Blood Flow to Bone Marrow During Development of Anemia or Polycythemia in the Rat

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We applied the radioactive microsphere method to follow the magnitude and time course (0 to 96 hours) of blood flow changes during development and recovery from anemia in awake rats. Blood flow was also monitored during a 96-hour period after polycythemia was induced (erythropoietin administered subcutaneously [SC]). The possible influence of innervation was also examined. After a blood loss of approximately 50% (hypovolemia), blood flow to the femoral marrow tripled within 12 hours and remained elevated for the entire 96-hour period. The relative increase in blood flow to the femoral bone was even greater. Similar findings were obtained in rats with phenylhydrazine (PHZ) hemolytic anemia (normovolemia). Denervation had no detectable effect on the increased blood flow to either marrow or bone. The augmented blood flow during hemolytic anemia was accompanied by a doubling of the oxygen consumption rate by the marrow, while the glucose uptake was not detectably altered. Erythropoietin supplements (3 × 1,000 IU/kg, SC, 6-hour intervals) increased blood flow to the marrow by approximately 25% after 48 hours, and at 72 hours the blood flow had reached a value twice that obtained under control conditions. These results indicate that blood flow to bone marrow is highly variable and hormonally and/or locally regulated. This may have practical consequences for marrow transplantation technology and for administration of drug therapy to patients with insufficient bone marrow hematopoiesis.

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and heat-sealed. Similarly, a PE 10 catheter for reference sampling of blood (groups A to E, see below) was placed into one brachial artery and advanced proximally so that its tip was just within the axillary region. This catheter was also used for measurement of mean arterial blood pressure. The other end of this catheter was led SC to the neck, filled with heparin, exteriorized adjacent to the infusion catheter, and heat-sealed. In this manner, atraumatic access to the catheters was ensured. The exact positions of the catheters were verified by dissection postmortem.

In each of the 32 rats in groups C and D (see below), one hind leg was denervated. The sciatic nerve on one side was exposed at the level of the sciatic notch and cut. Likewise, the ipsilateral femoral nerve was exposed just below the inguinal ligament and cut. The contralateral hind leg served as control.

All 155 rats were given ampicillin (15 mg/kg body weight, intramuscularly [IM]). Rats in groups A to F (see below) were returned to their cages to recover. Weight loss during the first 24 hours was at most 2.2% of initial body weight. Weight reduction per 24 hours was smaller during the remaining experimental period. Values for leukocytes, arterial blood gases, and arterial pH all remained within the normal range during the experimental period in all 155 rats.

**Blood Flow Measurements**

The microsphere method described by Heymann et al. was adopted to the present study as detailed by Iversen et al. Evidence for the validity and reliability of our version of this method has recently been presented. Briefly, microspheres (diameter, 16 μm; New England Nuclear, Boston, MA) labeled with either 109Ce, 51Cr, 19Ru, or 51Nb and stored in 0.9% NaCl containing 0.2% Tween 80, were ultrasonicated for 2 minutes. Shortly after and immediately before an infusion, the microspheres were vigorously mixed for 1 minute with a vortexer. The microspheres were then transferred to a 10-mL syringe and placed on the infusion pump (Harvard Instruments, Cambridge, MA). The infusion system was connected to a similar pump. Withdrawal of blood through this reference catheter in the brachial artery was stopped 2 minutes before the rats were killed. All marrow cells from one femur were sampled from one rat, and the whole body postmortem and then determined the marrow glucose uptake. The values for glucose uptake in these rats were similar to those in the six preintervention control rats, indicating that the measured values represent true uptake of glucose by the marrow cells.

**Determination of Oxygen Consumption Rate**

Oxygen consumption rate in group G rats (see below), six test rats, and six control rats was estimated from the changes in oxygen tension in an incubated marrow cell suspension as described by Opdahl et al. Immediately after each rat was killed, all marrow cells were removed from one femur as described below. All marrow cells were then suspended in Fisher’s medium (Gibco, Paisley, Scotland) containing 0.5% bovine albumin (Sigma, St Louis, MO) and adjusted to pH 7.35 with 10 mmol/L HEPES buffer (Sigma). A microliter of this suspension was enclosed in a chamber. The suspension was kept at 37°C and constantly stirred, its oxygen tension being measured continuously with a polarographic electrode (MSE, UK). In this way, one measurement could be obtained from each rat. The values for oxygen consumption rate were expressed as mmol O₂/min.

**Analyses of Blood Variables**

Hemoglobin (Hb), hematocrit (Hct), and reticulocytes were measured in duplicate with standard procedures. The values for reticulocytes were corrected according to the formula: Corrected reticulocytes (%) = actual reticulocytes (%) × (actual Hct/control Hct). When several measurements of either Hb, Hct, or reticulocytes were made from one rat, the person performing the measurements was ignorant about the order in which the samples had been collected.

Levels of erythropoietin were determined in plasma from groups A and E rats with a commercial kit (Medac, Hamburg, Germany) based on an enzyme-linked immunosorbent assay (ELISA) procedure as outlined by Goto et al.

**Experimental Protocol**

**Group A (bleeding and hypovolemic anemia).** Forty-eight hours after surgery, one batch of microspheres was infused into 30 rats (control infusion). Hypovolemic anemia was then induced by bleeding through the intracardiac catheter at a rate of 2 mL/min with a withdrawal pump (Harvard Instruments). The blood loss was made to be approximately 1.5% of the body weight and was typically between 5 and 7 mL. Microspheres were then infused into 30 rats and fixed 6, 17, and 96 hours, respectively. Into five of these latter rats, we also infused 0.19 MBq (5 μCi) ⁵⁷Fe-citrato (specific activity, 1.3 GBq/mg, 36 mCi/mg, IP; New England Nuclear, Dreieich, Germany) 5 hours before the bleeding. Five other rats (sham anemic) were bled in a similar manner as described above, but the blood was immediately reinfused. These five rats also received 0.19 MBq ⁵⁷Fe-citrato 5 hours before the reinfusion. Microspheres were
infused just before bleeding (control infusion) and then after 5 minutes, 24 hours, and 96 hours.

**Group B (hemohysis and normovolemic anemia).** Microspheres were infused into 20 rats 48 hours after surgery. The hemolyzing agent phenylephrine (Phz; 50 mg/kg body weight, IP) was then administered immediately. Microspheres were then infused into 10 of the rats 12 and 48 hours after the control infusion and into the remaining 10 rats after 36, 72, and 96 hours.

**Group C (denervation and hemohysis).** Twenty rats with one hind leg denervated were each given one batch of microspheres 96 hours after surgery. Normovolemic anemia was induced as described for group B and microspheres were administered into 10 of the rats 48 and 96 hours after the control infusion. The remaining 10 rats received microspheres after 12, 36, and 72 hours.

**Group D (denervation, bleeding and plasma transfusion; normovolemic anemia).** From each of 12 rats, we removed approximately 6 mL plasma 2 weeks before surgery. The plasma was stored at −70°C until use. These 12 rats had one hind leg denervated and were each given one batch of microspheres 96 hours after surgery. The rats were then bled as described for group A rats. Immediately following the bleeding, the blood loss was replaced with an equal amount of autologous plasma. Microspheres were then given to six of the rats 3, 6, and 12 hours after the control infusion. The other six rats received microspheres after 24 hours.

**Group E (polychrometeria).** Twenty rats were all given one infusion of microspheres 48 hours after surgery. Recombinant human erythropoietin (rHuEPO, Integrated Genetics, Boston, MA; 1,000 IU/kg body weight, SC) was then administered three times at 6-hour intervals. Ten rats received microspheres 6, 24, and 72 hours after the control infusion. The remaining 10 rats were given microspheres after 12, 48, and 96 hours.

**Group F (glucose uptake and hemohysis; normovolemic anemia).** Forty-eight hours after surgery, six control rats received one intraarterial bolus injection of 3H-deoxyglucose. In another 30 rats, normovolemic anemia was induced as described for group B. Deoxyglucose was injected either 5 minutes, or 12, 24, 48, or 96 hours after injection of Phz, with six rats in each group. The rats were killed 30 minutes after the deoxyglucose injection. The activity of 3H in plasma was then virtually zero.

**Group G (oxygen consumption rate and hemohysis; normovolemic anemia).** Forty-eight hours after the injection of ampicillin, six rats were killed, and the marrow cells within one femur from each rat were analyzed with respect to the oxygen consumption rate. Hemolytic anemia was induced as described for group B rats in six other rats 24 hours before the oxygen consumption rate was determined.

**Preparation of Samples**

Experiments were terminated by giving rats a lethal dose of pentobarbital.

One femur was removed from rats belonging to groups B, E, F, and G and both femurs from group A rats. Both tibiae were removed from rats in groups C and D. The bone was cleared of all visible soft tissue by dissection. The femurs and the tibiae were then weighed before the marrow was flushed out. Marrow cells were retrieved as completely as possible from the bones by flushing the bone and the marrow cavity showed only occasional and small fragments of marrow. Microscopic examination of the marrow content of 10 rats showed only red and no yellow marrow.

**RESULTS**

**Validation Studies**

**Group A.** Compared with the five sham-operated rats, the uptake of 59Fe into the marrow was approximately 60% higher in the anemic rats 96 hours after bleeding. No appreciable activity of 59Fe was detected in any of the flushed bones, indicating that the removal of marrow by flushing the bones had been complete. No significant differences were found in blood flow to bone between those femurs that had been flushed with collagenase and those that had not.

**Group B.** The arteries supplying denervated tibiae were only faintly stained with glyoxylic acid, in marked contrast to the brightly stained arteries from the contralateral innervated bones. Thus, catecholamine stores had been depleted, indicating that denervation had been fairly complete.
Hypovolemic Anemia

Group A. Approximately 12 hours after bleeding, both Hb and Hct were reduced by approximately 50% (Fig 1). During the remaining experimental period, Hb and Hct remained fairly constant. The number of reticulocytes was markedly elevated approximately 48 hours after bleeding (Fig 1).

Ninety-six hours after bleeding, the plasma concentration of erythropoietin had increased markedly to 140 (135 to 155) mU/mL, as compared with the control value of 7 (5.5 to 8.5) mU/mL.

Values for mean arterial blood pressure and heart rate are presented in Table 1. During bleeding, the mean arterial blood pressure decreased, from approximately 95 mm Hg to approximately 60 mm Hg. The rats then remained hypotensive throughout the observation period. A concomitant increase in heart rate from approximately 450 to 530 bpm persisted for approximately 24 hours after bleeding. The heart rate then gradually decreased.

Blood flow to the marrow had increased substantially by 6 hours after the bleeding, and it remained elevated approximately three times above the control level for the entire 96-hour period (Fig 2). A surprising increase (P < .05) in blood flow to the bone was even more pronounced in relative terms, but showed a more transient course (Fig 2).

Normovolemic Anemia

Group B. Data for Hb, Hct, and reticulocytes were similar to those presented for group A (Fig 1), but with a slower development of the anemia.

No major changes in either mean arterial blood pressure or heart rate were detected (Table 1).

The temporal changes in blood flow to the marrow and bone following Phz injection are depicted in Fig 3. In these rats, blood flow to the marrow increased more slowly, thus reflecting the slow development of anemia. However, marrow blood flow reached a higher peak value than in group A rats (50 v 35 mL/100 g x min).

Group C. The blood flow pattern for both the marrow and bone was similar in the innervated and denervated hind leg (Fig 4); consequently, denervation had no detectable effect on the initiation and maintenance of a high level of blood flow to marrow and bone. The tibial bones showed a more marked hyperemia than the femoral bones in group B rats (Fig 3 v Fig 4); however, no direct comparison has been performed of the blood flow rates to these bones.

Group D. Blood flow increased rapidly to marrow and bone in both the innervated and denervated hind leg (Fig 4). Thus, the sympathetic vasomotor activity did not play any appreciable role for the rapid induction of the hyperemic response to the acute normovolemic anemia. Neither mean arterial blood pressures nor heart rates were significantly different from the values obtained during control conditions in rats of groups C and D.

Injection of rHuEPO

Group E. Hb, Hct, and reticulocytes all increased within 72 hours after rHuEPO injections (Fig 1), indicating an
increased erythropoietic activity. A concomitant hyperemia was observed both in the marrow and in the bone (Fig 5). However, the blood flow increase was not significant until 48 hours after the first rHuEPO injection, and not marked before the 72-hour sampling. The plasma concentration of erythropoietin increased from 6.0 (4.5 to 7.5) mU/mL under control conditions to 9.5 (8.5 to 11.5) mU/mL at 48 hours, further to 55 (35 to 85) mU/mL at 72 hours, and finally to 145 (130 to 155) mU/mL at 96 hours.

Marrow Uptake of Glucose

Group F. Glucose uptake remained at control levels throughout the experimental period ($P > .05$), measurements being made just before or either 5 minutes, or 12, 24, 48, or 96 hours after Phz injection. The median uptake of glucose into the retrieved marrow cells from one femur was approximately $2.5 \times 10^{-7}$% of the injected dose (36 rats). Thus, the induced hemolytic, normovolemic anemia was not accompanied by a detectable change in marrow metabolic activity as measured by glucose uptake.

Marrow Oxygen Consumption Rate

Group G. The oxygen consumption rate of femoral cells (see group F above) doubled ($P < .05$) from 15.3 (12.4 to 18.6) to 32.4 (30.8 to 36.7) nmol O$_2$/min (six rats) during transition from the control to the anemic condition 24 hours after induction of hemolysis. The enhanced erythropoietic activity within the marrow thus appeared to be accompanied by an increase in both blood flow and oxygen consumption rate.

### DISCUSSION

#### Blood Flow to Marrow and Bone During Stimulated Erythropoiesis

We found that bone and bone marrow blood flow increased markedly in rats after induction of both a hypovolemic (bleeding) and a normovolemic (hemolytic) anemia and after polycythemia elicited by rHuEPO injections. The increase in blood flow occurred early after bleeding, being significant after 6 hours, when the Hb was still only slightly below normal. The start of the increased blood flow was intermediate in time under the hemolytic regimen, but late after the rHuEPO injections. This succession in time was reflected by the three reticulocyte curves, in that their half-maximum values were reached approximately 24 hours after the half-maxima of the respective marrow blood flow curves.

To our knowledge, this is the first report on changes in bone marrow blood flow occurring during perturbations of
MARROW BLOOD FLOW DURING ERYTHROPOIESIS

Blood flow to the tibial marrow and bone with intact and cut nerves. Results are from group C rats (hemolytic normovolemia), and group D rats (bleeding and plasma transfusion, normovolemic anemia).

steady-state hematopoiesis, with serial measurements obtained from one animal. Comparisons with previous data based on single measurements during steady-state, mostly on patients, are therefore of limited value.

Michelsen showed that blood flow to a denervated, perfused femur of a rabbit with Phz hemolytic anemia and accelerated erythropoiesis was approximately 2.5 times higher than the mean control values. Chen et al confirmed with the microsphere method that an increased marrow blood flow was present after 3 days and persisted for about 1 week after one large (25 mg/kg body weight, IP) dose of Phz to rabbits.

The mechanism behind the increased blood flow is unknown. Bone marrow contains sympathetic, noradrenergic, and vasoconstrictor nerves. In theory, the blood flow regulation is therefore possibly both hormonally/locally and neurally. However, a rapidly induced normovolemic, hemorrhagic anemia (group D, bleeding and plasma infusion) and a more slowly developing hemolytic anemia (group B) were accompanied by a rapidly and slowly occurring blood flow increase, respectively, that were not detectably different in the innervated and the denervated tibiae. This indicates that the augmented marrow and bone blood perfusion was not dependent on an intact innervation of bone and marrow in any of our experimental models. This conclusion is supported by our finding of a seemingly constant blood flow during the first 5 minutes after rapid bleeding (Fig 2), since a nervous regulatory mechanism would presumably have been apparent within this time period.

Erythropoietin might be a regulator of blood flow to bone marrow directly or indirectly. This suggestion has been made by earlier investigators for bone marrow and for spleen blood flow. When the increased marrow blood flow was similar in the bled and in the rHuEPO-injected groups of rats (A and E), 96 hours after the start of erythropoietic perturbations, we found similar and markedly greater than normal plasma concentrations of erythropoietin in the two groups. It could be that increased metabolism triggered by the enhanced erythropoiesis might lead to a larger blood flow, as is the case in, for example, skeletal muscle, putative chemical mediators here being K⁺, H⁺, phosphates, or endothelium-derived relaxing factors. Perhaps some more specific mediator, such as a cytokine or an intermediary of heme metabolism, is released in the marrow when erythropoiesis is accelerated. In any case, we observed a doubling of oxygen consumption rate in erythropoietically stimulated marrow, which indicates a higher metabolic rate. We cannot explain the lack of any detectable increase in the measured glucose uptake by the marrow, but it could mean that the erythroid cells compete with the myeloid cells for glucose as an energy substrate, so that the total glucose uptake remains approximately the same during increased

![Diagram of blood flow to the tibial marrow and bone](http://example.com/diagram)

Fig 5. Blood flow to the marrow and bone in group E rats given three rHuEPO injections (1,000 IU/kg, SC, 8-hour intervals). Symbols as in Fig 2.
erythropoiesis and decreased myelopoiesis. However, this hypothesis also rests on the assumption that anaerobic metabolism is more prevalent in myelopoietic than in erythropoietic cells.

We found concomitant changes in blood flow to marrow and to bone, the only exception being the late phase of hypovolemic anemia, when marrow blood flow stayed high, while bone blood flow had declined. The parallel blood flow increase could be due to the fact that the nutritional artery looses its muscular tunica media shortly after penetration to the marrow cavity. Branches given off thereafter to either the haversian canals of the bone or to the marrow would receive increased blood supply if the total blood flow is enhanced by dilatation of the muscular parts of the vessels.

Why the increase in blood flow to the bone after bleeding was transient is not easily explained. Chen et al did not detect any significant increase in cortical blood flow at all in anemic rabbits, and Michelsen’s results led him to postulate that the blood flow increase to the marrow was at least partly due to decreased venous resistance. New approaches therefore appear necessary to clarify this confusing picture. The microsphere method allowed accurate determination of blood flow to the bone marrow. This method does not give information as to which vessels contribute to any changes in vascular resistance. Furthermore, it does not give any information about events within the microcirculation, except for their contribution to overall vascular resistance.

The Microsphere Method

The validity and reliability of the microsphere method for estimation of cardiac output and organ blood flows in conscious rats have been firmly established. However, there are conflicting data concerning hemodynamic stability after infusion of high numbers of microspheres. While Tsuchiya et al reported a significant decrease in cardiac output and an increase in heart rate when the number of infused microspheres exceeded $1 \times 10^6$, Stanek et al observed a significant decrease in heart rate only when as many as $1.4 \times 10^6$ microspheres had accumulated within the rat. In the present study, we infused at most a total dose of $1.2 \times 10^6$ microspheres into a single rat (divided over several infusions). No changes in either arterial blood gases, heart rate, or mean arterial blood pressure were observed following any infusion. We therefore conclude that the infusion of microspheres did not interfere with the circulation in these rats.

Possible Clinical Implications

In bone marrow transplantation, for marrow failure, and in the future also probably as a remedy for inborn errors of metabolism, as many as possible of the intravenously introduced stem cells should “home to fertile soil” in the marrow. Here, pretreatment of the patient to secure a high blood flow to the bone marrow during the transplantation may be worthwhile. On the other hand, during chemotherapy of neoplasms without suspected marrow deposits, a sparse blood flow would be desirable. Some recombinant cytokines (colony-stimulating factors) have had unexpected granulocyte-mobilizing effects, which might depend on blood flow changes in the marrow, since earlier experiments suggested a relationship between the rate of bone marrow perfusion and the rate of cell release. To clarify these and other issues, the present work should be continued; for example, the possible effects of recombinant cytokines on bone marrow blood flow should be investigated.

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