Relationships Between Thrombopoiesis and Erythropoiesis: With Studies of the Effects of Preparations of Thrombopoietin and Erythropoietin

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The effects of administration of partially purified human urinary erythropoietin and rabbit thrombopoietin, and of endogenously produced erythropoietin and thrombopoietin on both red cell and platelet production were examined in mice. Partially purified thrombopoietin was prepared from rabbit plasma by sequential fractionation with ammonium sulfate precipitation, and DEAE and Sephadex G-100 chromatography. Preparations of thrombopoietin and partially purified human urinary erythropoietin (NIH No. H-11-TaLSL) were administered subcutaneously to normal mice, and the rate of incorporation of selenomethionine-$^{75}$Se into platelets was measured as an index of thrombopoietic activity of the infused material. Erythropoietin and thrombopoietin were assayed for erythropoietic activity by measuring the rate of appearance of $^{59}$Fe in the red cells of posthypoxic polycytemic mice. Preparations containing thrombopoietin had barely measurable erythropoietic activity, and 7 units of partially purified erythropoietin had little thrombopoietic activity. When endogenous levels of erythropoietin were increased by hypoxia, platelet production was not enhanced. Similarly, increased levels of thrombopoietin, induced in response to thrombocytopenia produced by platelet antiserum, did not alter red cell production. These data suggest that physiologically increased levels of thrombopoietin do not stimulate erythropoiesis, and that physiologically increased levels of erythropoietin do not stimulate thrombopoiesis. However, currently available, partially purified preparations of erythropoietin and thrombopoietin may be capable of stimulating both platelet and red cell production if used in sufficient quantities.

ERYTHROPOIETIN, the humoral regulator of erythropoiesis, is not generally believed to stimulate thrombopoiesis. However, under certain experimental conditions increased levels of erythropoietin are associated with thrombocytosis and increased platelet production. In addition, a preparation of human urinary erythropoietin has been reported to have thrombopoietic-stimulating activity. Furthermore, recently described techniques for the partial purification of thrombopoietin, a humoral regulator of platelet production, are similar to those used for the purification of erythropoietin, indicating po-
tential physicochemical similarities between the two humoral substances. These findings, in toto, suggest the possibility that, under certain circumstances, erythropoietin may have thrombopoietic-stimulating activity.

In order to define more clearly the potential effects of erythropoietin upon platelet production, the current studies examined the effect of the administration of partially purified human urinary erythropoietin and rabbit plasma thrombopoietin on both red cell and platelet production in mice. In addition, models in which levels of endogenous erythropoietin and thrombopoietin were increased by physiologic stimuli were evaluated for evidence that either of these humoral regulators altered both erythropoiesis and thrombopoiesis. The results suggested that thrombopoietin and erythropoietin are separate factors, and that the effects of each can be discriminated between in physiologic models. However, currently available preparations of partially purified erythropoietin and thrombopoietin may be capable of stimulating both platelet and red cell production if used in sufficient quantities.

MATERIALS AND METHODS

Measurement of Thrombopoietin

Thrombopoietic activity in fractions of plasma was determined by measuring their effect on incorporation of selenomethionine-75Se (75SeM, selenomethionine-75Se, Sethotope, E.R. Squibb & Sons, New Brunswick, N.J.; specific activity, 200 250 mCi/mg) into platelets, as described previously. Assays of thrombopoietic activity in mice were carried out using CD-1 mice (28 40 g), obtained from Charles River Laboratories, Wilmington, Mass. Their mean platelet count was 1407 x 10^9/liter ± 198 (1 SD), and hematocrit value was 0.43 ± 0.02 (1 SD). Mice were received at least 2 days before use, caged in groups of five, and fed standard laboratory food and water, ad libitum.

All preparations of material to be tested were administered subcutaneously during the 48-hr period preceding the intravenous injection of 1 μCi of 75SeM, which was given 8 hr after the last injection of test material. Incorporation of 75SeM into newly forming platelets, in vivo, was 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 (EDTA, 1.43 M, 5.3 g/100 ml of distilled H2O, buffered to pH 6.0 with THAM [Tris (hydroxymethyl) aminomethane], 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 (tubes used for platelet counts contained dried EDTA) 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 (hydroxymethyl) aminomethane (THAM) 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 H2O) then were 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. The 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 at 5500 g for 5 min and resuspended in 5 ml of Tris buffer, using a Vortex mixer. The resuspended platelets were poured onto a Millipore filter (1.2-μm pore size), held in a Millipore prefilter 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 × total plasma radioactivity × (total platelets on the filter)²/². The contamination of the filter by radioactive proteins was calculated for each sample and subtracted from the total activity. Per cent administered dose of ⁷⁵Selenium in circulating platelets was calculated for each sample as follows: cpm/plt (corrected) × total circulating platelets × 100/total cpm ⁷⁵Selenium injected, where total circulating platelets equaled whole blood platelet count per milliliter × 0.083 × body weight (kg).

The effects of various fractions of rabbit plasma proteins and erythropoietin on incorporation of ⁷⁵Selenium into platelets were measured as an index of the thrombopoiesis-stimulating activity of the infused protein fractions or erythropoietin.

**Measurement of Erythropoietin**

Erythropoietic activity in fractions of plasma or preparations of erythropoietin or thrombopoietin was determined by measuring their effect on incorporation of ⁵⁹Iron into erythrocytes, using a modification of the technique described by Lange, Simmons, and Dibelius. Female Swiss Webster mice (20–25 g) were obtained from Buckberg Laboratory Animals, Tompkins Cove, N.Y., at least 2 days before use, caged in groups of ten, and fed standard laboratory food and water, ad libitum. They were made polycythemic by exposure to low oxygen tensions (pO₂ = 40–60 mm Hg) for 14 days, in dimethyl silicone rubber membrane enclosures (General Electric Corp., Schenectady, N.Y.). Five days after removal from the enclosures, mice with hematocrit values of 0.65 or greater were used for assay. The mice received a single intraperitoneal injection of 0.5 ml of the material to be tested for erythropoietic activity. Forty-eight hours later, 1 μCi of ⁵⁹Iron was injected intraperitoneally and its uptake into red cells measured after another 48 hr as follows: 200 μl of blood were obtained from the retro-orbital venous plexus in a heparin-moistened micropipette and transferred to 400-μl plastic centrifuge tubes. Red cells were prepared for determination of radioactivity by centrifugation in a Beckman Microfuge at 10,000 g for 45 sec, followed by removal of supernatant plasma. Radioactivity of the red cells was determined in a gamma well scintillation counter. Samples were counted for 20 min at an energy range from 600 keV to infinity, and per cent administered dose of ⁵⁹Iron in circulating red blood cells calculated.

The per cent incorporation of ⁵⁹Iron into circulating red cells was used as an index of the erythropoiesis-stimulating activity of the various fractions of plasma (thrombopoietin) or erythropoietin. A standard log dose response curve was obtained using 0.1–0.5 units of erythropoietin (WHO standard B). The responses in the experimental models were expressed as units of erythropoietin activity, using this curve.

**Donor Plasma**

Plasma that contained thrombopoietic-stimulating activity was obtained from New Zealand white rabbits. Normal platelet-free plasma (PFP) was prepared from blood obtained by intracardiac puncture from unanesthetized animals and collected in siliconized glassware using acid-citrate-dextrose (ACD) as an anticoagulant (one part ACD: nine parts whole blood). After 24 hr, at which time the platelet counts were not significantly changed and the mean hematocrit value had fallen from 0.40 to 0.30, the animals were rendered acutely thrombocytopenic (platelet count < 30 × 10⁹/liter), by injection of 0.5–1.0 ml of anti-rabbit platelet serum. Antiplatelet sera to rabbit or mouse platelets were prepared, as previously described. PFP was prepared from blood collected 5–6 hr later. PFP, from normal or thrombocytopenic donors, was stored at 4°C for periods up to 24 hr. Plasma from normal or thrombocytopenic donors was fractionated at 4°C with saturated ammonium sulfate. Previous studies had shown that thrombopoiesis-stimulating factor(s) in the plasma of thrombocytopenic rabbits was present in the fraction of plasma proteins precipitated by 60%–80% levels of saturation of ammonium sulfate. Accordingly,

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*Second International Reference Preparation of human urinary erythropoietin, obtained from the WHO International Laboratories for Biologic Standards.*
the current experiments were performed using this fraction as the initial step in purification, and the material was lyophilized and stored at -20°C for periods up to 4 wk before use.

The precipitate produced by an ammonium sulfate saturation of 60%-80%, from normal or thrombocytopenic donors, was further fractionated by using DEAE-cellulose column chromatography, with 0.01 M citric acid and 0.02 M Na2HPO4 buffer, at pH 5.6. Two fractions were obtained following the application of a step-wise NaCl gradient, and were lyophilized. The protein fraction prepared by DEAE chromatography, which was found to contain thrombopoietic activity, was further fractionated by Sephadex G-100 chromatography, using the same buffer, at pH 5.6. Two fractions were obtained, and were lyophilized and stored at -20°C until use. A manuscript describing these purification steps is in preparation.

The fractions of plasma proteins (prepared by fractionation with DEAE-cellulose and Sephadex G-100 chromatography) were reconstituted to identical protein concentrations in citrate-phosphate buffer, and the NaCl concentrations of the preparations equalized. The pH was adjusted to 7.4 with 2 N NaOH before injection into mice.

Erythropoietin

Partially purified, lyophilized preparations of human urinary erythropoietin (NIH Pool No. H-11-TaLSL; 75 units/mg protein) were resuspended for injection in 0.9% NaCl, which contained 0.1% bovine serum albumin. For assays of thrombopoietic activity, the bovine serum albumin was omitted. The erythropoietin was collected and concentrated by the Department of Physiology, University of the Northeast, Corrientes, Argentina; and further processed and assayed by the Hematology Research Laboratories, Children's Hospital of Los Angeles, under Research Grant HE 10880 (National Heart and Lung Institute). It was authorized for distribution by the Erythropoietin Committee of the National Heart and Lung Institute.

Hypoxia

The effects of hypoxia upon platelet production were determined by measuring its effect upon the incorporation of 75SeM into mouse platelets. CD-1 mice (28-40 g) were placed in dimethyl silicone rubber membrane enclosures, in which the pH2 was maintained between 40-60 mm Hg (oxygen content 5%-7%). After 24 or 72 hr, 1 pCi of 75SeM was injected intravenously via the tail vein, and the hypoxia continued for an additional 16 hr. Levels of erythropoietin also were determined by assay of plasma for erythropoietin, as described above.

Thrombocytopenia

The effects of severe thrombocytopenia upon red cell production were determined by measuring its effect upon incorporation of 59Fe into circulating red cells of posthypoxic, polycythemic mice. Female Swiss Webster mice (20-25 g) were made polycythemic using dimethyl silicone rubber enclosures, as described above. Five days after removal from the enclosures, at which time the mean hematocrit value was 0.68, the mice were rendered severely thrombocytopenic (platelet count < 30 x 10^9/liter) by intravenous injection of guinea pig anti-mouse platelet serum. Controls received intravenous saline. The effect of increased endogenous production of thrombopoietin on red cell production was evaluated by the intraperitoneal injection of 1 µCi of 59Fe 6 hr later.

RESULTS

Effect of Fractions of Plasma From Thrombocytopenic Rabbits Upon Incorporation of 59Fe Into the Red Blood Cells of Polycythemic Mice

Protein fractions from plasma of thrombocytopenic rabbits, prepared as previously described, were administered to polycythemic mice in a single dose (total dose of protein was 0.03-0.17 g/kg). One µCi of 59Fe was administered intraperitoneally 24 hr after the injection of proteins, and per cent dose uptake was measured at 48 hr. The pooled data from five experiments are shown in Fig. 1. There was a significant increase in the level of 59Fe in red blood cells of
Fig. 1. Effect of fractions of plasma from thrombocytopenic rabbits upon incorporation of $^{59}$Fe into the red blood cells of polycythemic mice. Protein fractions were prepared by precipitation with ammonium sulfate (60%-80% saturation) and then fractionated sequentially with DEAE-cellulose and Sephadex gel chromatography. Each mouse received 1 μCi of $^{59}$Fe 48 hr following the single intraperitoneal injection of a protein fraction, and per cent dose uptake of $^{59}$Fe was measured at 48 hr. The data from five experiments are included. The mean ± 1 SE is shown, and the numbers in parentheses indicate the number of animals studied. There was a significant increase in the level of $^{59}$Fe in red blood cells of mice that received 0.1 ($p < 0.025$), 0.5 ($p < 0.005$), or 1.0 ($p < 0.0005$) units of erythropoietin (WHO standard B); DEAE-fraction II (total dose 0.05-0.06 g/kg); or Sephadex G-100 fraction II (total dose 0.03-0.05 g/kg). The latter two fractions contained thrombopoietic activity. The point labeled "DEAE (2 x)" represents the uptake of $^{59}$Fe in animals that received two to three times the usual dose (total dose 0.12-0.17 g/kg) required to demonstrate the presence of thrombopoietic activity in this protein fraction.

mice that received fraction II eluted from the Sephadex G-100 column ($p < 0.025$) and DEAE-fraction II ($p < 0.01$), both of which had been shown to contain thrombopoietic stimulating activity. However, the level of increased uptake of $^{59}$Fe indicated that the fractions of plasma, in the doses used, contained the equivalent of only approximately 0.1 unit of erythropoietin. DEAE-fraction II, prepared from nonanemic, thrombocytopenic rabbits, contained equivalent thrombopoietic-stimulating activity, but no detectable (< 0.1 unit) erythropoietin. Fraction I from the G-100 column did not contain either thrombopoietic-stimulating activity or detectable levels of erythropoietin.

Effect of a Preparation of Human Urinary Erythropoietin Upon Incorporation of $^{75}$SeM Into the Platelets of Normal Mice

Preparations of human urinary erythropoietin (NIH Pool No. H-11-TaLSL) were suspended in 0.9% NaCl and administered to mice during a 40-hr period. One microcurie of $^{75}$SeM was administered intravenously, 8 hr after the last of four equally divided doses of erythropoietin (total dose was 0.5–7 units/mouse); and per cent dose uptake was measured at 16 hr. There was a significant increase ($p < 0.005$) in the level of $^{75}$SeM only in the platelets of mice that received 7 units of erythropoietin (Fig. 2). The platelet counts of mice given erythropoietin did not increase or differ from those of mice given 0.9% NaCl. Furthermore, acute thrombocytopenia was not produced by the largest dose of erythropoietin.
Fig. 2. Effect of a preparation of human urinary erythropoietin upon incorporation of \(^{75}\)SeM into the platelets of normal mice. A preparation of human urinary erythropoietin (NIH Pool No. H-11-TaSL) was suspended in 0.9% NaCl. Each mouse received 1 \(\mu\)Ci of \(^{75}\)SeM intravenously after the last of four equally divided 0.5-ml doses of erythropoietin (total dose 0.5–7 U/mouse) or four 0.5-ml injections of 0.9% NaCl. Per cent dose uptake was measured at 16 hr. The mean \(\pm 1\) 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 had received 7 units of erythropoietin.

Absence of Inhibitors of Erythropoietin in the Fraction of Rabbit Plasma That Contained Thrombopoietin

The preparations of thrombopoietin or erythropoietin were not pure, as indicated by SDS-urea gel polyacrylamide electrophoresis. Each preparation contained many bands, several of which migrated in similar locations, suggesting the possibility that similar proteins or polypeptides were contained in the preparations. In order to determine if the inability to detect greater concentrations of erythropoietin in the thrombopoietin preparations was due to the presence of an inhibitor of erythropoietin, the following experiment was performed. The fraction from thrombocytopenic rabbit plasma (DEAE-II) that contained thrombopoietic activity (final concentration was 3 mg protein/ml) was incubated with a preparation of sheep plasma erythropoietin (Step III, 2.2 U/mg protein, Connaught Laboratories, Willowdale, Ont., Canada) (final concentration was 2 U/ml) for 1 hr at 20°C. The mixture then was assayed for erythropoietic activity. Each posthypoxic polycythemic mouse received a single dose of the incubation mixture, which contained 0.06 g/kg of the protein fraction that contained thrombopoietic activity and 1 unit of erythropoietin. There was no inhibition of erythropoietin activity by preparations that contained thrombopoietic activity.

Effects of Hypoxia Upon Platelet Production and Circulating Erythropoietin Levels

Since large doses of a preparation of erythropoietin had stimulated thrombopoiesis (as indicated by increased incorporation of \(^{75}\)SeM into platelets), experiments were performed to determine if an increase in endogenous production of erythropoietin would alter the rate of platelet production. In three separate
Fig. 3. Effect of hypoxia upon thrombopoiesis. In three separate experiments, mice were rendered hypoxic by the use of dimethyl silicone rubber membrane chambers. After either 24 or 72 hr of hypoxia, 1 μCi of 75SeM was injected intravenously and percent dose uptake was measured after an additional 16 hr of hypoxia. Each experiment showed no increase in platelet production, and the data were pooled. The mean ± 1 SE is shown, and the numbers in parentheses indicate the number of animals studied.

In another series of experiments, mice were rendered hypoxic for 40 hr, and samples of plasma were obtained and assayed for erythropoietin. Forty hours was selected for the time of collection of the blood samples because it equaled the total period of hypoxia experienced by the animals in some of the previously described experiments (24 hr before, plus 16 hr after injection of 75SeM). Therefore, this study indicated the level of circulating erythropoietin at the time of the thrombopoietin assay. The pooled results are shown in Fig. 4. There was a

Fig. 4. Effect of hypoxia upon circulating erythropoietin levels. Mice were rendered hypoxic by the use of dimethyl silicone rubber membrane chambers. After 40 hr of hypoxia, samples of plasma were assayed for erythropoietin, using posthypoxic mice. The mean ± 1 SE is shown, and the numbers in parentheses indicate the number of animals studied. There was a significant increase in the level of 55Fe in red cells of mice that received plasma from hypoxic mice (p < 0.025). The mean dose response (corrected to units of erythropoietin) indicated a circulating level of erythropoietin of > 1.0 U/ml in the plasma of hypoxic mice. Erythropoietin was not detected in the plasma of normal mice or in a preparation of bovine serum albumin. Bovine serum albumin was administered to one group of controls because it was present in solutions of erythropoietin.
significant increase \((p < 0.025)\) in the levels of \(^{59}\text{Fe}\) in red cells of mice that received plasma from hypoxic mice. The mean dose response, corrected to units of erythropoietin, indicated a circulating level of >1.0 U/ml of erythropoietin in the plasma of the hypoxic mice. Thus, although hypoxia produced a significant elevation of the level of circulating erythropoietin, there was no effect on platelet production.

**Effect of Thrombocytopenia Upon Red Cell Production**

Posthypoxic polycythemic mice are sensitive to the effects of erythropoietin. Therefore, the effect of increased levels of thrombopoietin (produced by thrombocytopenia) on red cell production was tested using the following model. Posthypoxic, polycythemic mice were rendered acutely thrombocytopenic with intravenous anti-mouse-platelet serum. Platelet counts fell acutely to less than \(30 \times 10^9/\text{liter}\), but hematocrit values remained unchanged. Six hours later, \(^{59}\text{Fe}\) was administered in order to measure the effect of acute thrombocytopenia on erythropoiesis. Six hours was chosen because thrombopoietin levels have been shown to be elevated in rabbits, rats, sheep, and mice, 2–12 hr following the induction of acute thrombocytopenia.\(^{17,21}\) Erythropoiesis was not stimulated by acute, severe thrombocytopenia. \(^{59}\text{Fe}\) incorporation in 12 nonthrombocytopenic, polycythemic mice was \(1.2\% \pm 0.5\%\) and \(1.6\% \pm 0.4\%\) in ten thrombocytopenic, polycythemic animals. However, the production of acute thrombocytopenia in posthypoxic, polycythemic mice did result in a marked increase in incorporation into platelets of \(^{55}\text{SeM}\), which was administered 48 hr after induction of acute thrombocytopenia. This study demonstrated that the ability of polycythemic mice to increase platelet production, in response to acute thrombocytopenia, was not impaired. de Gabriele and Penington\(^2\) also have reported that polycythemia did not block the normal response to acute thrombocytopenia.

**DISCUSSION**

Previous studies of the potential role of erythropoietin in altering platelet production have produced conflicting results. An effect of erythropoietin on thrombopoiesis has been suggested by the presence of thrombocytosis in clinical states associated with increased erythropoiesis.\(^{6,7,24,25}\) A relationship apparently has been observed between reduced arterial oxygen saturation and increased thrombopoietic activity in plasma of children with cyanotic congenital heart disease;\(^{26}\) but thrombopoietic-stimulating activity was measured using an assay based upon changes in circulating levels of platelets, which may not reflect alterations in platelet production and which may be difficult to interpret.\(^{1,23}\) Recently, Jackson, Simone, and Edwards\(^{28}\) reported that production of anemia, with concomitant blockade of erythropoiesis by actinomycin D, resulted in increased megakaryopoiesis and thrombocytosis. They suggested that erythropoietin may be capable of stimulating thrombopoiesis under appropriate conditions. Shreiner and Levin measured the effect of a partially purified preparation of human urinary erythropoietin upon thrombopoiesis.\(^{9,29}\) Although the rate of thrombopoiesis was increased by large doses of erythropoietin, contamination of the preparation of erythropoietin by thrombopoietin could not be ruled out.
In contrast to these studies, other investigators have concluded there is little or no relationship between erythropoietic and thrombopoietic activity. People living at high altitudes have normal platelet counts. de Gabriele and Penington injected partially purified human urinary erythropoietin into rats and did not detect a rise in platelet counts. In addition, Van Dyke reported that erythropoietin from human, sheep, and rabbit sources did not increase the platelet counts of primates. Shaikh and Erslev observed that decreased erythropoietin levels, produced by hypertransfusion of red blood cells, did not result in decreased synthesis of platelets, as measured by uptake of \(^{75}\text{SeM}\) into platelets. Although the platelet counts were decreased in the mice, they concluded that the decrease was due to increased blood volume and dilution of platelets, caused by the hypertransfusion of red blood cells. Similar results were reported in rats, using \(\text{Na}_2^{35}\text{SO}_4\).

In order to define more clearly the possible influence of erythropoietin on platelet production, the present studies attempted to determine whether stimulation of erythropoiesis and thrombopoiesis could be produced independently. Two approaches were used. The first was the administration of partially purified preparations of erythropoietin or thrombopoietin. Our data indicated that some fractions of plasma, which contained thrombopoietic-stimulating activity, were capable of slightly stimulating red cell production and that large doses of preparations of partially purified human urinary erythropoietin stimulated thrombopoiesis. Inhibitors did not appear to account for the divergence of these activities, since incubation of erythropoietin with thrombopoietin did not produce a decrease in the activity of the erythropoietin.

The second approach was manipulation of circulating levels of endogenously produced erythropoietin or thrombopoietin in experimental animals. When endogenous levels of erythropoietin were increased by 40-88 hr of hypoxia, platelet production was not altered. Rand, Anderson, Lukis, and Creger studied the effect of hypoxia on platelet levels in rats. Experimental animals were kept hypoxic for 10-20 days and then underwent acute phlebotomy. As the hematocrit value rose with hypoxia, the platelet counts decreased, but the decrease appeared to be related to the rise in total plasma solids, suggesting that the plasma platelet concentration remained constant. After hemorrhage, platelet counts rose as expected, and the authors concluded that platelet production was neither increased nor decreased in the hypoxic rat. However, other investigators have obtained divergent results in apparently similar experiments, using isotopic techniques. Cooper and Cooper reported increased production of platelets in mice during the first 24 hr of hypoxia. This increase was followed by apparently decreased production of platelets during the next 4 days, but blood volumes were not reported. Shreiner and Levin, using \(^{75}\text{SeM}\), found that platelet production was increased during the first 24 hr of hypoxia, but was normal at 7 days. Plasma from hypoxic mice did not contain detectable thrombopoietic activity when injected into normal recipients. Interestingly, Merino observed that some people, exposed to high altitudes for approximately 3 wk, demonstrated a slight decrease in circulating platelet counts. Birks, Klassen, and Gurney suggested that platelet production was decreased by hypoxia for periods of longer than 7 days. Their conclusions were based upon the observation that circulating platelet counts decreased in mice sub-
jected to hypoxia. However, measurements of platelet production or blood volume were not performed. They obtained the same results in splenectomized animals, indicating the thrombocytopenia was not secondary to splenomegaly. De Gabriele and Penington reported that hypoxia, of 22 days duration, produced a marked rise in hematocrit value, but very little change in the platelet count. In addition, when hypoxia was continued for 6 wk after splenectomy, platelet counts remained unchanged. The authors suggested that slight reductions of the platelet count were due to a change in blood volume.

These varied results may not be as inconsistent as they appear, because the investigators used different techniques, species, and experimental conditions to obtain their data. For instance, the apparent increase in platelet production detected within the first 24 hr of hypoxia may have been due to nonspecific, stressful stimulation, independent of increased elaboration of erythropoietin. During this early period, animals frequently lose weight and some die. Since changes in blood volume could produce erroneous interpretations of results in experiments that measured the effect of hypoxia on platelet production, we determined blood volumes, with Fe-labeled red cells, in hypoxic animals. Although the animals lost weight, blood volumes decreased proportionately, and the ratio of blood volume to body weight remained constant (i.e., normal) after 48 hr of hypoxia. The values obtained were comparable to those of other investigators. Perhaps noteworthy is the general agreement that platelet levels are increased after 24–48 hr of hypoxia, but normal or decreased after longer periods of time.

The kidney, necessary for the production of erythropoietin, also has been proposed as a site of thrombopoietin production. Krizsa, using an assay based upon changes in platelet counts, reported that the serum of nephrectomized rats did not contain thrombopoietic activity in response to hemorrhage, in contrast to the normal response of rats that had undergone splenectomy, adrenalectomy, or hypophysectomy. He concluded that the kidney was necessary for the production of a posthemorrhagic thrombopoietic factor. However, removal of the kidneys did not prevent the production of a thrombopoietic factor after induction of acute thrombocytopenia, suggesting that at least two different mechanisms were involved in the elaboration of humoral factors that produced thrombocytosis in recipient animals. In addition, Jackson et al. who interpreted their experiments as indicating that under appropriate conditions erythropoietin was capable of stimulating thrombopoiesis, observed that bilateral nephrectomy prevented alterations in megakaryocytopoiesis; they proposed that a substance elaborated by the kidney, in response to acute anemia, accelerated platelet production. McDonald demonstrated thrombopoietic activity in cultures of human embryonic kidney cells and suggested that the kidney may be one site of thrombopoietin production. However, de Gabriele concluded that the kidney was not the site of thrombopoietin production because normal recovery of platelet levels occurred, following induction of thrombocytopenia by platelet antiserum, in nephrectomized rats. Furthermore, anephric humans do not become thrombocytopenic (Evatt and Sapir, unpublished observations).

Our data demonstrate that physiologically increased levels of thrombopoietin do not stimulate erythropoiesis, and that physiologically increased levels of
erythropoietin do not stimulate thrombopoiesis, indicating that thrombopoietin and erythropoietin are different humoral factors. However, under certain circumstances (e.g., anemia), erythropoietin or another factor produced by the kidney may be capable of stimulating more than one type of precursor cell. In contrast, the kidney is not necessary for the production of the thrombopoiesis-stimulating factor that results from acute thrombocytopenia. Currently available, partially purified preparations of erythropoietin and thrombopoietin are capable of stimulating both platelet and red cell production if used in sufficient quantities.

Contrary to what has been reported previously, McDonald has recently observed that the kidney is necessary for the appearance in plasma of thrombopoietin, following production of acute thrombocytopenia.

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