Platelet-derived Growth Factor

I. HIGH YIELD PURIFICATION AND EVIDENCE FOR MULTIPLE FORMS*

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Platelet-derived growth factor has been purified from human outdated platelet-rich plasma with a 21% overall yield. This five-step procedure represents a 500,000-fold purification over serum. 6.4 ng/ml (2 x 10^{-10} M) of purified platelet-derived growth factor stimulated DNA synthesis in quiescent, density arrested cultures of Swiss 3T3 cells at a level equivalent to that produced by 5% calf serum. The growth-promoting activity and specific binding to 3T3 cells has been shown to be associated with four entities of molecular weights: 31,000, 29,000, 28,500, and 27,000, as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis under nonreducing conditions. In the presence of 2-mercaptoethanol, the four molecular species are converted to three inactive chains of lower molecular weights: 17,500, 16,000, and 14,400.

Other methods of chemically cleaving the disulfide bonds were investigated: reductive cleavage by S-sulfonation, reductive cleavage with diithiothreitol, and performic acid oxidation. In all cases, mitogenic activity was reduced by 80-100%. Attempts to restore mitogenic activity after reduction were unsuccessful. Two-dimensional peptide mapping of the nonreduced and reduced multiple forms was also investigated. The four nonreduced moieties gave basically identical maps. The maps of the reduced 17,500- and 16,000-dalton chains were also essentially identical, but the map of the reduced 14,400-dalton chain was significantly different. We propose that platelet-derived growth factor consists of two polypeptide chains: a 14,400-dalton chain and either a 17,500- or a 16,000-dalton chain.

Most diploid cells in vitro require serum for optimal growth, and the final cell density will be proportional to the serum concentration used (Holley and Kiernan, 1968). This serum requirement can be subdivided into at least two categories: nutrients and cofactors used within the cell and regulatory macromolecules (growth factors) that act at "hormone-like" levels. In an attempt to define the regulatory macromolecules in serum, a great deal of effort has been devoted to fractionation of serum (Holley and Kiernan, 1971; Temin et al., 1972; Antonades et al., 1975). The complex nature of serum and its multiplicity of actions has made interpretation of its role in the growth of cells in culture difficult to interpret. But what has become clear is that platelet-derived growth factor, which is released by the platelet during coagulation, is the major macromolecule in serum stimulating DNA synthesis and cell growth in connective tissue cells (Ross et al., 1974; Kohler and Lipton, 1974; Westermark and Wasteson, 1975). The study of PDGF has been of great interest, not only as a means of better understanding the regulation of cell proliferation, but also because of its potential role in the pathogenesis of atherosclerosis (Ross and Glomset, 1973, 1976).

We report here the purification of human PDGF from outdated platelets and evidence for four molecular forms which are all biologically active in terms of mitogenicity and binding to responsive 3T3 cells. In addition, we report the closely related structure of the four forms as determined by peptide mapping and propose a molecular model for native PDGF based on the mapping data.

EXPERIMENTAL PROCEDURES

[3H]Thymidine Assay for Mitogenic Activity—Confluent Swiss 3T3 cells were routinely used for assay of [3H]thymidine incorporation into DNA. The Swiss 3T3 cells were plated in 12-well dishes (Linbro) at a density of approximately 4.5 x 10^4 cells/well in 1.5 ml of Dulbecco’s modified Eagle’s medium with l-glutamine and pyruvate (Gibco). The cells were allowed to grow to confluency (4–5 days, approximately 2 x 10^5 cells/well). On the day of test sample addition, 15 ml of penicillin/streptomycin (100 U/ml stock, Gibco) was added before test samples (50–400 µl). Test samples were in either 1 M acetic acid or phosphate-buffered saline. [3H]Thymidine (100 µl of 27.8 µCi/ml in serum-free medium, 76 Ci/mmol) was added at 17 h after test sample addition. At 21 h the cells were harvested as follows: medium was aspirated; each well was washed twice with 1 ml of ice-cold 5% trichloroacetic acid; 0.8 ml of 0.25 M NaOH was added to each well to solubilize trichloroacetic acid-insoluble material; after mixing, 0.6 ml of NaOH solution was removed and added to 5 ml of Aquasol for counting in a Packard 2600 scintillation counter. Each sample was assayed in duplicate and at three doses to accurately evaluate the degree of purification and recovery of mitogenic activity. Duplicate measurements routinely agreed within 5%. When samples required dialysis before assay, bovine serum albumin was added first to the dialysis bag to give a final concentration of 2 mg/ml. Samples in volatile buffers and most dialyzed samples were lyophilized and then resuspended in 1 M acetic acid for testing. Test fractions were always compared to the response to 5% calf serum as a standard reference.

SDS-Gel Electrophoresis—SDS-slab gel electrophoresis was performed (Laemmli, 1970) using a 18% separating gel and a 4.5% stacking gel of 1.5-mm thickness. Samples, both nonreduced and reduced, were boiled for 5 min in SDS sample buffer before loading. Depending on the protein load, the gels were either stained with Coomassie brilliant blue for 5–10 µg or silver-stained (Switzer et al., 1979) for 0.1–1.0 µg. When necessary, stained gels were dried onto Whatman No. 3MM paper and then exposed to Kodak X-Omat RP-5 film, using Chronex

1. The abbreviations used are: PDGF, platelet-derived growth factor; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; PRP, platelet-rich plasma.
A-lactalbumin (14,400) from Pharmacia. The 15% SDS gels were calibrated with phosphorylase b (94,000), bovine serum albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), soybean trypsin inhibitor (20,100), and alpha-lactalbumin (14,400) from Pharmacia.

Elution from SDS Gels—Material for elution was loaded in analytical sample wells for 1.5-mm thick slab gels. At the end of the run, the lanes to be eluted were sliced into a predetermined number of fractions using previously determined Rf values. The fraction cuts were extended into adjacent lanes run simultaneously for staining and ultimately served as the reference points when the protein-staining profile was correlated with elution of mitogenic activity. The gel section to be stained was fixed, the gel sections for elution were finely end over end for 18 h at 4°C. The elution buffer was changed 2 times, followed each time by an 18-h end over end mixing at 4°C. Eluates from each fraction were pooled separately and lyophilized. The fractions were reconstituted in 200 μl of 0.5 M bovine serum albumin and chilled to 4°C for acetone precipitation to remove the SDS. 1.8 ml of cold acetone was added, and samples were mixed and then centrifuged at 3000 rpm for 15 min. Each pellet was washed twice with cold 90% acetone followed by centrifugation. The samples were air-dried and then solubilized for assay of mitogenic activity.

Chemical Cleavage of the Disulfide Bonds—For SDS gels, samples were boiled in SDS sample buffer containing 2% 2-mercaptoethanol for 5 min. S-Sulfonation was performed in 0.5 M ammonium carbonate, pH 8.1, for 24 h at room temperature as previously described (Pechere et al., 1958). Samples were completely reduced with a 50-fold molar excess of diithiothreitol in 0.5 M Tris, pH 8.1, 6 M guanidine, 0.02 M EDTA at 4°C in a nitrogen atmosphere and after reduction some samples were alkylated with iodoacetamide at 4°C for 1 h in a nitrogen atmosphere (Königsberg, 1972). Performic acid oxidation was performed in 88% formic acid at 4°C for 20 min as described previously (Hirs, 1956).

Iodination of PDGF and Binding of PDGF to 3T3 Cells—PDGF was iodinated using the iodine monochloride method as described (Bowen-Pope and Ross, 1982). 125I-PDGF was incubated with confluent, quiescent cultures of Swiss 3T3 cells at 4°C for 4 h with gentle shaking (Bowen-Pope and Ross, 1982), washed 3 times with phosphate-buffered saline containing 1 mg/ml of bovine serum albumin, and then eluted from the cell surface with SDS sample buffer. The eluted material was prepared as described earlier for SDS-PAGE.

Peptide Mapping of 125I-Radiolabeled PDGF—The multiple components of PDGF resolved by SDS-gel electrophoresis and stained with Coomassie brilliant blue were individually cut from the gel for radioiodination using Bolton-Hunter reagent, digestion with Proteinase K, and subsequent two-dimensional mapping on cellulose thin layer chromatography plates as previously described (Sage et al., 1981).

Reagents—All glassware and plasticware used in the purification and subsequent analysis were siliconized using either Aerosol or Surfase from Pierce, CM-Sephadex, Sephacryl S-200, heparin-Sepharose, and phenyl-Sepharose were obtained from Pharmacia. Recrystallized acrylamide and bisacrylamide from Bethesda Research Laboratories were used for the peptide mapping studies. For analytical polyacrylamide gels, reagents (electrophoresis purity) were obtained from Bio-Rad. Pentex grade recrystallized bovine serum albumin was purchased from Miles. Purified mitochondrial malate dehydrogenase was provided by Dr. Ralph Bradshaw.

Protein Concentration—Throughout the purification procedure, protein content was monitored by absorbance at 280 nm. Protein concentrations were calculated by assuming that A280 = 1.0 for a 1 mg/ml solution in a 1-cm path cuvette.

RESULTS AND DISCUSSION

High Yield Purification of Platelet-Derived Growth Factor—The purification scheme for PDGF from outdated platelet-rich plasma is presented in the miniprint supplement. This procedure (Table II), unlike others previously published (Antoniades et al., 1979; Heldin et al., 1979, 1981), provides a high yield with an overall recovery of 21% and employs the more readily available outdated platelet-rich plasma as starting material. The earliest procedures reported (Antoniades et al., 1979; Heldin et al., 1979) both utilized elution after SDS-PAGE. These procedures provided overall yields of 1.5-5% and have the additional potential problem of perturbation by residual SDS of subsequent analyses. The more recent report (Heldin et al., 1981) provided a 6% yield without the use of SDS-PAGE but used fresh platelets as the starting material. The advantage of using fresh platelets is that plasma proteins are excluded from the starting material; however, fresh platelets are not generally available to most laboratories in large amounts. Although outdated platelet-rich plasma is more readily available, its use as a starting material requires the processing of both platelets and plasma proteins. This is necessary because approximately 90% of the growth-promoting activity has already been released into the plasma when the material is received from the blood bank (data not shown). The purification scheme described in the miniprint supplement purifies PDGF away from these plasma proteins without the use of SDS-PAGE and without sacrificing high yield.

Multiple Forms of Purified Platelet-Derived Growth Factor—SDS-PAGE analysis of nonreduced PDGF from various stages in the purification is shown in Fig. 1. This Coomassie-stained gel illustrates the complexity of proteins removed during the purification procedure. The group of proteins with molecular weights of about 30,000 which are responsible for the PDGF activity can only be distinguished at the purity of the phenyl-Sepharose step. With the resolution of the Coo-
massie-stained 15% SDS-PAGE, the phenyl-Sepharose-purified fraction appeared to be composed of three different molecular weight entities represented by a faint band at 31,000, a broad band at 29,000, and a third band at 27,000. These three molecular entities represent greater than 90% of Coomassie blue stain on the gel as determined by densitometric scan.

However, with the increased sensitivity of the silver stain technique (Switzer et al., 1979), analysis of the phenyl-Sepharose-purified PDGF in the same 15% SDS-PAGE system indicated four components in the nonreduced state with molecular weights of 31,000, 29,000, 28,500, and 27,000. Fig. 2 shows silver-stained gels of two different preparations of PDGF in which the proportions of the four components varied. Preparation B contained more of the 31,000-dalton moiety and less of the 28,500-dalton component than preparation A. The proportions of the four molecular weight components were quite variable from preparation to preparation, but the following decreasing order of predominance based on silver staining was routinely observed: 29,000; 27,000; 28,500; 31,000.

Analysis of the purified material by gradient SDS-gel electrophoresis and by the acidic electrophoresis system used for histones (Panyim and Chalkley, 1969) failed to give better resolution than that achieved with 15% SDS-PAGE and silver staining. Previous reports (Heldin et al., 1977, 1979; Ross et al., 1979) have noted the broad distribution of PDGF mitogenic activity when analyzed by SDS-gel electrophoresis, molecular sieving, or isoelectric focusing. The specific molecular entities identified in this report may explain this previously observed heterogeneity.

Reduction of the purified PDGF with 2% 2-mercaptoethanol, followed by analysis in the 15% SDS-PAGE system, indicated three principal molecular weight bands: 14,400; a broad band at 16,000; and 17,500. Preparation A, which was deficient in the 31,000-dalton nonreduced component, was also deficient in the 17,500-dalton reduced component.

Reduced PDGF was biologically inactive. Several different methods of chemically cleaving the disulfide bond were investigated as shown in Table I: reductive cleavage by S-sulfonation, reductive cleavage with dithiothreitol, and performic acid oxidation. In all cases mitogenic activity was reduced by 80-100%. Additional studies suggest that any residual activity may reflect incomplete unfolding of the PDGF molecule and therefore inaccessibility of disulfide bonds (data not shown). An attempt was also made to promote reassociation of chains reduced with dithiothreitol (Table I). In none of the cases investigated was mitogenic activity restored.

**Association of the Multiple Forms with the Biological Activity of PDGF**—The observation of multiple components in preparations of purified PDGF led us to question which of these components was responsible for the biological activity of PDGF. The experiment shown in Fig. 3 addresses the question of mitogenic activity as assayed by [3H]thymidine incorporation into DNA. Two preparations, containing differing

![Fig. 2 Silver-stained SDS-PAGE of two preparations of PDGF varying in their proportion of four molecular weight components.](image-url)
ent proportions of the four components, were analyzed simultaneously by 15% SDS-PAGE. A portion of each was silver-stained to show the protein profile and a portion was cut into 1.5-mm slices and further minced and eluted to determine mitogenic activity. The preparation shown in the top of Fig. 3 contained decreased amounts of the 27,000-dalton moiety by silver staining. Analysis of the mitogenic activity (Fig. 3) indicated that it closely followed the protein profile seen on staining, providing evidence that, within the limitations of the technique used, all four forms possess growth-promoting activity.

Binding to 3T3 cells was also investigated to determine the contribution of each of the multiple components. This experiment is illustrated in Fig. 4. Three separate preparations of phenyl-Sepharose-purified material, each containing different proportions of the four components, were iodinated using the iodine monochloride method (Bowen-Pope and Ross, 1982) and the iodinated PDGF was bound to confluent, quiescent cultures of 3T3 cells at 4 °C for 4 h with gentle shaking (Bowen-Pope and Ross, 1982), washed twice with phosphate-buffered saline containing 1 mg/ml of bovine serum albumin and solubilized in SDS sample buffer. The native, iodinated, and cell-bound PDGF were then analyzed in the 15% SDS-PAGE system. The native preparations were silver-stained (A, in Fig. 4), and the iodinated PDGF (2) and cell-bound PDGF (3) were analyzed by autoradiography. By silver staining, preparation A in Fig. 4 was principally composed of the 29,000-, 28,500-, and 27,000-dalton moieties with very little of the 31,000-dalton component present; preparation B was primarily composed of the 29,000- and 28,500-dalton components with a M<sub>r</sub> = 38,000 contaminant; and preparation C contained primarily the 29,000- and 27,000-dalton components with a small proportion of the 31,000-dalton moiety. Analysis by densitometric scanning of the autoradiographs of the iodinated PDGF indicated that the multiple components were

| TABLE I |
| ~ chemical cleavage of disulfide bonds in PDGF and attempts to restore mitogenic activity by reassociation |

Disulfide bonds of heparin-Sepharose-purified PDGF were chemically cleaved by S-sulfonation, performic acid oxidation, and reduction with dithiothreitol and assayed for mitogenic activity on Swiss 3T3 cells as described under "Experimental Procedures." Before assay the various samples were dialyzed against 1 mM acetic acid, pH 2.2, to remove excess reagents except three of the reduced samples which were dialyzed under the following conditions in an attempt to promote reassociation of the reduced chains: (b) neutralized with an equal volume of 1 M propionic acid and then dialyzed against 0.1 M sodium acetate, pH 5.5, followed by 0.02 M sodium acetate, 0.1 M sodium chloride, pH 5.5; (c) phosphate-buffered saline, pH 7.4; and (d) 5 mM ammonium bicarbonate, pH 8.

| Condition | Incorporation of 3H-thymidine into Swiss 3T3 cells | Activity Recovered |
|-----------|-----------------------------------------------|--------------------|
| Heparin-Sepharose-purified PDGF | 107.5 ± 7.3 | 100 |
| S-Sulfonation of PDGF | 20.7 ± 1.2 | 19.3 |
| Performic acid oxidation of PDGF | 13.85 ± 1.1 | 12.9 |
| PDGF incubated in formic acid oxidation buffer without hydrogen peroxide | 128.5 ± 4.5 | 119.5 |
| Reduced and alkylated PDGF | 1.54 ± 0.14 | 1.4 |
| Alkylated PDGF | 129.8 ± 1.9 | 120.7 |
| PDGF incubated in reduction buffer without dithiothreitol | 120.5 ± 24 | 113.9 |
| Attempts to reassociate reduced chains | | |
| a. Reduced PDGF, dialyzed, pH 2.2 | 1.08 ± 0.09 | 1.6 |
| b. Reduced PDGF, dialyzed, pH 5.5 | 1.05 ± 0.3 | 0.98 |
| c. Reduced PDGF, dialyzed, pH 7.4 | 1.48 ± 0.27 | 1.4 |
| d. Reduced PDGF, dialyzed, pH 8.0 | 2.97 ± 0.24 | 2.8 |

**FIG. 3.** Elution of mitogenic activity from SDS gels of PDGF preparations varying in their proportion of the 27,000-dalton component. 15% SDS gels of two preparations of phenyl-Sepharose-purified PDGF were simultaneously run for silver staining and elution to determine mitogenic activity on Swiss 3T3 cells as described under "Experimental Procedures." The solid lines indicate the densitometric scan of the stained gel shown below the scan. The bar graphs indicate the percentage of total mitogenic activity recovered from the eluted fractions. Overall recovery from the gels was approximately 10%.

**FIG. 4.** Binding of three preparations of 125I-PDGF to Swiss 3T3 cells and analysis on SDS gels. Three preparations of phenyl-Sepharose-purified PDGF (A, B, and C), that varied in their proportion of the four molecular weight components defined in Fig. 2 were iodinated and then bound to 3T3 cells for analysis on 15% SDS-PAGE as described under "Experimental Procedures." Native PDGF (1) was silver-stained and 125I-PDGF (2) and cell-bound 125I-PDGF (3) were analyzed by autoradiography of the dried SDS gels. The two lanes of silver-stained native PDGF represent two different protein loads on the gels.

iodinated in approximately the same proportions seen on silver staining. More importantly, in all three preparations the identical complement of components that were iodinated were bound to 3T3 cells. In preparation B which contained the M<sub>r</sub> = 38,000 contaminant, none of the contaminant bound to the cells. Thus, as assessed by mitogenic activity and binding to the responsive cell line 3T3, biological activity was found to be associated with all four purified moieties of PDGF.

**Peptide Homologies Among the Multiple Forms of PDGF**—To further evaluate the interrelationship of the mul-
For multiple forms of PDGF, two-dimensional peptide mapping was performed. Each of the four components was cut from Coomassie-stained 15% SDS gels. They were then iodinated in the gel using the Bolton-Hunter reagent, digested in the gel with the enzyme Proteinase K (specific for peptide bonds adjacent to the carboxylic groups of aliphatic and aromatic amino acids) and mapped on cellulose thin layer chromatography plates (Sage et al., 1981). Fig. 5 shows the autoradiographic maps of the four nonreduced components with molecular weights of 31,000 (A), 29,000 (B), 28,500 (C), and 27,000 (D). The 29,000 and 28,500-dalton components were not resolved on the Coomassie-stained 15% SDS gel and, therefore, separate bands for mapping were cut based on $R_f$ values. The low protein:gel ratio required for silver staining prevented adequate iodination necessary to obtain good maps when mapping was attempted on silver-stained 15% SDS gels in which these two bands were resolved. The maps in Fig. 5 illustrate striking similarities among the four components. The arrows indicate the major spots that disappear in the next lower molecular weight form: arrows in map A are spots not present in maps B, C, or D; the arrow in maps B and C indicates the spot not present in map D. No significant differences were detected between maps B and C. This may be a function of the inability to resolve the separate components or because any differences between maps B and C do not cause a significant alteration in peptide fragment migration in this system. Fig. 5 also shows that the peptide map of mitochondrial malate dehydrogenase (map F), a protein similar in size and charge to PDGF, is clearly distinct from the peptide maps produced by any of the forms of PDGF. This indicates that the close relationships between the four forms of PDGF do not result from their similar size and charge.

The reduced chains were also analyzed by two-dimensional peptide mapping and the results are shown in Fig. 6. The 17,500- and 16,000-dalton chains resulted in similar peptide maps. The arrows on map A have been used to indicate two peptides present in the 17,500-dalton chain (A) not present in the 16,000-dalton chain (B). The 14,400-dalton chain (C), however, differs markedly from the other two in that it lacks many of the major spots present in the other two reduced chains (arrows on map B have been used to indicate these). As would be expected, a combined peptide map of the reduced components did not provide a map identical to the nonreduced 31,000-dalton moiety (data not shown). Since the disulfide bonds have been cleaved, more peptide fragments have been

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**Fig. 5. Two-dimensional peptide maps of the four molecular weight components of nonreduced PDGF, a blank gel, and mitochondrial malate dehydrogenase.** Individual bands of phenyl-Sepharose-purified PDGF were cut from Coomassie-stained 15% SDS gels, iodinated in the gel using Bolton-Hunter reagent, digested with the enzyme Proteinase K and mapped on cellulose thin layer chromatography plates for autoradiographic analysis as described under "Experimental Procedures." Electrophoresis was performed along the horizontal axis and chromatography along the vertical axis. The four molecular weight components of purified PDGF defined in the legend to Fig. 2 and analyzed here by autoradiography were: A, 31,000; B, 29,000; C, 28,500; and D, 27,000. The arrows indicate the major spots not present in the next lower molecular weight form: arrows in map A are spots not present in maps B, C, or D; and the arrow in maps B and C indicates the spot not present in map D. Peptide maps were also run of a blank gel (E) and mitochondrial malate dehydrogenase (F), a chemically similar molecule to PDGF.

**Fig. 6. Two-dimensional peptide maps of the three reduced chains of purified PDGF.** Peptide maps of the three chains isolated after reduction of purified PDGF with 2% 2-mercaptoethanol were run as described in the legend to Fig. 5: A, 17,500-dalton chain; B, 16,000-dalton chain; and C, 14,400-dalton chain. The arrows have been used to indicate the spots that are not present in the next lower molecular weight chain: arrows in map A indicate spots not present in map B; arrows in map B indicate spots not present in map C.
generated. In addition, since the —SH groups of the reduced chains were not blocked, reformation of disulfide bonds different from the native molecule cannot be excluded. However, the similarities in the location and concentration of spots in the reduced and nonreduced maps are striking.

The similarities between the maps of the 17,500- and 16,000-dalton reduced chains and the dissimilarity between these and the map of the 14,400-dalton reduced chain provide strong evidence for a two-chain structure joined by disulfide bonds proposed earlier for native PDGF (Heldin et al., 1979, 1981). The data would further suggest that the multiplicity seen in nonreduced PDGF is primarily the result of modification of the higher molecular weight chain (M_r = 17,500). The broadness of the 16,000- and 14,400-dalton bands and potentially our inability to resolve separate entities within these bands could account for the fact that we see less multiplicity in the reduced chains. The fact that the summation of the molecular weights of the reduced chains is greater than the molecular weights of the nonreduced forms can probably be explained by the difficulty in assessing molecular weights in nonreduced molecules because of variability in three-dimensional configuration (Reynolds and Tanford, 1970). Based on these data, we propose a molecular model for native PDGF in which the reduced 14,400-dalton chain is combined with either the 16,000- or 17,500-dalton chain. Further, to maintain biological activity, it is essential that the integrity of the disulfide bonds between these chains be preserved. The multiple forms of the PDGF molecule may be due to differential glycosylation, the result of proteolytic cleavage of the 31,000-dalton PDGF molecule, or, more likely, if PDGF is like many other growth factors, all four molecular forms may be proteolytic cleavage products of a higher molecular weight precursor.

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Purification and Characterization of PDGF

1. High-Yield Purification and Evidence for Multifunctionality

**TABLE II**

| Purification of Platelet-Derived Growth Factor from Outdated Platelet-Rich Plasma |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Reaction                        | Specific Activity | Units of Growth Promoting Activity | Percentage of Activity Remaining | Individual Material Steps |
| 5% Cell Serum Standard Conditions | 3250             | 1.0             |                  |                  |                  |
| Human Platelet Factors Isolated Using Differential Centrifugation | 1075             | 3.0             | 100              |                  |                  |
| Sepharose 4-50 Column              | 1.0              |                  |                  |                  |                  |
| Phenyl-Sepharose Chromatography | 0.8              | 165             | 40               | 10              |                  |
| Hydrophobic Chromatography        | 0.5              | 6500            | 28               | 70              |                  |
| Hydrophobic Chromatography        | 0.3              | 37,375          | 23               | 81              |                  |
| Hydrophilic Chromatography        | 0.2              | 507,808         | 21               | 90              |                  |

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