNA synthesis and mitosis, suggests other physiological roles for this new class of TGFs. These may include participation in various repair phenomena in response to tissue injury or some other evolutionarily primitive role yet to be discovered. Although the finding of TGFs originated in studies of mechanism of malignant transformation of cells (2), further investigation of their physiological role may be relevant to mechanisms of pathogenesis of proliferative diseases other than cancer, such as atherosclerosis and diseases of connective tissue (25). Also, it may be possible to use these polypeptides in a therapeutic manner to repair tissue damage, as in wound healing of various types.

All of these problems will require further laboratory investigation. The following would appear to be key problems: (*i*) the determination of the primary structures and, in particular, any sequence homologies of TGFs from both neoplastic and non-neoplastic tissues; (*ii*) the control of synthesis, processing, and release of TGFs as well as their interaction with receptors; and (*iii*) the mechanism of synergism between EGF and certain TGFs, including functional relationships between TGFs and the known phosphoprotein kinases.

The present findings add evidence to the concept that neoplasia may result from a quantitative, rather than qualitative, alteration in non-neoplastic biochemical processes and suggest that study of a set of peptides that are found in many non-neoplastic tissues may be of importance to understanding and control of neoplasia. Finally, the finding of potential TGF activity in so many tissues that ordinarily do not express the neoplastic phenotype also suggests that cellular control mechanisms that can suppress the expression of these peptides remain to be discovered.

**Note Added in Proof.** Recent experiments indicate that there are two distinct classes of cellular TGFs in both neoplastic and non-neoplastic tissues. One class, similar to previously described extracellular TGFs (2–4), competes with EGF for membrane receptors and is not potentiated by EGF in the soft-agar assay. The other class of TGFs, as reported here, is potentiated by EGF to induce the formation of large colonies in the soft-agar assay and does not compete with EGF in a receptor binding assay. The functional levels of each of these two classes of TGFs in various tissues remain to be determined.

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