Thyroxine Suppresses Thrombocytopoiesis and Stimulates Erythropoiesis in Mice (43507)

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Abstract. **Thyroxine has been shown *in vitro* to stimulate erythropoiesis by two mechanisms: a direct, β_2-adrenergic receptor-mediated stimulation of red cell precursors, and an indirect, erythropoietin-mediated mechanism. Clinical reports have suggested that excess thyroxine also exerts depressive effects on thrombocytopoiesis, but the most sensitive methods of assessing platelet production, i.e., percentage of ^{35}S incorporation into platelets and determination of megakaryocyte size and number, are not appropriate for analysis of platelet production in human patients. The purpose of this study was to use a mouse model to investigate the effects of the hyperthyroid state on erythropoiesis and thrombocytopoiesis, and to assess *in vivo* the two mechanisms by which thyroxine has been described to stimulate erythropoiesis *in vitro*. We found that thyroxine administration significantly depressed platelet production and stimulated erythropoiesis in mice. Both the D- and L-isomers of thyroxine in appropriate doses produced this depression of thrombocytopoiesis, and the effect was dose dependent for both isomers. Daily administration of thyroxine:increased blood volume; decreased the peripheral platelet count, total circulating platelet count and mass, percentage of ^{35}S incorporation into platelets, and megakaryocyte number and size; and concurrently increased indices of red cell production (packed cell volume, red blood cell count, total circulating red blood cell count and mass, and reticulocyte count). Additionally, propranolol, a nonspecific β-blocker, partially reversed the suppression of platelet production by L-thyroxine, lending credence to the assertion that the direct, β_2-adrenergic receptor-mediated stimulation of the erythroid cell line by thyroxine reported to exist *in vitro* may also be important *in vivo*.** [P.S.E.B.M. 1992, Vol 201]

An inverse relationship between erythropoiesis and thrombocytopoiesis has been described in mice responding to an erythropoietic stimulus, i.e., hypoxia (1). Long-term hypoxia has been demonstrated to increase erythropoiesis while concurrently decreasing thrombocytopoiesis (2). Platelet counts (1–3), percentage of ^{35}S incorporation into platelets (1–4), colony-forming unit-megakaryocyte populations (5), megakaryocyte precursor cells (5, 6), and megakaryocyte concentrations in the bone marrow and spleen (7) have all been shown to be suppressed in mice held in an environment with low oxygen tensions, whereas packed cell volumes of these animals were increased (1-8). In hypoxic mice, increased erythropoietin (EPO) titers (9) and normal thrombopoietin levels (2) have been demonstrated, thereby supporting the hypothesis (1–2) that the marked stimulation of the erythroid cell line results in competition between precursor cells of the erythrocytic and megakaryocytic cell lines (stem cell competition). In this hypothesis, increased erythropoiesis leads to decreased numbers of cells in the megakaryocytic cell lineage, which leads to decreased thrombocytopoiesis.

Thyroid hormones are known to stimulate erythropoiesis *in vitro* (10–12), and clinical states of thyroid dysfunction suggest that thyroxine levels are related to *in vivo* erythropoiesis. In cultures of mammalian marrow, thyroxine increased proliferation of cells in the erythroid series (10, 11). The mechanism of this response was proposed to be at least partially mediated

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by a β_2-adrenergic receptor, as addition of propranolol (a $\beta_1\beta_2$-adrenergic receptor antagonist) or butoxamine (a selective β_2-adrenergic receptor antagonist) prevented erythroid stimulation *in vitro* (10). Interestingly, this direct stimulatory effect did not seem to be dependent upon calorigenic potential; i.e., the D-isomer of thyroxine, which has no calorigenic activity, stimulated *in vitro* erythropoiesis, as did the L-isomer (11). There is compelling evidence that thyroxine exerts both a direct (β_2-adrenergic) and indirect (EPO-mediated) effect on erythroic precursors (10–14). Because the *in vitro* data have been obtained using cultures of whole marrow, this direct effect can only be assigned with certainty to the level of the bone marrow; to assert with certainty that the β-adrenergic receptor is on the erythroid cell precursors would require that similar experiments be done on purified cultures of erythroid cell precursors.

Clinical data also support the hypothesis of direct and indirect action of thyroxine by the state of the erythron in thyroid-deranged patients; in one review, about 20% of cats with hyperfunctional thyroid adenomas had an erythrocytosis (15), and in a study of 56 dogs with hypothyroidism, a normocytic, normochromic anemia was observed in about half of the subjects (16). In humans, hyperthyroidism is associated with increased erythropoiesis and an increased plasma volume, whereas up to 60% of hypothyroid patients are reported to be anemic (17).

The effects of thyroxine on the thrombocytopoietic system are poorly understood. The earliest reports of such effects were in the form of case reports of a hemorrhagic tendency in hyperthyroid patients (18–20). Thrombocytopenia in Graves' disease has been proposed to be due to several factors: an autoimmune phenomenon, secondary to hypersplenism, or the result of direct damaging effects of thyroid hormones on platelets (21). Results of a recent study (22) suggested, despite the historical association between autoimmune thrombocytopenia and Grave's disease (23), that the cause of thrombocytopenia in hyperthyroidism is primarily metabolic, although in rare cases, an autoimmune thrombocytopenia may be superimposed. Use of platelet volume or size to characterize blood disorders allows detection of more subtle changes in thrombocytopoiesis (24) than platelet count alone. Ford *et al.* (25) have described an increased mean platelet volume in human hyperthryoid patients, and conversely, van Doormal *et al.* (26) have reported that small-sized platelets predominate in the hypothyroid state.

These latter reports suggest the need for a study in which very sensitive parameters of thrombocytopoiesis can be utilized to evaluate the hyperthyroid state. In mice, platelet counts have been described as a less sensitive index of thrombocytopoiesis than ^{35}S uptake into platelets (27), or megakaryocyte size and number, and percentage of small acetylcholinesterase-positive cells in bone marrow. It was the goal of this work to determine the relationship between thrombocytopoiesis and the known erythropoietic stimulation induced by the thyroid hormone, and to evaluate the two proposed mechanisms (i.e., direct and indirect) of erythroid stimulation in relation to the effects of thyroxine on the thrombocytopoietic system. The results support the theory that thyroxine concurrently stimulates erythropoiesis and suppresses thrombocytopoiesis *in vivo*, and that the suppression of thrombocytopoiesis by L-thyroxine can be partially blocked by treatment with the nonspecific β-adrenergic blocker propranolol.

Materials and Methods

Animals. Male 6- to 8-week-old C3H/HENHSD (C3H) mice purchased from Harlan Sprague-Dawley (Indianapolis, IN) were used in this work.

Pharmaceuticals. Thyroxine (L- and D-isomers) from Sigma Chemical Co. (St. Louis, MO) in the sodium salt form was dissolved in $1 \times 10^{-3} M$ NaOH (pH 10.3) and administered subcutaneously; NaOH ($1 \times 10^{-3} M$) was used as the control substance (28). Propranolol (Inderal; Ayerst Pharmaceutical, New York, NY) was obtained in the intravenous injectable form and diluted with appropriate volumes of 0.9% saline before intraperitoneal injection. Saline (0.9%) was used as the control material for injections of propranolol.

Experimental Design. In the dose-response experiments, the total daily dose of L- or D-thyroxine was divided into two injections and administered subcutaneously; mice were treated on Days 0 and 1, and were sacrificed on Day 3. In the time course experiments, mice were given a single daily subcutaneous injection of thyroxine at a dose of 25 μg/mouse/day. In experiments with propranolol, a daily dose of 4 mg/kg of the β-blocker was divided into two intraperitoneal injections. One injection was administered 1 hr before the daily dose of 25 μg of thyroxine and the other dose of propranolol was given approximately 6 hr later the same day.

^{35}S Incorporation. Single intravenous injections of 30 μCi of ^{35}S ($Na_2{}^{35}SO_4$) were given 24 hr before sacrifice, and blood (0.5–1.0 ml) was collected from sodium pentobarbital-anesthetized mice (0.5 ml of 5 mg/ml of sodium pentobarbital administered intraperitoneally 5–10 min before collection) via cardiac puncture. Platelets were collected by differential centrifugation at 450*g* and were washed free of red cells and plasma. The percentage of ^{35}S incorporation into platelets was then calculated using the method described by McDonald (29).

Platelet Size. Samples of blood for platelet-sizing analysis were collected by cardiac puncture into 1.0 ml of 3.8% sodium citrate solution following anesthesia with sodium pentobarbital. The blood samples were then centrifuged at room temperature for 4.5 min at 160*g* to allow for collection of the platelet-rich plasma

fraction that was used for size analysis using an Electrozone Celloscope (Particle Data, Inc., Elmhurst, IL) (26, 30) with a 128 multichannel analyzer. The instrument was calibrated using 2.02-μm diameter latex particles. When samples were used for both platelet-sizing analysis and ^{35}S incorporation, the platelet-rich plasma was added back to the packed red blood cells (RBC) and diluted with 1% EDTA–0.538% NaCl solution for a total volume of 3.0 ml, then recentrifuged at 450*g*.

Megakaryocyte Size and Number. A femur from each mouse was removed and fixed in 10% phosphate-buffered formalin. Bones were embedded in glycol methacrylate, and multiple sections, each of 2-μm thickness, were cut at 100-μm intervals throughout the femur. The sections were stained with hematoxylin and eosin. For megakaryocyte size, a minimum of 200 megakaryocyte profiles were identified and analyzed using a digitizing image analysis system (Analytical Systems, Atlanta, GA). The method of Weibel (31) was used to correct for optically lost caps, and corrections were made for tissue shrinkage, as described previously by Cullen and McDonald (32). For analysis of megakaryocyte number, sections were examined by light microscopy at ×400, and the number of megakaryocytes per high-powered field was counted in a minimum of 10 marrow fields per femur. The average number per high-powered field was then corrected for optically lost caps and converted to number per unit volume, as described previously (32).

Blood Volume Determination and Related Calculations. ^{59}Fe-Labeled erythrocytes were used in the determination of the effect of exogenous thyroxine administration on blood volumes (3). Donor mice were given 0.5 μCi each of ^{59}Fe as ferric chloride; forty-eight hours later, these mice were sacrificed and the ^{59}Fe-labeled erythrocytes were collected by cardiac puncture into citrate anticoagulant. The labeled red cells were isolated from the citrated plasma by centrifugation, and the packed red cells were resuspended in 0.9% saline. Control animals treated with daily subcutaneous injections of 1×10^{-3} *M* NaOH and animals treated with daily subcutaneous injections of 25 μg/day of L-thyroxine were injected intravenously with 0.1 ml of ^{59}Fe-labeled red blood cells 15–20 min before sacrifice. Blood (100 μl) was diluted in 2.0 ml of water, and the radioactivity of the resulting solution was counted in a gamma counter. Blood volume is expressed as ml/100 g body weight (%).

Blood volumes were then used in the calculation of absolute measurements of peripheral red blood cells and platelets (i.e., total circulating red blood cell count [TCRBCC], total circulating red blood cell mass [TCRBCM], total circulating platelet count [TCPC], and total circulating platelet mass [TCPM]) (3). TCRBCC was calculated by multiplying the peripheral red blood cell count by the total milliliters of blood/mouse, and TCRBCM was calculated by multiplying the TCRBCC by the average erythrocyte size. Similarly, TCPC represents the product of peripheral platelet count and total blood volume in milliliters, and TCPM was calculated by multiplying the TCPC and the average platelet size (3).

Other Hematologic Evaluations. A sample of blood collected from the retro-orbital sinus was utilized for analyses of platelet count (performed manually using a hemocytometer chamber and phase-contrast microscopy), red and white blood cell counts (performed with a Coulter counter; Coulter Inc., Hialeah, FL), and reticulocyte count (performed manually on new methylene blue-stained smears). Packed cell volumes (PCV) were performed by standard techniques.

Statistics. Student's *t* test was used for statistical analysis of data. One mouse was considered to be a single data point in determining degrees of freedom.

Results

Dose Response of Thyroxine. The percentage ^{35}S incorporation into platelets of mice was evaluated following administration of doses of 25–200 μg/mouse of both L- and D-thyroxine (Fig. 1). The total dose was divided into four subcutaneous injections, given two each on Days 0 and 1 of the experiment. The vehicle (1×10^{-3} *M* NaOH) was used as the control substance. A dose of 30 μCi of ^{35}S was administered intravenously on Day 2, and mice were sacrificed on Day 3. Both thyroxine isomers produced significant depression of thrombocytopoiesis (D-isomer, $P < 0.005$; L-isomer, $P < 0.0005$), as indicated by reduced ^{35}S incorporation into platelets. The L-isomer produced greater suppression of ^{35}S uptake than the D-isomer at each dose evaluated, although no statistical significance in these

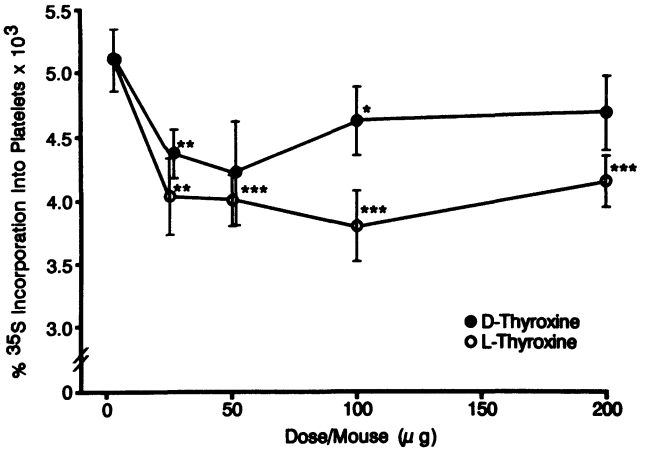

Figure 1. The effect of various doses of D- and L-thyroxine on ^{35}S incorporation into platelets of mice. Each data point represents a group of 10 mice, except at 25-μg and 100-μg doses of the D-isomer, where nine mice were used per point. The 0 dose value represents data from mice given 1×10^{-3}*M* NaOH (vehicle). Vertical bars indicate SE. Values were significantly different from control: * $P < 0.05$; ** $P < 0.005$; *** $P < 0.0005$.

values was found. Control data are represented as Day 0 in Figure 1.

Time Course of Thyroxine Effects. Daily administration of 25 μg/mouse/day of L-thyroxine also resulted in significant suppression of platelet production (Fig. 2); both platelet count ($P < 0.05$ to $P < 0.005$ on Days 6 and 8) and percentage of ^{35}S incorporation into platelets ($P < 0.05$ to $P < 0.005$ on Days 2–8) were decreased over the course of 8 days of thyroxine administration (Fig. 2, A and B). Control animals were dosed daily with a like volume of 1×10^{-3} *M* NaOH. Control animals were sacrificed on each day of the experiment; animals sacrificed on different days did not differ statistically, and were, therefore, pooled as controls (Day 0 in Fig. 2). Additionally, platelet size was found to be increased ($P < 0.05$) after 8 days of treatment (Fig. 2C), and reticulocyte counts (Fig. 3A, $P < 0.005$), PCV (Fig. 3B, $P < 0.025$), and red blood cell counts (Fig. 3C, $P < 0.05$) were also increased.

Table I shows that megakaryocyte size, expressed as mean megakaryocyte diameter, was significantly decreased in mice by treatment with L-thyroxine when compared with control levels after 2–6 days ($P < 0.05$ to $P < 0.005$) of treatment. Likewise, megakaryocyte concentrations were significantly lower when compared with control levels after thyroxine treatment of 4–8 days duration ($P < 0.05$).

Blood Volumes and Related Calculations. Daily administration of 25 μg/mouse/day of L-thyroxine significantly ($P < 0.05$) increased the blood volume of mice after 6 days of L-thyroxine administration (Table II). Daily administration of this dose of thyroxine also significantly depressed TCPC and TCPM ($P < 0.0005$), whereas TCRBCC and TCRBCM were increased ($P <$

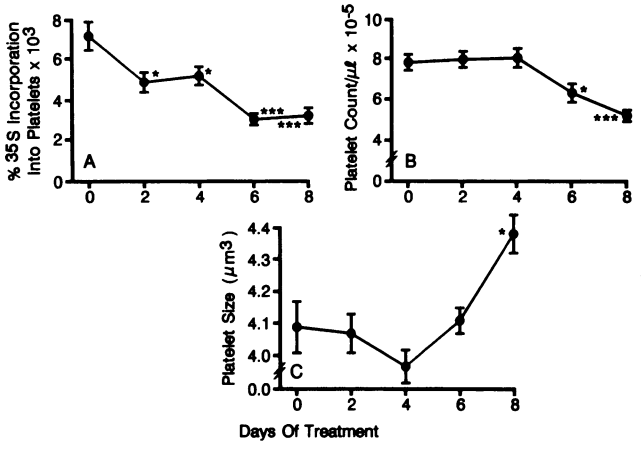

Figure 2. The effects of daily administration of L-thyroxine (25 μg/day) on percentage of ^{35}S incorporation into (A) platelets of mice, (B) platelet count, and (C) platelet size. The Day 0 value represents data from mice administered 1×10^{-3} *M* NaOH for 2 to 8 days. All mice, including controls, were sacrificed at the same time. In all panels, each data point represents the average of six mice and vertical bars indicate SE. Values were significantly different from control: * $P < 0.05$; *** $P < 0.005$.

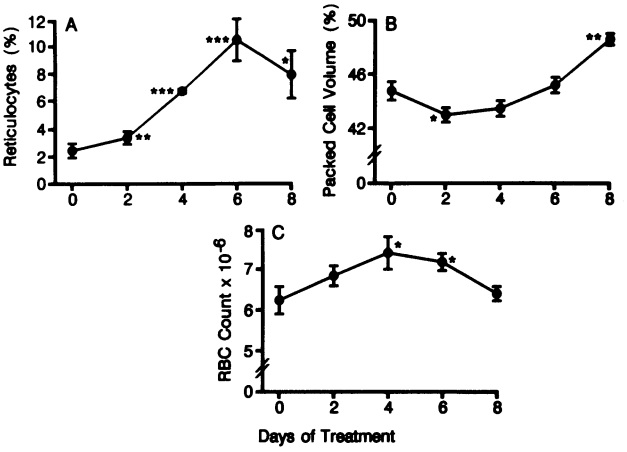

Figure 3. The effect of daily thyroxine administration on erythropoietic indices. These data are from the same mice as in Figure 2. (A) Percentage of reticulocytes; (B) Packed cell volumes; (C) RBC counts. The Day 0 value represents data from mice administered 1×10^{-3} *M* NaOH for 2 to 8 days. In all panels, each data point represents six mice and vertical bars indicate one SE. Values were significantly different from control: * $P < 0.05$, ** $P < 0.025$; *** $P < 0.005$.

Table I. Effects of L-Thyroxine on Megakaryocyte Size and Number[a]

Days of treatment	Megakaryocytes/ $mm^3 \times 10^{-3}$[b]	Megakaryocyte diameter[c] (μm)
0	1.83 ± 0.13 (12)	35.69 ± 0.99 (11)
2	1.81 ± 0.05 (7)	32.37 ± 0.99 (9)[d]
4	1.35 ± 0.05 (7)[d]	30.39 ± 1.77 (8)[d]
6	1.39 ± 0.12 (10)[d]	29.97 ± 1.41 (10)[e]
8	1.47 ± 0.09 (9)[d]	33.34 ± 0.91 (10)

[a] Values are given as mean ± SE (number of mice/treatment). L-Thyroxine (25 μg) was injected subcutaneously per mouse per day.
[b] Megakaryocyte number was corrected for errors due to section thickness, 5% tissue shrinkage, and optically lost profiles (32).
[c] Megakaryocyte size, obtained from perimeter measurements of approximately 200 section profiles for each mouse, was corrected for the 5% tissue shrinkage that occurs during histological processing (32).
[d] Values were significantly lower than for Day 0: $P < 0.05$.
[e] Values were significantly lower than for Day 0: $P < 0.005$.

0.05 and $P < 0.005$, respectively) by this treatment (Table III).

Effects of Concurrent Thyroxine and Propranolol Administration. In these experiments, 25 μg of L-thyroxine per day were administered subcutaneously in a single dose, and two doses of propranolol were administered intraperitoneally before and after the thyroxine injection (total dose of 16 mg/kg). This injection regimen was followed for 4 days (Days 0–3); control animals received 1×10^{-3} *M* NaOH and 0.9% saline on an identical injection schedule. The animals were then sacrificed and the percentage of ^{35}S incorporation into platelets was evaluated. As in previous experiments, both L- and D-isomers of thyroxine significantly (L-

Table II. Effects of L-Thyroxine on Blood Volume of Mice[a]

Days of treatment	Number of mice	Blood volume (% body wt)
0	4	5.52 ± 0.16
2	4	5.84 ± 0.40
4	4	5.85 ± 0.27
6	4	6.40 ± 0.33[b]
8	4	6.13 ± 0.35

[a] Values are given as mean ± SE. L-Thyroxine (25 μg) was injected subcutaneously per mouse per day. Blood volume was measured by the ^{59}Fe-labeled erythrocyte dilution technique (3).
[b] Blood volumes were significantly higher than values found on Day 0: $P < 0.05$.

isomer, $P < 0.0005$; D-isomer, $P < 0.05$) suppressed ^{35}S incorporation into platelets (Fig. 4), with the L-isomer producing a greater suppression than the D-isomer. Propranolol partially prevented the suppression of platelet production by L-thyroxine ($P < 0.05$) when administered concurrently with the hormone (L-Thy versus L-Thy/Prop). However, ^{35}S incorporation values did not return to control levels (Fig. 4) after concurrent thyroxine and propranolol treatments.

Discussion

This work demonstrates that *in vivo* stimulation of the erythroid cell line by thyroxine is associated with decreased thrombocytopoiesis. While indices of erythropoiesis (reticulocyte count, RBC count, and PCV) increased after thyroxine administration (Fig. 3), thrombocytopoietic activity decreased as measured by platelet count, percentage of ^{35}S incorporation into platelets (Fig. 2), and megakaryocyte number and diameter (Table I). The increased platelet size observed after thyroxine administration (Fig. 2) is presumed to be a compensatory effort to maintain platelet mass in the face of marked thrombocytopenia. This finding is in agreement with reports of increased platelet volume in human hyperthyroid patients (22, 25).

Hyperthyroidism has been reported to cause an increase in blood volume in humans (17), and this report is in agreement with our finding of increased blood volume in mice after administration of exogenous thyroxine. Reticulocyte count, which is expressed as a percentage of total red cells, is independent of blood volume. However, this increase in blood volume accounts for the early decrease in the PCV (Fig. 3), since an increase in blood volume before a large number of new RBC enter the peripheral circulation will cause a dilutional effect. This confounding factor is eliminated by considering absolute parameters of red cell and platelet production (TCRBCC, TCRBCM, TCPC, and TCPM; Table III). These data show that, independent of changes in blood volume, red cells in the peripheral circulation are increased in number and mass after several consecutive days of thyroxine administration, whereas platelet numbers and platelet masses are decreased in the same animals. A consistent feature of these data is that red blood cell appearance in the peripheral blood peaks after 6 days of administration, and then moderates (although remaining significantly elevated as compared with controls). This likely represents a plateau effect of the stimulation of red cell production by thyroxine, the mechanism for which we have not investigated.

There are a number of hypotheses that could explain the observed inverse relationship between erythropoiesis and thrombocytopoiesis. The two effects could be independent; i.e., stimulation of erythropoiesis by thyroxine occurs via the well-documented direct and indirect mechanisms already described (10–14), whereas the hormone simultaneously exerts an independent, direct suppressive effect on some cell population in the marrow, thereby producing the observed peripheral changes in thrombocytes. However, given the similar pattern of erythroid and platelet changes already reviewed in naturally occurring thyroid derangement and in hypoxia, it seems likely that the two effects occur by a related mechanism.

Two mechanisms have been described for erythroid

Table III. Effects of L-Thyroxine on Blood Platelet and RBC Indices[a]

Days of treatment	Platelet count × 10^{-6}	TCPC × 10^{-8}	TCPM × 10^{-9} (μm^3)	PCV (%)	RBC count × 10^{-6}	TCRBCC × 10^{-7}	TCRBCM × 10^{-11} (μm^3)
0	1.05 ± 0.06	13.9 ± 0.6	5.5 ± 0.2	44.8 ± 0.1	7.09 ± 0.11	9.5 ± 0.1	6.00 ± 0.08
2	0.84 ± 0.07	12.0 ± 0.6	4.5 ± 0.2	41.9 ± 0.9[b]	6.80 ± 0.22	9.8 ± 0.7	6.06 ± 0.05
4	0.73 ± 0.05[b]	10.7 ± 0.8[b]	4.1 ± 0.3[b]	42.6 ± 0.5[c]	6.68 ± 0.07[b]	9.7 ± 0.2	6.21 ± 0.10
6	0.62 ± 0.02[c]	9.6 ± 0.3[d]	4.1 ± 0.1[c]	47.3 ± 0.3[c]	7.00 ± 0.18	11.1 ± 0.4[b]	7.49 ± 0.03[c]
8	0.53 ± 0.02[d]	7.9 ± 0.4[d]	3.2 ± 0.2[d]	46.8 ± 0.4[c]	7.00 ± 0.25	10.5 ± 0.4[b]	7.10 ± 0.03[b]

[a] Values are given as mean ± SE; four mice were used in each treatment group. L-Thyroxine (25 μg) was injected subcutaneously per mouse per day. TCPC and TCPM were calculated as previously described utilizing platelet counts, blood volumes, and sizes of platelets (3). TCRBCC and TCRBCM were calculated as outlined in Materials and Methods.
[b] Values were significantly different from values for Day 0: $P < 0.05$.
[c] Values were significantly different from values for Day 0: $P < 0.005$.
[d] Values were significantly different from value for Day 0: $P < 0.0005$.

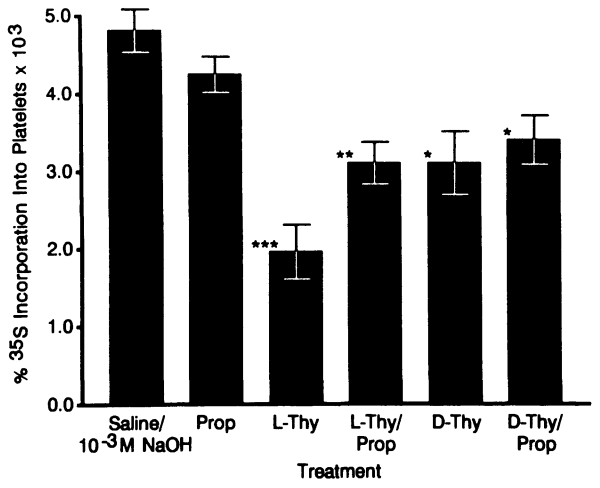

Figure 4. The effects of 14 days of concurrent thyroxine (25 μg/day) and propranolol (16 μg/day) on ^{35}S incorporation into platelets of mice. Twenty-one mice were used for the saline-control group and five mice were used for all other treatment groups. Vertical bars indicate 1 SE. Values were significantly different compared with control (saline/10^{-3} *M* NaOH-treated) mice: * $P < 0.05$; ** $P < 0.005$; *** $P < 0.0005$. Also, L-thyroxine and L-thyroxine/propranolol were significantly ($P < 0.05$) different from one another (not indicated with asterisks on graph).

stimulation by thyroxine (10–12). First, a direct stimulatory effect of thyroxine *in vitro*, which has been characterized as a β_2-adrenergic effect (10). This effect appears to be independent of calorigenic potential (11). The indirect effect of thyroxine is thought to be via the release and action of EPO (14), presumably in response to increased oxygen utilization by cells stimulated by thyroxine. In this work, both L- and D-isomers of thyroxine increased erythropoiesis and suppressed platelet production *in vivo*, although the L-isomer consistently had a greater effect than the D-isomer. This finding is consistent with the hypothesis that *in vivo*, the L-isomer exerts both direct and indirect stimulatory effects on the erythroid cell line. The latter has been described *in vitro*, whereas the D-isomer, which is incapable of increasing metabolic rate and oxygen consumption of somatic cells, exerts only a direct (β-adrenergic) effect. Therefore, the D-isomer has a weaker stimulation of RBC production, which leads to a smaller suppressive effect on thrombocytopoiesis.

Concurrent administration of propranolol would be expected to negate the direct effect of thyroxine, and allow only indirect stimulation of the erythron by thyroxine. Indeed, administration of propranolol significantly ($P < 0.05$) reversed the depression of platelet production produced by L-thyroxine administration (Fig. 4, L-Thy versus L-Thy/Prop). Propranolol did not completely prevent the reduced platelet production caused by D-thyroxine, as would be predicted by this model. This effect could be explained by a difference in binding affinities between the L- and D-isomers at the β-adrenergic site, different receptor sites for the L- and D-isomers on the red cell precursors, or (less likely) by contamination of the D-isomer with the L-isomer. Additional unelucidated mechanisms of erythron stimulation by the D-isomer are possible. For clarification, future experiments should utilize a selective β_2-blocker in addition to propranolol. The data support the hypothesis that the L-isomer of thyroxine stimulates RBC production by both direct and indirect mechanisms, and that this erythroid stimulation is associated with decreased platelet production.

Recent studies (33) have demonstrated a relationship at the molecular level between transcription factors for the erythrocytic and megakaryocytic cell populations. These data further support the idea of a close association between red cell and platelet production in the marrow. Since two experimental states that stimulate erythropoiesis (hypoxia and thyroxine administration) both result in decreased thrombocytopoiesis, and in light of this recent molecular data, a reasonable hypothesis is that there is a common pool of progenitor cells for megakaryocyte and erythroid precursor production. In this hypothesis, an acute and intense demand for production of red cells would result in a depletion of the pool of progenitor cells, leading to decreased thrombocytopoiesis.

The definitive answer to this question may lie in the documentation of erythropoietin and thrombopoietin levels in experimentally and/or naturally occurring hypoxic- and thyroid-deranged patients. At this time, sensitive *in vitro* thrombopoietin assays that require microquantities of serum are not available for use in a determination of the hormone. To conduct further studies of changes in platelet production in naturally occurring hyperthyroidism, feline patients would be a reasonable study group, since feline hyperthyroidism is most often due to adenomatous hyperplasia of the thyroid tissue (34). This is in contrast to canine and human patients, in whom neoplastic (thyroid adenocarcinoma in canines) or autoimmune (Graves' disease in humans) diseases are often the cause of increased thyroid hormone levels; these diseases can have broader systemic effects and could be confounding factors in such an analysis. Furthermore, the reason for the difference in platelet size in hyperthyroid patients should be evaluated more fully to characterize changes in thrombocytopoiesis that occur in this condition (24–26).

This work was intended to explore the relationship between erythroid stimulation by thyroxine (reported to have two distinct mechanisms *in vitro*) and thrombocytopoiesis. We conclude that thyroxine administration, like hypoxia, depresses thrombocytopoiesis while concurrently stimulating erythropoiesis. These data further support the concept (33) that the precursor cells of the erythroid and megakaryocytic lineages bear a close association; the changes described herein are consistent

with a common progenitor population that is depleted by the indirect (EPO-mediated) and direct (β_2-adrenergic) stimulation of the erythroid cell line in this *in vivo* system.

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1. Langdon JR, McDonald TP. Effects of chronic hypoxia on platelet production in mice. Exp Hematol **5**:191–198, 1977.
2. McDonald TP, Cottrell M, Clift R. Effects of hypoxia on thrombocytopoiesis and thrombopoietin production of mice. Proc Soc Exp Biol Med **160**:335–339, 1979.
3. McDonald TP, Cottrell M, Clift R. Effects of short-term hypoxia on platelet counts of mice. Blood **51**:165–175, 1978.
4. McDonald TP. A comparison of platelet production in mice made thrombocytopenic by hypoxia and by platelet specific antisera. Br J Haematol **40**:299–309, 1978.
5. Rolovic Z, Basara N, Biljanovic-Paunovic L, Stojanovic N, Suvajdzic N, Pavlovic-Kentera V. Megakaryocytopoiesis in experimentally induced chronic normobaric hypoxia. Exp Hematol **18**:190–194, 1990.
6. McDonald TP, Cullen WC, Cottrell M, Clift R. Effects of hypoxia on the small acetylcholinesterase-positive megakaryocyte precursor in bone marrow of mice. Proc Soc Exp Biol Med **183**:114–117, 1986.
7. Cullen WC, McDonald TP. Effects of isobaric hypoxia on murine medullary and splenic megakaryocytopoiesis. Exp Hematol **17**:246–251, 1989.
8. McDonald TP. Platelet production in hypoxic and RBC-transfused mice. Scand J Haematol **20**:213–220, 1978.
9. McDonald TP, Lange RD, Congdon CC, Toya RE. Effect of hypoxia, irradiation, and bone marrow transplantation on erythropoietin levels in mice. Radiat Res **42**:151–163, 1970.
10. Popovic WJ, Brown JE, Adamson JW. The influence of thyroid hormones on *in vitro* erythropoiesis. J Clin Invest **60**:907–913, 1977.
11. Golde DW, Bersch N, Chopra IJ, Cline MJ. Thyroid hormones stimulate erythropoiesis *in vitro*. Br J Haematol **37**:173–177, 1977.
12. Dainiak N, Hoffman R, Maffei IA, Forget BG. Potentiation of human erythropoiesis *in vitro* by thyroid hormone. Nature **272**:260–262, 1978.
13. Sainteny F, Larras-Regard E, Frindel E. Thyroid hormones induce hemopoietic pluripotent stem cell differentiation toward erythropoiesis through the production of pluripoietin-like factors. Exp Cell Res **187**:174–176, 1990.
14. Chopra IJ. Triiodothyronines in Health and Disease. Heidelberg, Germany: Springer-Verlag, p120, 1981.
15. Meric SM. The laboratory tests that confirm a diagnosis of feline hyperthyroidism. Vet Med **84**:964–968, 1989.
16. Peterson ME, Ferguson DC. Thyroid disease. In: Ettinger SJ, Ed. Textbook of Veterinary Internal Medicine, 3rd ed. Philadelphia: WB Saunders, p1642, 1989.
17. Wintrobe MM. Clinical Hematology. Philadelphia: Lea & Febiger, pp690–697, 1981.
18. Jackson AS. Acute hemorrhagic purpura associated with exophthalmic goiter. JAMA **96**:38–39, 1931.
19. Woodruff PW. The behavior of blood platelets in thyrotoxicosis. Med J Aust **2**:190–197, 1940.
20. Bechgaard P. Tendency to hemorrhage in thyrotoxicosis. Acta Med Scand **124**:79–91, 1946.
21. Kurata Y, Nishioeda Y, Tsubakio T, Kitani T. Thrombocytopenia in Graves' disease: Effect of T_3 on platelet kinetics. Acta Haematol **63**:185–190, 1980.
22. Panzer S, Haubenstock A, Minar E. Platelets in hyperthyroidism: Studies on platelet counts, mean platelet volume, 111-Indium labeled platelet kinetics, and platelet associated immunoglobulins G and M. J Clin Endocrinol Metab **70**:491–496, 1990.
23. Jim RT. Thyrotoxicosis associated with thrombocytopenia. Hawaii Med J **43**:156–158, 1984.
24. Bessman JD, Gilmer PR, Gardner FH. Use of mean platelet volume improves detection of platelet disorders. Blood Cells **11**:127–135, 1985.
25. Ford HC, Toomath RJ, Carter JM, Delahunt JW, Fagerstrom JN. Mean platelet volume is increased in hyperthyroidism. Am J Hematol **27**:190–193, 1988.
26. van Doormal JJ, van der Meer J, Oosten HR, Halie MR, Doorenbos H. Hypothyroidism leads to more small-sized platelets in circulation. Thromb Haemost **58**:964–965, 1987.
27. McDonald TP. A comparison of platelet size, platelet count and platelet ^{35}S incorporation as assays for thrombopoietin. Br J Haematol **34**:257–267, 1976.
28. Hoenig M, Ferguson DC. Impairment of glucose tolerance in hyperthyroid cats. J Endocrinol **212**:249–251, 1989.
29. McDonald TP. Bioassay for thrombopoietin utilizing mice in rebound thrombocytosis. Proc Soc Exp Biol Med **144**:1006–1012, 1973.
30. McDonald TP. Effect of thrombopoietin on platelet size in mice. Exp Hematol **8**:527–532, 1980.
31. Weibel ER. Stereologic Methods. I. Practical Methods for Biological Morphometry. New York: Academic Press, 1980.
32. Cullen WC, McDonald TP. Comparison of stereologic techniques for the quantification of megakaryocyte size and number. Exp Hematol **14**:782–788, 1986.
33. Romeo PH, Prandini MH, Joulin V, Mignotte V, Prenant M, Vainchenker W, Marguerie G, Uzan G. Megakaryocytic and erythrocytic lineages share specific transcription factors. Nature **344**:447–449, 1990.
34. Meric SM. Recognizing the clinical features of feline hyperthyroidism. Vet Med **84**:956–963, 1989.