Partial Purification of Thrombopoietin
From the Plasma of Thrombocytopenic Rabbits

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Partially purified thrombopoiesis-stimulating activity was prepared from the plasma of thrombocytopenic rabbits using ammonium sulfate precipitation and DEAE cellulose, Sephadex, and carboxymethyl cellulose chromatography. The protein fraction precipitated by an ammonium sulfate saturation of 60%-80%, previously shown to contain thrombopoiesis-stimulating activity, was used as starting material. Column chromatography was carried out at room temperature at pH 5.6. Under these conditions, thrombopoiesis-stimulating activity (thrombopoietin) was retained by DEAE cellulose (0.03 M citrate-phosphate buffer) and carboxymethyl cellulose (0.003 M citrate-phosphate buffer), and eluted with 0.4 M NaCl. Thrombopoietin was retarded by Sephadex G-100; the ratio of the elution volume to the void volume was 1.32:1. Immunoelectrophoretic analysis of partially purified thrombopoietin indicated that following removal of most of the albumin by DEAE chromatography, only proteins with the mobilities of beta-globulins and albumin and traces of other anodally migrating proteins were detectable in the fractions that contained thrombopoiesis-stimulating activity. Thrombopoietin was not dialyzable and was stable from at least pH 5.6 to 7.5. It was approximately 1000-fold purified following sequential chromatography with DEAE and carboxymethyl cellulose. Although the three fractions described reproducibly stimulated thrombopoiesis, as measured by increased levels of selenomethionine-75Se (75SeM) in the circulating platelets, platelet counts did not increase.

There is increasing evidence that an important mechanism by which platelet production is regulated depends on a humoral substance (thrombopoietin) that affects the production of platelets by megakaryocytes. Thrombopoietin, or plasma thrombopoiesis-stimulating activity, can be detected reliably by the use of selenomethionine-75Se (75SeM) or Na,35SO4. Both isotopes are incorporated into the cytoplasm of megakaryocytes, and labeled platelets subsequently appear in the circulation after their production and release from megakaryocytes. There is a relationship between the level of megakaryocytopoiesis and incorporation of Na235SO4 or 75SeM in platelets.

Other investigations based on these isotopic techniques have shown that certain fractions of plasma or serum from thrombocytopenic donors stimulate thrombopoiesis. Utilization of fractions, rather than whole plasma or serum, decreases the potential for nonspecific stimulation of thrombopoiesis by foreign proteins and permits the valid use of donors and recipients of different species.
Using as starting material proteins precipitated by an ammonium sulfate saturation of 60%–80% and previously demonstrated to stimulate thrombopoiesis, the current studies demonstrated that sequential fractionation with DEAE cellulose, Sephadex, and CM cellulose can be used to partially purify thrombopoietin from the plasma of thrombocytopenic rabbits. Thrombopoietin was purified approximately 1000-fold following chromatography with DEAE cellulose and carboxymethyl cellulose.

**MATERIALS AND METHODS**

Thrombopoietic activity in fractions of plasma was determined by measuring their effect on incorporation of $^{75}$SeM (Sethotope, E. R. Squibb & Sons, New Brunswick, N.J.; specific activity, 200–250 mCi/mg) into newly forming platelets, as described previously. Assays of thrombopoietic activity in mice were carried out using CD-I mice (25–40 g), obtained from Charles River Laboratories, Wilmington, Mass. Their mean platelet count was $1407 \times 10^9/liter \pm 198 \times 10^9/liter$ (1 SD). Mice were received at least 2 days before use, caged in groups of 5, and fed standard laboratory food and water, ad libitum.

All preparations of material to be tested were administered subcutaneously, in 4 equally divided doses at 9:30 a.m. and 4:30 p.m., during the 48-hr period preceding the intravenous injection of 1 $\mu$Ci of $^{75}$SeM, which was given 8 hr after the last injection of test material. Levels of $^{75}$SeM in circulating platelets, in vivo, were measured 16 hr later.

Animals were anesthetized with ether, and approximately 1 ml of blood was obtained by cardiac puncture with a plastic syringe that contained 0.02 ml of buffered EDTA (0.143 M; 5.3 g/100 ml in distilled H$_2$O, pH 6.0, Fisher Scientific Co., Fair Lawn, N.J.). Blood samples were transferred to siliconized tubes, and blood for platelet counts and hematocrit values was collected into capillary tubes from this specimen. Microhematocrit values were determined by the method of Strumia, Sample, and Hart, and platelets were counted by the method of Bull, Schneiderman, and Brecher using a Coulter electronic counter. Five milliliters of Tris buffer (THAM, 72.8 g; NaCl, 18 g; 0.2 N HCl, 240 ml; and 4.8% EDTA, 40 ml; in 4 liters of distilled H$_2$O) then was added to each sample, and platelet-rich plasma was prepared by centrifugation of the mixture at 160 g for 20 min at room temperature. The volume and platelet count of the platelet-rich plasma were measured. Platelet-rich plasma contained approximately 75% of the platelets present in the initial sample of whole blood.

Platelets were prepared for determination of radioactivity using the Millipore filter method. Platelets were sedimented by centrifugation and resuspended in 5 ml of Tris buffer using a Vortex mixer. The resuspended platelets were poured onto a Millipore filter (1.2 $\mu$m pore size) held in a Millipore prefiler and Swinnex holder. Complete transfer of platelets onto the Millipore filter was accomplished by rinsing the tube with 5 ml of Tris buffer. The platelets on the filter were washed immediately with 30 ml of Tris buffer. Radioactivity of the entire filter and of 1 ml of platelet-free plasma was determined by the use of a gamma-well scintillation counter (Picker Nuclear Autowell II, Intertech, Inc., North Haven, Conn.). Samples were counted for 30 min at an energy range from 0.350 to 0.450 MeV.

A correction factor for radioactive mouse plasma trapped within the filter was determined, as previously described for mouse platelets and plasma. Radioactive plasma protein trapped on the filter was equal to 0.0023 $\times$ plasma radioactivity $\times$ total platelets on the filter. Contamination of the filter by radioactive proteins was calculated for each sample and subtracted from the total activity. Percent administered dose of $^{75}$SeM in circulating platelets (plts) was calculated for each sample as follows: cpm/plt (corrected) $\times$ total circulating plts $\times$ 100/cpm $^{75}$SeM injected, where total circulating platelets equals whole blood platelet count per milliliter $\times$ 0.083 $\times$ body weight (kg).

Platelet antiserum was prepared in guinea pigs by serial injections of an emulsion of washed rabbit platelets and complete Freund's adjuvant. The antiserum was adsorbed with washed rabbit red cells and frozen. Guinea pig anti-rabbit platelet serum, thus prepared, did not produce anemia, hemolysis, or leukopenia when injected into rabbits.

*Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or the U.S. Department of Health, Education, and Welfare.
**Donor Plasma**

Plasma that contained thrombopoiesis-stimulating activity was obtained from New Zealand white rabbits. Normal platelet-free plasma (PFP) was prepared from blood collected in siliconized glassware, using acid-citrate-dextrose (ACD) as an anticoagulant (1 part ACD: 9 parts blood) and obtained by intracardiac puncture from unanesthetized animals. After 24 hr, at which time the platelet counts were not significantly changed and the mean hematocrit level had fallen from 0.40 to 0.30, the animals were rendered acutely thrombocytopenic (platelet count less than 30 x 10⁴/liter) by injection of 0.5–1.0 ml of anti-rabbit platelet serum; PFP was prepared from blood collected 5–6 hr later. For some experiments, rabbits not bled previously were made acutely thrombocytopenic. PFP was stored at 4°C for periods up to 24 hr. Plasma, pooled either from at least 2 normal or 2 thrombocytopenic donors, was fractionated at 4°C with saturated ammonium sulfate. Precipitates, obtained at ammonium sulfate saturations of 0%–40%, 40%–60%, and 60%–80% were prepared by centrifugation at 27,000 g at 4°C for 10 min. Previous studies⁴ had shown that thrombopoiesis-stimulating factor(s) in the plasma of thrombocytopenic rabbits was present in the plasma proteins precipitated by 60%–80% levels of ammonium-sulfate saturation. Accordingly, this fraction was reuspended in Tris buffer, pH 7.4, desalted at room temperature using G-25 Sephadex gel chromatography, and lyophilized and stored at −20°C for periods up to 4 wk before use in the current experiments.

**Partial Purification of Thrombopoietin**

The precipitate produced by an ammonium sulfate saturation of 60%–80% was further fractionated using diethylamino-ethyl (DEAE) cellulose column chromatography (DE-52, Whatman Laboratories, Springfield Mill, Maidstone, Kent, England). Desalted, lyophilized protein (750–850 mg) from the 60%–80% ammonium sulfate fraction, prepared from normal or thrombocytopenic donors, was dissolved in distilled water; the protein was equilibrated at room temperature with 0.001 M citric acid–0.02 M Na₂HPO₄ buffer at pH 5.6 (41 ml of 0.01 M citric acid and 59 ml of 0.02 M Na₂HPO₄/100 ml), by column chromatography with G-25 Sephadex. DEAE cellulose was prepared by suspension in the same citrate-phosphate buffer, and the pH adjusted to 5.6 with 85% phosphoric acid. Then, the DEAE cellulose was washed in a Buchner funnel using the citrate-phosphate buffer until both the pH (5.6) and conductance remained constant. The 60%–80% fraction then was applied to a DEAE cellulose column (0.9 x 15 cm; Pharmacia Fine Chemicals, Uppsala, Sweden) at 80 cm of water pressure. After application of the sample, the column was initially washed with the buffer; the remaining protein was eluted subsequently with citrate-phosphate buffer to which sufficient NaCl had been added to produce a final concentration of 0.4 M NaCl. The fractions were lyophilized.

The initially retained fraction, obtained from the plasma of thrombocytopenic donors, was demonstrated to contain thrombopoiesis-stimulating activity (see Results) and was further fractionated by Sephadex gel chromatography. Sephadex G-100 was suspended at room temperature in 0.01 M citric acid–0.02 M Na₂HPO₄, buffer at pH 5.6 (as used above). Lyophilized protein (400–600 mg), prepared by DEAE fractionation, was suspended in 7 ml of distilled water at room temperature and applied to a G-100 column (2.5 x 100 cm; Pharmacia Fine Chemicals), in an ascending direction, at a pumped flow rate of 1 ml/min at 20°C. The fractions were lyophilized and stored at −20°C until use.

The initially retained fraction from DEAE cellulose chromatography (DEAE-II) was also fractionated by carboxymethyl (CM) cellulose gel chromatography (CM 32, Whatman Laboratories). Lyophilized protein (400–600 mg) from fraction DEAE-II, prepared from thrombocytopenic donors, was dissolved in distilled water, and the protein was equilibrated at room temperature with 0.001 M citric acid–0.002 M Na₂HPO₄, buffer at pH 5.6 (41 ml of 0.001 M citric acid and 59 ml of 0.002 M Na₂HPO₄/100 ml), by column chromatography with G-25 Sephadex. CM cellulose was prepared by the following procedure. The dry gel was suspended in 15 vol of 0.5 N NaOH, allowing at least 30 min for equilibration; the gel then was washed in a Buchner funnel using distilled water until the effluent reached a pH of approximately 8. Thus prepared, the gel was suspended in 15 vol of 0.5 N HCl for at least 30 min and washed with distilled water until the pH of the filtered effluent was approximately 7. Then the CM cellulose was washed with 0.01 M citric acid–0.02 M Na₂HPO₄ buffer at pH 5.6 until the pH remained constant. A column (0.9 x 15 cm) was prepared with the gel and equilibrated with 0.001 M citric acid–0.002 M Na₂HPO₄ buffer until conductance remained constant and equilibrated with the starting buffer. Fraction DEAE-II was applied to the column at 80 cm of water pressure. After application of the sample, the column was initially washed with buffer, and the remaining protein was
eluted with citrate-phosphate buffer to which sufficient NaCl had been added to produce a final concentration of 0.4 M NaCl. The fractions were lyophilized. Each fraction was adjusted to pH 7.4 with 2 N NaOH, and the NaCl concentration adjusted to approximately 0.9% NaCl before injection into mice.

Immunologic Analysis of Protein Fractions

Immunoelectrophoresis of the fractions prepared was carried out with the technique of Grabar and Williams,23 using 0.05 M sodium barbital buffer, pH 8.6, and 2% agar. Antisera, prepared in goats, against the following rabbit plasma proteins were employed: albumin (Nutritional Biochemicals Corp., Cleveland, Ohio); gamma globulin (Cappel Laboratories, Downingtown, Pa.); immunoglobulins (IgA, IgG, and IgM; Cappel Laboratories); and whole serum (Cappel Laboratories).

RESULTS

Effects of Fractions of Plasma Obtained by DEAE Cellulose Chromatography on Incorporation of $^{75}$SeM Into the Platelets of Mice

Experiments were performed to determine the thrombopoiesis-stimulating activity of fractions of plasma obtained by sequential fractionation with ammonium sulfate and DEAE cellulose gel chromatography. Protein fractions of plasma from normal or thrombocytopenic rabbits, prepared by precipitation with 60%-80% ammonium sulfate saturation, were placed on a column of DEAE cellulose, suspended in a citrate-phosphate buffer at pH 5.6. The initially eluted, unretained peak (DEAE-I) and the second peak (DEAE-II), eluted by the addition of 0.4 M NaCl to the citrate-phosphate buffer (Fig. 1), were tested for thrombopoietic activity in mice (see Materials and Methods). Each of the protein fractions was administered subcutaneously, in 4 equally divided doses. One microcurie of $^{75}$SeM was administered intravenously 8 hr after the last dose of the protein fractions. A total dose of 0.05-0.06 g/kg of protein was used in five different experiments. There was a significant increase ($p < 0.002$) in the level of $^{75}$SeM only in the platelets of mice that received fraction DEAE-II from the plasma of thrombocytopenic rabbits (Fig. 2). In five separate experiments, fraction DEAE-II from thrombocytopenic rabbits, which had not been bled previously, produced similarly increased levels of $^{75}$SeM. No detectable dose–response relationship was noted in the range tested. Platelet counts of mice that received protein fractions that contained the thrombopoiesis-stimulating factor(s) did not increase or differ from platelet counts of control mice.

Fig. 1. DEAE cellulose chromatography of a fraction of rabbit plasma proteins, initially prepared by precipitation with 60%-80% ammonium-sulfate saturation. DEAE cellulose was swelled in 0.03 M citrate-phosphate buffer at pH 5.6. The initial fraction of proteins (DEAE-I) was not retained. The second fraction (DEAE-II) was eluted by 0.4 M NaCl in citrate-phosphate buffer.
Fig. 2. Effect of fractions of plasma from normal or thrombocytopenic rabbits on incorporation of $^{75}$SeM into mouse platelets. A protein fraction, prepared by precipitation with 80%-80% ammonium-sulfate saturation was additionally fractionated into DEAE-I and DEAE-II with DEAE cellulose chromatography (see Fig. 1). Each mouse received 1 $\mu$Ci of $^{75}$SeM, intravenously, 8 hr after the last of 4 equally divided 0.5-ml doses of proteins (total dose 0.05-0.06 g/kg); percent dose uptake was measured at 16 hr. The mean ±1 SE is shown, and the numbers in parentheses indicate the number of animals studied. There was a significant increase ($p < 0.002$) in the level of $^{75}$SeM only in the platelets of mice that received fraction DEAE-II from the plasma of thrombocytopenic rabbits. $p$ Values were calculated on the basis of Student's t test.

When the second, initially retained, peak (DEAE-II) was eluted with an NaCl gradient (0.01 M–0.04 M) in citrate-phosphate buffer at pH 5.6, several small peaks were detected (Fig. 3). These peaks were grouped into two pools, which were designated DEAE-IIa and DEAE-IIb, as indicated in Fig. 3. The fractions were administered subcutaneously and assayed for thrombopoietic activity, as indicated above. A total dose of 0.01–0.035 g/kg of protein was used in 4 different experiments. There was a significant increase ($p < 0.005$) in the level of $^{75}$SeM in the platelets of mice that received either fraction DEAE-IIa or DEAE-IIb from the plasma of thrombocytopenic mice (Fig. 4). Platelet counts of the groups that received thrombopoiesis-stimulating factor(s) did not differ from those of control mice.

Fig. 3. DEAE cellulose chromatography of a fraction of rabbit plasma proteins. A fraction of rabbit plasma, prepared by precipitation with 60%-80% ammonium-sulfate saturation, was placed on DEAE cellulose, swollen in 0.03 M citrate-phosphate buffer at pH 5.6. The initial peak (DEAE-I) was not retained. Subsequent peaks were eluted with a NaCl gradient (0.01 M–0.4 M) in citrate-phosphate buffer. DEAE-IIa and DEAE-IIb were collected by combining the indicated peaks.
Fig. 4. Effect of fractions of plasma from thrombocytopenic rabbits on incorporation of \textsuperscript{75}SeM into mouse platelets. 
A protein fraction, prepared by precipitation with 60\%-80\% ammonium-sulfate saturation, was additionally separated into protein fractions, DEAE-I, DEAE-II, and DEAE-llb with DEAE cellulose chromatography. Four equally divided, 0.5-ml doses of protein (total dose 0.01-0.035 g/kg) were administered. The mean ±1 SE is shown, and the numbers in parentheses indicate the number of animals studied. There was a significant increase ($p < 0.005$) in the level of \textsuperscript{75}SeM in the platelets of mice that received either DEAE-IIa or DEAE-llb from the plasma of thrombocytopenic rabbits. 

Effects of Fractions of Plasma Obtained by Sephadex G-100 Chromatography on Incorporation of \textsuperscript{75}SeM Into the Platelets of Mice

Protein fraction DEAE-II, obtained from the plasma of thrombocytopenic rabbits by precipitation with 60\%-80\% ammonium sulfate saturation and additional fractionation with DEAE cellulose, was demonstrated to contain thrombopoietic activity (see above). DEAE-II then was placed on a Sephadex G-100 column, and 2 elution peaks were obtained, G-100-I and G-100-II (Fig. 5). These protein fractions then were assayed for thrombopoietic activity, as described above, and the results are shown in Fig. 6. A total dose of 0.03-0.05 g/kg was used in 6 different experiments. There was a significant increase ($p < 0.05$) in the level of \textsuperscript{75}SeM only in the platelets of mice that received fraction G-100-II from plasma of thrombocytopenic mice. Platelet counts of mice that received thrombopoiesis-stimulating factor(s) did not differ from those of control mice.

Effects of Fractions of Plasma Obtained by CM Cellulose Chromatography on Incorporation of \textsuperscript{75}SeM Into the Platelets of Mice

Protein fraction DEAE-II, which was obtained from thrombocytopenic rabbits and contained thrombopoietic activity, was suspended in 0.001 M citric acid–0.002
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Fig. 6. Effect of fractions of plasma from thrombocytopenic rabbits on incorporation of $^{75}$SeM into mouse platelets. A protein fraction was precipitated with 60%–80% ammonium-sulfate saturation and then fractionated sequentially by DEAE cellulose and Sephadex G-100 gel chromatography. Four equally divided, 0.5-ml doses of proteins (total dose 0.03–0.05 g/kg) were administered. The mean ±1 SE is shown, and the numbers in parentheses indicate the number of animals studied. There was a significant increase ($p < 0.05$) in the level of $^{75}$SeM only in the platelets of mice that received fraction G-100-II from the plasma of thrombocytopenic rabbits.

$M$ Na$_2$HPO$_4$ buffer at pH 5.6. The initial peak (CM-I) was not retained; the second peak (CM-II) was eluted with 0.4 $M$ NaCl in the citrate-phosphate buffer (Fig. 7). Each of the protein fractions was assayed for thrombopoietic activity. A total dose of 0.003–0.006 g/kg of protein was used in 5 separate experiments. The pooled data are shown in Fig. 8. There was a significant increase ($p < 0.005$) in the level of $^{75}$SeM only in the platelets of mice that received fraction CM-II from thrombocytopenic mice. Platelet counts of mice that received thrombopoiesis-stimulating factor(s) did not differ from those of control mice.

Immunologic Studies

Immunoelectrophoresis of the plasma fraction prepared by ammonium-sulfate precipitation demonstrated multiple precipitin lines, primarily albumin and beta-globulins. Immunoglobulins (IgG, IgA, and IgM) were not detectable. Almost all albumin was removed from the initially retained fraction (DEAE-II) by DEAE cellulose chromatography. The remaining proteins had the electrophoretic mobility of beta-globulins, although other anodally migrating proteins also may have been present. Protein fraction CM-II contained beta-globulins and possibly other anodally migrating proteins.
Fig. 8. Effect of fractions of plasma from thrombocytopenic rabbits on incorporation of $^{75}$SeM into mouse platelets. A protein fraction was precipitated with 60%-80% ammonium-sulfate saturation and then fractionated sequentially with DEAE cellulose and CM cellulose chromatography. Four equally divided, 0.5-ml doses of protein (total dose 0.003–0.006 g/kg) were administered. The mean ± 1 SE is shown, and the numbers in parentheses indicate the number of animals studied. There was a significant increase ($p < 0.005$) in the level of $^{75}$SeM only in the platelets of mice that received fraction CM-II from the plasma of thrombocytopenic rabbits.

DISCUSSION

A factor(s) that stimulates the production of platelets has been detected in the blood of thrombocytopenic animals and humans. Furthermore, recent studies have demonstrated that certain fractions obtained from the plasma of thrombocytopenic animals have thrombopoiesis-stimulating activity (thrombopoietin).

Table 1 summarizes the various steps we have used to fractionate plasma proteins and partially purify thrombopoietin. The fraction obtained following chromatography with carboxymethyl cellulose represents an approximately 1000-fold purification. As little as 135 ng (a mean total dose of 0.0045 mg/g body weight) reproducibly and significantly stimulated thrombopoiesis in mice. McDonald et al. have reported that thrombopoietin, prepared from the plasma of thrombocytopenic sheep or rats by DEAE chromatography, can be detected at a dose range of 0.08-0.8 mg/g.

As anticipated, most of the protein retained by DEAE was not retained by carboxymethyl cellulose. However, thrombopoiesis-stimulating activity was bound by both chromatographic materials at pH 5.6, although the biologic activity was retained only weakly by carboxymethyl cellulose at low ionic strength. This indicates that proteins with thrombopoiesis-stimulating activity were close to their isoelectric points at this pH. These data also suggest heterogeneity of the distribution of positively and negatively charged residues on the surface of the molecules under study that resulted in their selective interaction with the oppositely charged ion exchange resin.

Immunologic analysis indicated that following chromatography with DEAE

| Table 1. Purification of Thrombopoietin From the Plasma of Thrombocytopenic Rabbits |
|---------------------------------|-----------------|----------------|
| Step                            | Dose (mg/g body wt)* | Purification Factor |
| Plasma                          | 2.4–7.2          | 1              |
| (NH$_4$)$_2$SO$_4$ (60%-80% sat)| 0.8–2.4          | 3              |
| DEAE-II†                        | 0.05–0.06        | 87             |
| G-100-II†                       | 0.03–0.05        | 120            |
| CM-II†                          | 0.003–0.006      | 1067            |

*Total dose of protein that produced an increased level of $^{75}$SeM in the platelets of normal mice (only rabbits were utilized in the experiments in which whole plasma was administered).
†See Results for definition of these fractions.
cellulose and CM cellulose, no immunoglobulins were detectable. Proteins with the mobility of beta-globulins and albumin, and other anodally migrating proteins remained present. Thrombopoietin is nondialyzable and stable from at least pH 5.6 to 7.5. Following precipitation with ammonium sulfate and dialysis, it is stable for 3–6 mo at −70°C (Levin and Tang, unpublished observations).

The nature of thrombopoietin remains unknown. Since similar techniques are used to prepare crude preparations of erythropoietin and thrombopoietin,16–18,29 it is tempting to speculate that at least some 'poietins have a similar basic structure, appropriate modifications of which result in specificity for one cell type (i.e., in the case of thrombopoietin, a committed precursor of megakaryocytes or megakaryocytes themselves). Our data (Figs. 3 and 4) suggest that different molecular species of thrombopoietin may exist, as has been shown to be the case for erythropoietin.32 Presumably, the presence of variable amounts of sialic acid accounts for these findings.33

Increased levels of isotope in platelets in the circulation from 16 to 24 hr after administration of Na₂³⁵SO₄ or ⁷⁵SeM to animals that have received thrombopoietin15,16,34 suggest that at least some of the isotopic label is incorporated into mature megakaryocytes, which then rapidly release labeled platelets. MacPherson has suggested that thrombocytopenia (presumably via the mechanism of increased levels of thrombopoietin) affects differentiated megakaryocytes and also causes increased numbers of megakaryocytes to develop from the precursor pool.15 The rapid fall in platelet count following a four-fifths hepatectomy also has been interpreted by Siemensma et al.36 as indicative of an effect of thrombopoietin on mature megakaryocytes, since their study suggested that the liver is a source of thrombopoietin. Therefore, currently available data are compatible with an action of thrombopoietin both on diploid precursors of megakaryocytes37 and on maturing megakaryocytes. A combined effect would explain both the rapid appearance of labeled platelets in the circulation following administration of thrombopoietin and the increase in labeled platelets that persists for approximately 3 days in rodents.5,10,34

Interpretation of potential responses of megakaryocytes to thrombopoietin has been made more difficult by the inconsistency of detection of increased platelet counts following administration of thrombopoietin.1,6,16,28,31,42,46 Until more is understood about the chemical nature and modes of actions of thrombopoiesis-stimulating factors, we believe that the term "thrombopoietin" should be restricted to thrombopoiesis-stimulating activity derived from the blood, which presumably has physiologic significance. The importance of this distinction is emphasized by the study of McDonald, et al.,47 which did not demonstrate increased incorporation of ⁷⁵S in the platelets of mice, in which endogenous thrombopoiesis had been suppressed by platelet hypertransfusion, following administration of thrombopoiesis-stimulating activity obtained from culture medium from human embryonic kidney cells; despite the data that indicate such animals provide an assay system sensitive enough to detect thrombopoietin in the plasma of both thrombocytopenic and normal donors.8,10

The nature of the sensor that regulates thrombopoietin levels is unknown, but it appears that the concentration of platelets in the blood, per se, cannot be the sole variable to which the bone marrow eventually responds. The persistence of
thrombocytopenia in experimental models and clinical states in which there is splenomegaly and an increased splenic platelet pool, without alteration of platelet life span or evidence of bone marrow depression, suggests that total platelet mass may be a key factor in regulating megakaryocytopoiesis.48-52 Erslev et al.53 have suggested that platelet production may be adjusted to maintain total platelet surface area at a normal value, rather than platelet number, function, or mass. In addition, it is necessary to account for the variety of nonspecific stimuli, such as inflammation, surgery, hemorrhage, and malignancy, which produce thrombocytosis without preceding thrombocytopenia.4 Nevertheless, the concept that a function of platelets is monitored in order to regulate thrombopoiesis is attractive. The production of red cells is regulated by the adequacy of their function of transporting oxygen to tissues. Mahmood and Robinson54 recently postulated that the functional capacity of granulocytes to control infection by phagocytosing microorganisms and their products, such as endotoxin, determines the level of colony-stimulating activity and, hence, of granulopoiesis. Regardless of the feedback mechanisms by which platelet number, mass, or function regulate thrombopoiesis, it is noteworthy that sustained thrombocytosis produced by hypertransfusion of platelets does not totally suppress thrombopoiesis in rodents.8,10

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