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# Purification and Partial Characterization of Bovine Pituitary Fibroblast Growth Factor

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A purification procedure and partial characterization of bovine pituitary fibroblast growth factor (FGF) are described. The steps of the published methods [3,4] which yield inhomogeneous material, were retained, with modifications. The final isolation, with an additional purification of ~20-fold, was achieved by electrophoresis in polyacrylamide gels at acid pH. The mitogenic peptide has a molecular weight of 14,500–15,000 as determined on SDS gels, chromatographs as a monomer in nondenaturing conditions, and is active at the picomolar level in effecting the incorporation of $^{3}$H-thymidine in Balb/c 3T3 cells. A preliminary amino acid composition is presented.

**Key words: pituitary fibroblast growth factor, silver staining, amino acid analysis**

The bovine fibroblast growth factors (FGFs) have been identified as mitogenic substances that exert a variety of effects on cells derived from embryonic mesoderm [1]. Brain and pituitary are the two major sources of these activities [2], and purification of FGF from both of these tissues has been reported [3, 4]. Brain FGF was also reported to be composed of fragments of myelin basic protein (MBP) [5], but our investigations have shown that this association is not correct [6]. Brain preparations, in fact, contain two distinct mitogenic entities characterized by acidic and basic pIs. The latter has many properties in common with, and may be identical to, the pituitary form of FGF [7]. However, confirmation of this identity will require further chemical and structural characterization of both molecules.

In addition to resolving the question of the relationship of the activities in brain and pituitary preparations, it was also of interest to us to obtain a purified mitogen for examining its biochemical and molecular mechanism of action, particularly relative to those of other polypeptide growth factors [8]. The pituitary, rather than brain, appeared a more promising source of FGF because the latter preparation contained two mitogens, was composed largely of MBP fragments, which have no relationship

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to the mitogenic activity [6, 7], and was approximately tenfold less potent in a Balb/c 3T3 bioassay in our hands [7].

This report describes the purification and initial characterization of bovine pituitary fibroblast growth factor. Although the mitogenic activity isolated by the published procedure [3, 4] is highly active, we found it necessary to carry out a twentyfold or further purification to obtain homogeneous material. The general properties (size and charge) reported for the impure preparation are basically the same for the pure mitogen.

## METHODS

### Balb/c 3T3 Cell Bioassay

Biological activity was monitored by measuring incorporation of (methyl-$^3$H)-thymidine into DNA of Balb/c 3T3 fibroblasts as described previously [6] with minor modifications [7]. One unit of activity is defined as 50% of maximal stimulation by a sample of FGF or 10% calf serum (CS).

### Preparation of Pituitary FGF

All procedures were performed at 0–4°C. The columns indicated are appropriate for a typical preparation of approximately 1 kg starting material. Biological activity was monitored in the Balb/c 3T3 assay.

**Homogenization and extraction.** Frozen pituitaries, obtained from Pel Freeze Biologicals, Rogers AR, or as the gift of Dr D.A.K. Roncari, University of Toronto, were thawed, trimmed, and homogenized in a 4-liter Waring blender with 3 × 15 sec bursts in two volumes of 0.15 M ammonium sulfate, 0.25 mM phenylmethylsulfonyl flouride (PMSF), and 1 mM EDTA per kg tissue (wet weight) with 5 mg each leupeptin and pepstatin A (Sigma Chemical Company, St. Louis). The pH of the homogenate was adjusted to 4.5 with 2 N HCl, stirred for 2 hr, and then spun at 12,000 g for approximately 1 hr. The supernatant was retained and adjusted to pH 6.5 to 7.0 with 1 N NaOH.

**Ammonium sulfate precipitation and dialysis.** The first ammonium sulfate precipitation of the neutralized crude extract was performed by slowly adding 250 g/liter of the salt, stirring for an additional 15 min, and then centrifuging as above for approximately 45 min. The second precipitation of the resultant supernatant was accomplished by the addition of 290 g/liter ammonium sulfate followed by stirring and centrifugation as in the first treatment. This pellet (P-II), which was stored at 4°C overnight, was redissolved in distilled $H_2O$ containing PMSF and EDTA and dialyzed extensively against the same solution for 1½ days in dialysis tubing with a 6,000–8,000 MW cut off (Spectropor 1, Spectrum Medical Industries, Inc., Los Angeles). The final P-II was clarified by centrifugation for 45 min as described above.

**Carboxymethyl-Sephadex-C50 stepwise fractionation.** The final P-II was adjusted to pH 6.0 and a conductivity of 2–2.5 mmho by adding 0.2 M sodium phosphate buffer, pH 6.0, and 0.1 M $NaH_2$ $PO_4$ and the sample loaded onto a column (4 × 12 cm) of carboxymethyl-Sephadex-C50 (CM-Sephadex) (Pharmacia Fine Chemicals, Uppsala, Sweden) in 0.1 M sodium phosphate, pH 6.0. All solutions in this step included 0.25 mM PMSF and 1 mM EDTA. The column was washed extensively with this buffer and then with the same phosphate buffer containing 0.15

M NaCl. The active fraction (F-III) was eluted with 0.1 M phosphate buffer, pH 6.0, containing 0.6 M NaCl, dialyzed extensively against distilled $H_2O$ as for P-II (omitting PMSF and EDTA from the two final dialysis changes), and then lyophilized.

**Sephadex G-75 column chromatography.** A column (2.5 × 90 cm) of Sephadex-G-75 superfine (10–40 $\mu$ resin) was developed in 0.2 M ammonium bicarbonate, 0.5 mM EDTA, 0.125 mM PMSF, at a flow rate of 10–12 ml/hr. The lyophilized F-III fraction was dissolved in 6–8.5 ml of the column buffer and centrifuged before loading on the column. Fifteen-minute fractions were collected. The final active fractions were pooled and lyophilized directly for the next step.

**CM-Sephadex-C50 gradient elution.** A column (1 × 10 cm) of CM-Sephadex was equilibrated with 0.1 M sodium phosphate, 0.1 M NaCl, pH 6.0, at a flow rate of 6.5–7.5 ml/hr. The Sephadex G-75 pool was redissolved in loading buffer, dialyzed against the same for equilibration, and centrifuged to remove particulate matter. The sample was loaded, and the column washed first with loading buffer and then with 0.15 M NaCl in the sodium phosphate buffer. A gradient from 0.15 to 0.6 M NaCl (240–280 ml total volume) was developed to elute the FGF activity. Fractions were pooled, dialyzed against 0.2 M ammonium bicarbonate, and lyophilized 2–3 times to remove salts.

The final purification step was elution from acid electrophoresis gels, as described below.

## Protein Determination

Protein concentration was determined by the method of Lowry et al [9] for all steps through the first CM-Sephadex column. Concentrations in the active pools from the Sephadex G-75 and the final CM-Sephadex columns were estimated by absorption at 220 nm (1 unit at 220 nm was equivalent to ~90 $\mu$g/ml based on a BSA standard), or by amino acid analyses of acid hydrolysates on a Durrum D-500 amino acid analyzer for the final CM-Sephadex FGF pool. Estimates by absorption at 220 nm and by amino acid analysis were very similar.

## SDS Polyacrylamide Electrophoresis

SDS polyacrylamide gels, 16.5%, were prepared and developed according to the method of Laemmli [10]. Protein bands were visualized by the silver-staining method, described previously [11], or by Coomassie Blue R. For SDS gel electrophoresis of acid gel slices, the appropriate segments were thawed and equilibrated for 15–20 min at room temperature in a buffer containing 10% glycerol, 2% SDS, 0.0625 M Tris (pH 6.8), and 2.5 mM dithiothreitol (DTT). The slices were inserted with forceps at the bottom of stacking gel wells and electrophoresed as described above. In some cases, SDS gel samples were also prepared in this equilibration buffer at 50% v/v, instead of the usual buffer containing $\beta$-mercaptoethanol.

## Acid Gel Electrophoresis

Acid gel disk electrophoresis was performed by the type "G" modified Reisfeld method of Rodbard and Chrambach [12] as described previously [7]. Ten percent, 7.5–8.0 cm, polyacrylamide resolving gels and 3.0% stacking gels were polymerized with light in the presence of riboflavin-5′ phosphate. Samples were dissolved in 10% glycerol and a 1:10 dilution of upper running buffer. Gels were electrophoresed at approximately 4°C and 10 mamp for 2 hr. This was the amount of time it took for a

cytochrome c marker, which ran near the buffer front, to migrate close to the bottom of the gel. Sample lanes to be tested for biological activity were cut into 0.25-cm segments and eluted by shaking overnight at 4°C in 1.0 ml of PBS and 2 mg/ml BSA. Adjacent lanes containing FGF to be further analyzed were also sliced and frozen at −20°C, while determination of the segments containing activity was made. Gels were stained with Coomassie Blue R.

### Elution of Samples from Acid and SDS Gels

Eluted material was obtained from four separate gel electrophoresis experiments. Three samples were obtained from acid gels. Fifty micrograms of fraction CM-C (see Fig. 2) were loaded, and the peak activity slices were determined by analysis of the bioactivity of gel slices obtained from a parallel lane. The fourth sample was sliced from an SDS gel run that had been loaded with 140 μg of CM-D (see Fig. 2). A parallel lane (25 μg loaded) was stained with Coomassie Blue R in order to determine the slices with the appropriate 15,000-MW band. The selected slices (0.25 × 0.15 × 2.5 cm) from all four gel runs were thawed, cut into smaller pieces, and transferred to 16-ml round bottom polypropylene tissue culture tubes (Falcon Labware, Oxnard, California). Gel fragments (two slices per incubation) were shaken gently for 16–20 hr at 37°C in 1.0 ml autoclaved distilled water with 1 mM benzamidine and 50 μg/ml PMSF added. The first eluate was transferred to another plastic tube, and the gel slices were washed once with 200 μl of autoclaved water, which was combined with the first supernatant. Forty microliters of the eluted sample was analyzed in SDS polyacrylamide gels.

### Amino Acid Analysis

All glassware and instruments were rinsed with distilled water, and precautions were taken to avoid contamination of samples. Twenty-five and 50 μg of fraction CM-D were run on a 12-cm, 16.5% SDS polyacrylamide gel and stained with 0.25% Coomassie Blue R in a solution of methanol:acetic acid:water (5:4:1). The gel was destained with several changes of a fresh solution of methanol:acetic acid: water (2:1:7) until background was negligible. The 15,000-MW band was cut from the two lanes in the gel, along with a parallel slice from a blank lane, and each was processed separately. Samples were rinsed further with 2–3 changes of a few milliliters of destainer with 2–3 hr between rinses. Slices were cut into smaller fragments, ~1 $mm^3$, and transferred to acid-washed 16 × 150 mm tubes, 0.5–1.0 ml 6 N HCl was added, tubes were evacuated, and samples hydrolyzed for 24 hr at 110°C. After hydrolysis, samples were transferred to 5-ml acid-washed glass conical tubes and the residual gel in hydrolysis tubes was rinsed two times with ~300 μl of 6 N HCl and combined in the conical tubes. These tubes were spun for 10 min at 1,800g, and the clarified supernatant was transferred for rotary evaporation. Compositions were determined on a Durrum D-500 amino acid analyzer using a 1,000 pmol standard. The glycine content from the blank slice was subtracted from reported values.

## RESULTS

The procedure for purification of bovine pituitary FGF adopted in these studies is based on the method of Gospodarowicz and colleagues as described in their original papers [3, 4]. The introduction of protease inhibitors in these studies, in particular

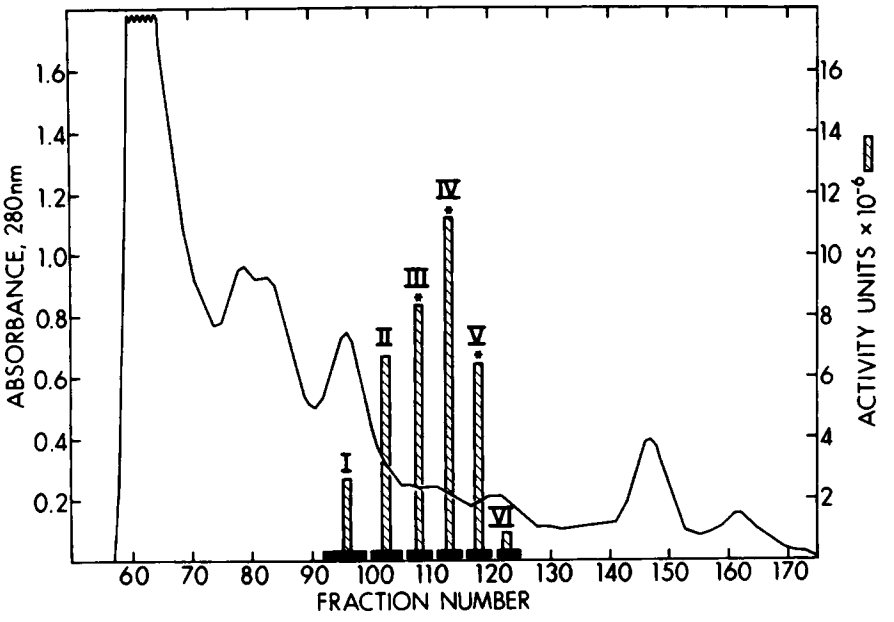


Fig. 1. Elution profile of the Sephadex G-75 gel filtration of pituitary FGF. The fraction (~300 mg), corresponding to F-III [3], was loaded in 7.6 ml column buffer and eluted at a flow rate of 10 ml/hr with 15-min fractions collected. Absorption at 280 nm (———) was read and fractions pooled. Biological activity was measured and total units (bars) were estimated. The starred pools (III–V) were combined for the next step.

leupeptin and pepstatin in the initial extraction at pH 4.5, was an important modification which yielded better overall recovery of FGF activity and higher specific activity material.

An example of a typical Sephadex G-75 gel filtration of the active fraction from the stepwise CM-Sephadex fractionation (F-III of reference [3] is shown in Figure 1. There was a major peak of activity as measured by incorporation of (methyl-$^3$H)-thymidine into Balb/c 3T3 cells that eluted in a similar position as cytochrome c, suggesting a molecular weight in the range of 13,000, as originally reported [3]. Generally a shoulder of activity (fractions 90–100) eluted prior to the major peak, and smaller amounts of activity were also observable in all of the higher-MW fractions. The major activity peak (pools II–V) represented 80–90% of the total activity recovered from gel filtration columns. To obtain the most enriched fraction for further purification, it was important to avoid contamination by material found in the large peak absorbing at 280 nm preceding the FGF (fractions 90–105). Therefore, although pool II contained as much activity as pool V, it was not included in the pool of activity prepared for the next step.

The final CM-Sephadex column (step E) in the pituitary FGF purification was slightly modified from the earlier procedure [3] in that a wash with 0.15 M NaCl was introduced before initiation of the gradient. This served to remove some of the major protein contaminants that eluted early in the direct 0.1–0.6 M NaCl gradient applied originally [3]. Figure 2 shows the 0.15–0.6 M NaCl gradient portion of the profile. The biological activity eluted very broadly from the column and was not associated

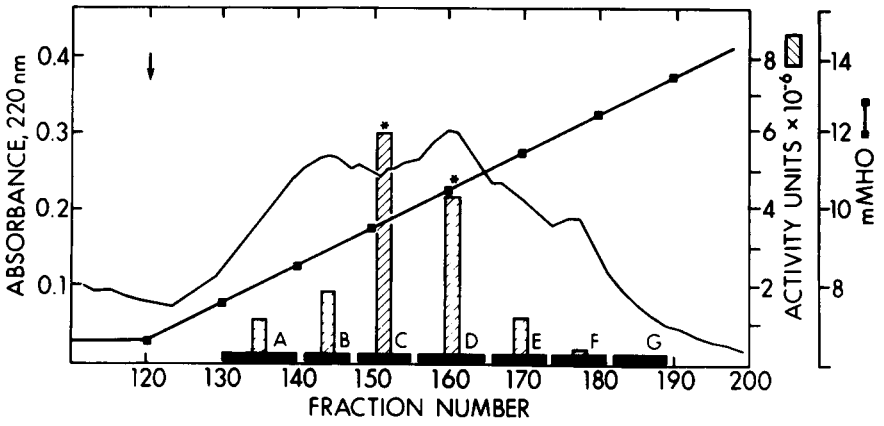


Fig. 2. Elution profile of a portion of CM-Sephadex-C50 chromatography of pituitary FGF (step E). Pools III–V of the Sephadex G-75 column (Fig. 1) (19.15 mg protein) were loaded in 9.7 ml of 0.1 M sodium phosphate, 0.1M NaCl, pH 6.0, to the CM-Sephadex column ( 1 × 10 cm), which was washed extensively with this buffer and then with 0.15 M NaCl in the phosphate buffer (not shown). The linear gradient was developed with 120 ml each 0.15 M and 0.6 M NaCl in the phosphate buffer, at a flow rate of 6.5 ml/hr with 20-min fractions collected. The arrow indicates the start of the gradient. Fractions were pooled as shown and biological activity (bars) determined. The pools were dialyzed with 0.2 M ammonium bicarbonate and lyophilized. The starred fractions were used for further purification. Absorption at 220 nm (———), conductivity at 0°C (■——■) are shown.

**TABLE I. Sephadex G-75 and CM-Sephadex Gradient Purification Fractions of Pituitary FGF**

| Fraction | Total protein (mg) | ng/ml/unit[a] | Total units × $10^{-6}$ |
|---|---|---|---|
| G-75[b] (III–V) | 19.15 | 0.737 | 26 |
| CM[c]-A | 0.392 | 0.35 | 1.12 |
| CM-B | 0.355 | 0.192 | 1.82 |
| CM-C[d] | 0.395 | 0.066 | 5.98 |
| CM-D[d] | 0.542 | 0.125 | 4.33 |
| CM-E | 0.330 | 0.28 | 1.18 |

[a]One unit is defined as 50% of maximal stimulation of incorporation of $^3$H-thymidine into Balb/c 3T3 cells by FGF.
[b]Sephadex G-75 fraction from chromatography shown in Figure 1.
[c]CM-Sephadex gradient fraction from chromatography shown in Figure 2.
[d]CM pools utilized in further characterization of pituitary FGF.

with a distinct peak absorbing at 220 nm; however, careful pooling revealed that fractions 148–155 (CM-C) and 156–165 (CM-D) were significantly more active than the other pools.

The recoveries from the final stages of this purification are shown in Table I. The combined pool (III–V) from the Sephadex G-75 step (Fig. 1) was active at 0.74

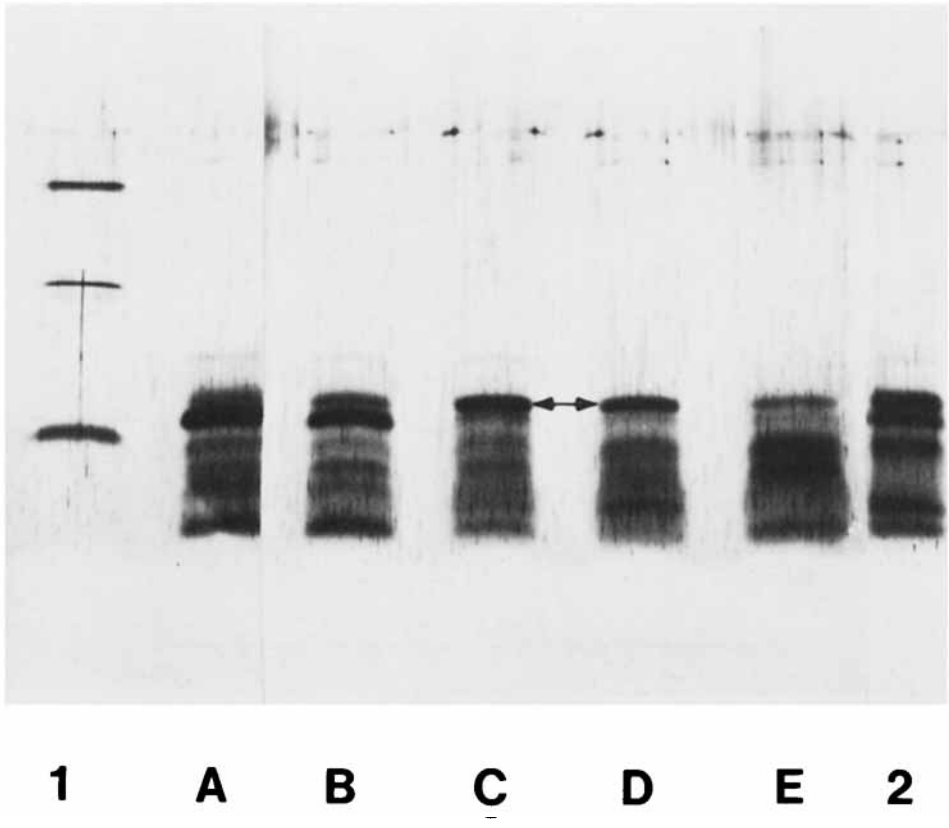


Fig. 3. SDS polyacrylamide gel electrophoresis of the fractions from the CM-Sephadex chromatography (Fig. 2). The SDS polyacrylamide gel (16.5%, 8 cm) was developed and silver-stained as described in the Methods. Lane 1, 300 ng each of molecular weight standard (from top to bottom): ovalbumin, chymotrypsinogen, and cytochrome c; lanes A–E, 1 $\mu$g each of CM-Sephadex pools A–E (Fig. 2); lane 2, 1 $\mu$g of another preparation of pituitary FGF prepared by the same methods but with active pools combined.

ng/ml/unit, and approximately $26 \times 10^6$ units were loaded onto the subsequent column. Data from pools A–E of the CM-Sephadex column (Fig. 2) are included for further comparisons. It can be seen that CM-C (0.066 ng/ml/unit) had the highest specific activity, with CM-D being somewhat less potent. Total recovery of activity from these two fractions was about 40% of the load sample (Sephadex G-75 pools III–V) and 15–30% of total activity from the initial crude extract. This represented approximately a 10,000 to 15,000-fold purification. The protein amounts indicated were based on absorption at 220 nm prior to dialysis and 80% was recovered when 0.2 M ammonium bicarbonate was used as the dialysate. That only approximately 300 $\mu$g of CM-C and 430 $\mu$g of CM-D were left for further analysis indicates the limiting protein available at this level of purification.

Figure 3 shows the silver-stained pattern of pools CM-A–E analyzed on 16.5% SDS polyacrylamide gels. All of the fractions were still heterogeneous, but one particular band, indicated by the arrow, appeared to purify in relationship to the

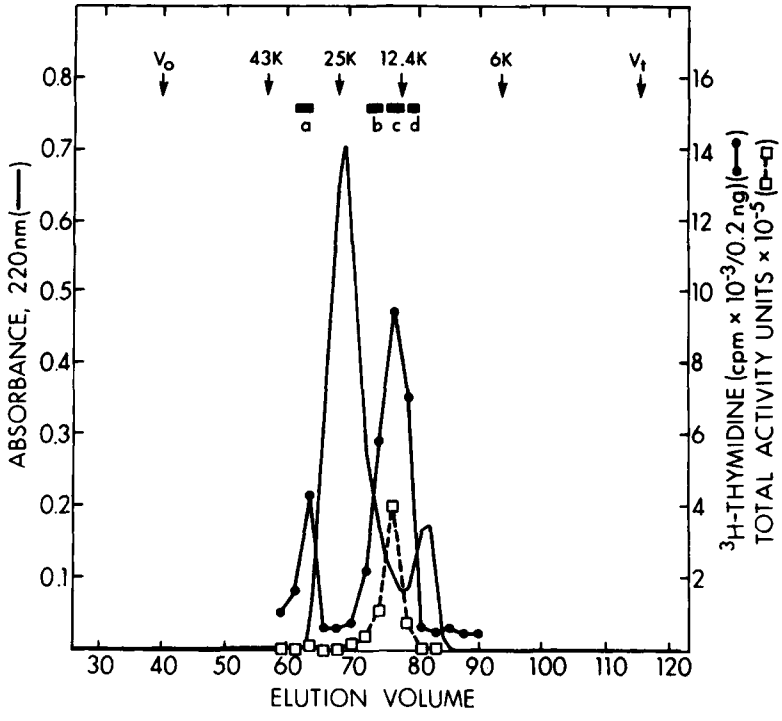


Fig. 4. Elution profile of the Sephadex G-75 gel filtration of repurified pituitary FGF. A column (1 × 150 cm) of Sephadex G-75 (10–40 μm) was eluted with 0.1 M ammonium bicarbonate, 0.6 M NaCl, at a flow rate of 4.2 ml/hr. A sample of 1 mg pituitary FGF, a repurified fraction, was loaded in 0.6-ml column buffer. Fifteen-minute fractions were collected and absorption at 220 nm (——) read. Aliquots of fractions (0.2 ng) were tested for biological activity in the 3T3 cell assay (●——●) and total units (□------□) were estimated from the dose-response curve of the load sample. Pools a–d were dialyzed against 0.2 M ammonium bicarbonate and lyophilized for further analysis. Standardization of the column was determined with ferritin (Vo), ovalbumin (43K), chymotrypsinogen (25K), cytochrome c (12.4K), relaxin (6K), and tritiated $H_2O$ (Vt).

specific activity of the pools. It represented the most prominent species in fraction CM-C and proportionately less so in fraction CM-D, with further reductions in surrounding pools. Lane 2 is material also purified by these methods, but in this preparation, the active pools were combined. The identified band was also a major component of this preparation. The molecular weight from these SDS gels is estimated to be 14,500–15,000.

Further evidence that this protein copurifies with the biological activity was provided by the chromatography of less pure FGF (repurified material from pooling of side fractions from several other preparations and procedures) on a column of Sephadex G-75 (Fig. 4). It was essential to use 0.5–0.6 M NaCl in the column buffer composed of 0.1 M ammonium bicarbonate for recovery of protein and activity. Two apparent protein peaks were observed; the major peak of activity, with a molecular weight estimated at 14,000, eluted exactly between them. There was also a small amount of another mitogenic component with a higher molecular weight, ~30,000, but it represented less than 5% of the activity loaded on the column. The fractions

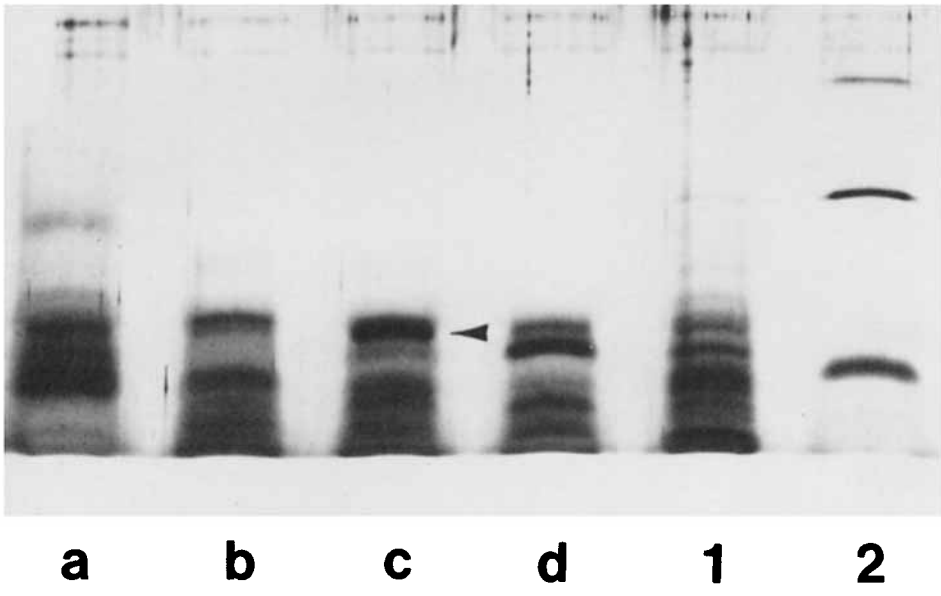


Fig. 5. SDS polyacrylamide gel electrophoresis of the fractions from the Sephadex G-75 gel filtration column (Fig. 4). A 16.5% SDS polyacrylamide slab gel (8 cm) was developed and protein bands revealed by silver staining. Lanes a–d, 1 $\mu$g each of the Sephadex G-75 fractions a–d (Fig. 4); lane 1, 1 $\mu$g of load sample of Sephadex G-75 column; lane 2, standards, 300 ng each as described in Figure 3.

indicated were pooled, and 1 $\mu$g from each was analyzed on SDS polyacrylamide gel electrophoresis (16.5%) (Fig. 5). It can be seen that the same protein band identified in Figure 3 copurified with the biological activity, being the most prominent band in fraction c (lane c). Fraction a, the activity pool with a higher molecular weight, also has a band corresponding to a molecular weight of ~15,000 (lane a), but it is not known if there is any relationship of this protein to the observed activity.

In order to purify the mitogenic component recovered from the final CM-Sephadex column (step E) (Fig. 2), fractions CM-C or CM-D were electrophoresed on acid pH gels. The pattern of CM-C revealed by Coomassie Blue R staining (Fig. 6, lane B) also showed a major protein component. It migrated near the lysozyme marker and was estimated to be 30–35% of the material loaded onto the gel. In this experiment, CM-C was applied to two parallel lanes and sliced: One (6 $\mu$g loaded) was used to elute for testing in the Balb/c 3T3 bioassay, and the other (3 $\mu$g loaded) to analyze in SDS gels. The activity profile from this sliced and eluted lane (Fig. 7) displayed a single mitogenic component that appeared to migrate with the major staining band on the acid gel. Quantitative (80–100%) recovery of activity was obtained from this elution.

From the second parallel lane, the peak activity fractions (20–24) were selected and analyzed by SDS gel (16.5%) electrophoresis. The pattern revealed by silver staining of the gel is shown in Figure 6. The major species (indicated by an arrow) that copurified with the acid gel peak of activity corresponded to the band suggested to be the mitogenic component in the impure samples (see Figs. 3, 5). Although there

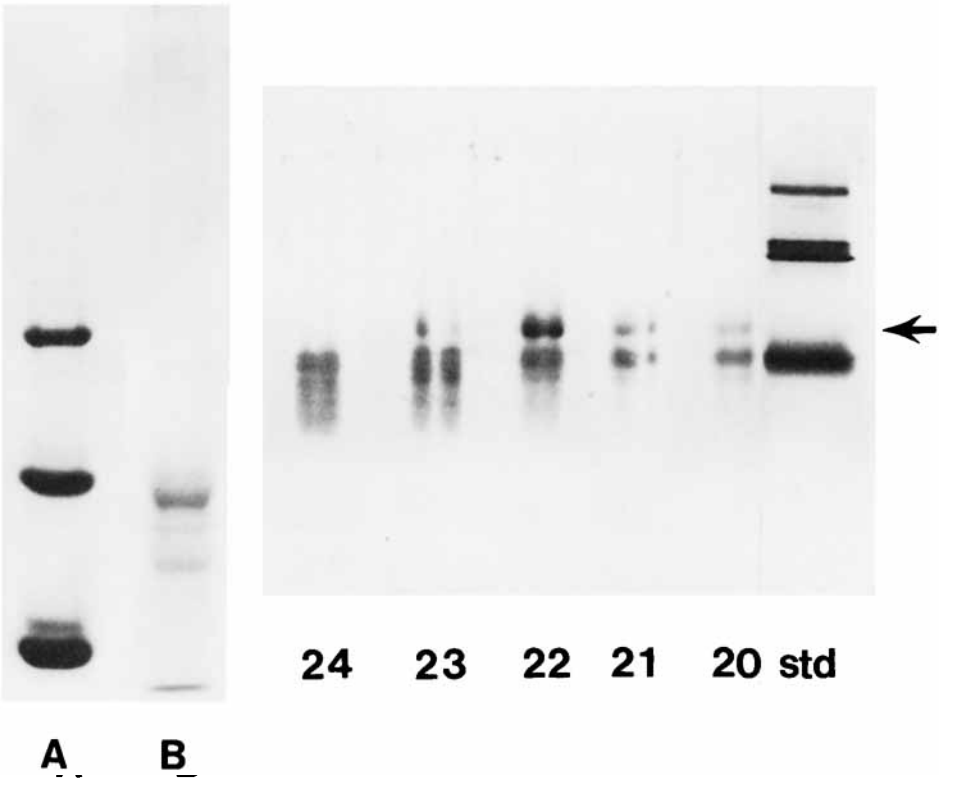


Fig. 6. Acid gel electrophoresis of purified pituitary FGF (lanes A and B) and second-dimensional SDS gel electrophoretic analysis of active fractions (lanes 24—standard). Left: Lane B) Fifteen micrograms of CM-C (see Fig. 2), and (lane A) marker proteins (from top to bottom: chymotrypsinogen, 10 $\mu$g; lysozyme, 10 $\mu$g; and cytochrome c, 20 $\mu$g) were electrophoresed on a 10% (8 cm) acid gel and proteins stained with Coomassie Blue R. Right: The peak activity acid gel slices from a lane run in parallel (3 $\mu$g loaded) to the samples shown in lanes A and B (left) and the slice activity profile (see Fig. 7) were equilibrated with the sample buffer and electrophoresed on a 16.5% SDS polyacrylamide gel. The silver-stained pattern of slices 20–24, corresponding to the same segments assayed in Figure 7, are shown, with the major component indicated by an arrow. Molecular weight markers (std) are the same as shown in Figure 3.

were other contaminating proteins in these acid gel slices, none of them were enriched across the activity peak. Identical results were obtained with fraction CM-D. It should be noted that FGF is inactivated in SDS; the possibility of analyzing SDS gels directly for mitogenic activity was therefore excluded.

Figure 8 shows the results of similar acid gel experiments where material eluted from peak activity slices were analyzed by SDS gel electrophoresis. Lanes A and C represent the most active fractions from different acid gel runs, and lane B is the trailing fraction (equivalent to slice 21 in Fig. 6 or 7) from the same acid gel experiment as in lane C (Fig. 8). Lane D contains material eluted from slices of the band identified as FGF from an SDS gel electrophoresis run of CM-D. Although some minor contaminants were detectable, the major species in all of these samples was the 15,000-MW component.

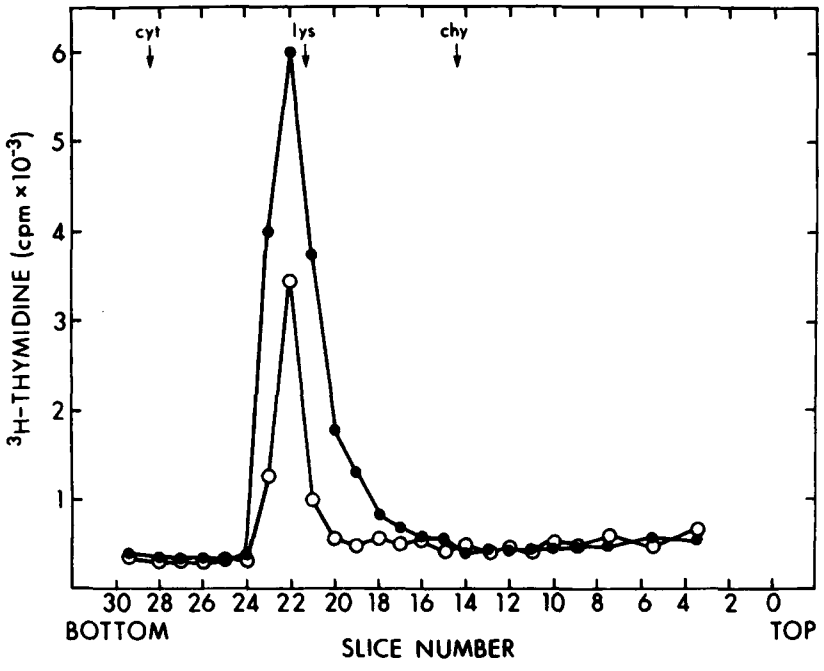


Fig. 7. Activity profile of acid gel electrophoresis of purified pituitary FGF. Six micrograms of pituitary FGF (fraction CM-C, Fig. 2) were electrophoresed on the acid gel described in Figure 6 (left). Gels were cut into 0.25-cm slices and eluted in 2.0 mg/ml BSA in PBS for determination of biological activity. Migration of the sample was from right to left and activity is expressed in cpm of $^3$H-thymidine incorporated into Balb/c 3T3 cells. Sample volume added to assay: 0.2 μl (●——●), 0.04 μl (○——○) of 1.0 ml total per eluted slice.

A preliminary amino acid composition of the 15,000-MW band was obtained by hydrolyzing the band cut directly from an SDS gel (Table II). It was not possible to obtain a value for arginine because it was obscured by an enormous ammonia peak, resulting from the polymerization of the SDS gels with ammonium persulfate. Methionine was not detectable, but it may have been oxidized during the electrophoresis or on subsequent manipulations. A comparison of this amino acid composition with that published by Gospodarowicz [3] reveals no particular similarities even when those values are increased to the correct molecular weight range.

## DISCUSSION

Essentially, homogeneous bovine pituitary FGF has been obtained by the addition of an acid gel electrophoresis step to a slightly modified version of the original preparation scheme [3]. This material has a molecular weight of 14,500–15,000 as determined by SDS gel electrophoresis, and elutes in monomeric form with a similar size on gel filtration. In addition, the active mitogen retains other properties associated with pituitary FGF in that it is acid- and heat-labile [4], has a basic pI [4], and is resistant to reducing agents [13] (data not shown).

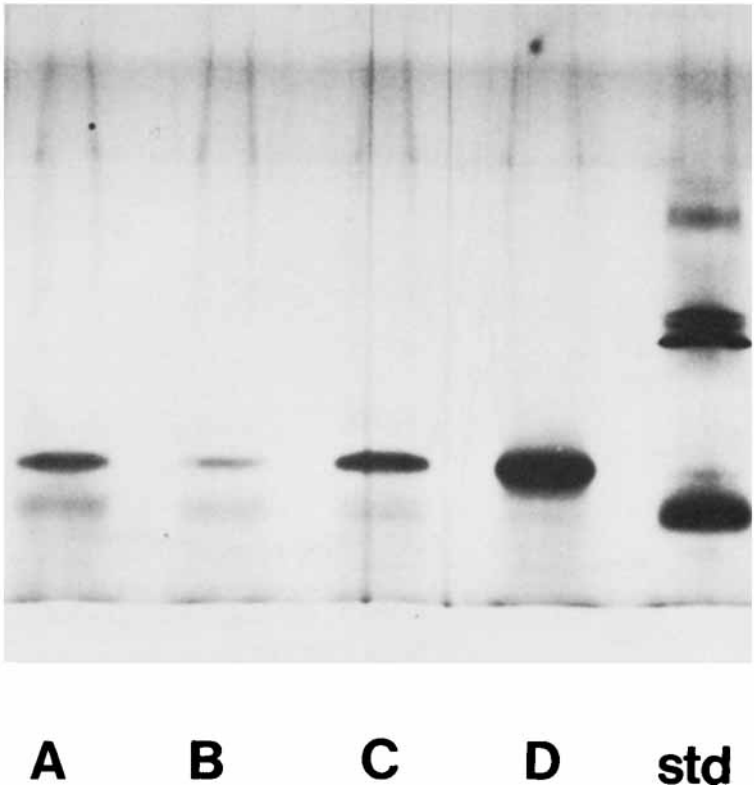


Fig. 8. SDS polyacrylamide gel electrophoresis of pituitary FGF eluted from acid pH and SDS gels. SDS gels (16.5%, 8 cm) were developed and silver-stained as described in the text. Lane A) Peak activity fraction from acid gel electrophoresis (8 cm long gel) of 50 μg CM-C (see Fig. 2), eluted as described in the Methods. Lanes B and C) The active fractions from two acid gel experiments (12 cm long), 50 μg CM-C (see Fig. 2) loaded to each, were eluted as described; lane C, the peak activity slices from the combined gel experiments; lane B, the trailing active slices from the combined acid gels. Lane D) The 15,000-MW band was sliced out of an SDS gel electrophoresed with 140 μg of CM-D (see Fig. 2) and eluted as described. Molecular-weight markers (std) are the same as in Figure 3.

When purified by us by the methods of Gospodarowicz [ 3, 4], the pituitary FGF preparation was highly active in the 3T3 cell bioassay (0.4–1.0 ng/ml/unit, not shown). However, by electrophoretic criteria it contained multiple components and required twentyfold or more further purification. Even with the modifications introduced in the procedures presented here, the most active material (CB-C) from the final CM-Sephadex column was heterogeneous and did not coelute with any major component absorbing at 220 nm. Numerous attempts to purify FGF further, including many other chromatographic and electrophoretic methodologies [14], were unsuccessful, which may be due in part to the fact that recovery of FGF from columns or dialysis is a major problem. This property of adhering to support media may explain the broad elution pattern of FGF in purification on the CM-Sephadex gradient column, although some inherent heterogeneity in the FGF molecule cannot be ex-

**TABLE II. Amino Acid Composition of Pituitary FGF***

| Amino Acid | Observed | Integer | Literature[a] A | Literature[a] B |
|---|---|---|---|---|
| Aspartic acid | 14.1 | 14 | 5 | 10 |
| Threonine | 5.6 | 6 | 4 | 8 |
| Serine | 14.8 | 15 | 5 | 10 |
| Glutamic acid | 13.4 | 13 | 7 | 17 |
| Proline | 10.1 | 10 | 5 | 10 |
| Glycine | 14.0 | 14 | 8 | 16 |
| Alanine | 10.4 | 10 | 8 | 16 |
| Half-cystine | ND[b] | — | 2 | 4 |
| Valine | 4.6 | 5 | 4 | 8 |
| Methionine | 0 | — | — | — |
| Isoleucine | 1.4 | 1 | 3 | 6 |
| Leucine | 12.7 | 13 | 3 | 6 |
| Tyrosine | 7.2 | 7 | 2 | 4 |
| Phenylalanine | 8.8 | 9 | 2 | 4 |
| Histidine | 3.0 | 3 | 2 | 4 |
| Lysine | 16.2 | 16 | 12 | 24 |
| Arginine | —[c] | — | 5 | 10 |
| Tryptophan | ND | — | — | — |
| Total | | 136 | | 154 |

*Average of two 24-hr hydrolysates.
[a]Taken from reference 3. Column A, as reported; column B, those values × 2. This correction gives 144 residues (without arginine), which is comparable to the 136 determined in this study.
[b]Not determined.
[c]Arginine obscured by the ammonia peak.

cluded. The nonspecific interactions appear to be primarily ionic, because high salt concentrations in gel filtration or dialysis of dilute protein solutions in 0.2 M ammonium bicarbonate buffer tended to overcome this problem.

Final isolation of FGF, by gel electrophoresis at acid pH, yielded the 14,500-MW protein that copurified with the biological activity and was judged to be greater than 90% pure. However, even the purest material obtained contained some minor contaminants, and some caution should be expressed concerning the definitive association of this component with the fibroblast growth activity. The specific activity of the final material (estimated to be 0.01–0.03 ng/ml/unit, equivalent to 1-2 × $10^{-12}$ M) would appear to support the premise that pituitary FGF has been correctly identified.

With the identification of the active pituitary FGF molecule and with the small amounts of purified material obtainable from gels (estimated to be several nmols/kg pituitary), further studies of the chemical nature and the molecular features of the synthesis and mechanism of action of this growth regulatory agent will be possible. It will also be interesting to examine the relationship of pituitary FGF to other growth factors, including the basic brain FGF [7] and the other mitogens observed in pituitary FGF preparations, and to determine whether different tissues or cells have related molecules.

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