

The effects of erythrocythemia on blood viscosity, maximal systemic oxygen transport capacity and maximal rates of oxygen consumption in an amphibian

S.S. Hillman, P.C. Withers, M.S. Hedrick, and P.B. Kimmel

Department of Biology, Portland State University, P.O. Box 751, Portland, Oregon 97207, USA

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Summary. 1. Graded erythrocythemia was induced by isovolemic loading of packed red blood cells in the toad, *Bufo marinus*. Blood viscosity, hematocrit, hemoglobin concentration, maximal aortic blood flow rate and maximal rates of oxygen consumption were determined after each load.

2. Blood viscosity was related to hematocrit in the expected exponential manner; $\ln \eta = 0.43 + 0.035 \text{ Hct}$ (Fig. 2).

3. Maximal blood flow rates in the dorsal aorta were inversely proportional to blood viscosity and fit predictions of the Poiseuille-Hagen flow formula (Fig. 3). The effect of increased blood viscosity was to reduce aortic pulse volume, but not maximal heart rate (Figs. 4, 5).

4. Maximal systemic oxygen transport capacity (aortic blood flow rate \times hemoglobin concentration \times O₂ binding capacity of hemoglobin) was linearly correlated with the maximal rate of oxygen consumption (Fig. 6).

5. These data indicate that optimal hematocrit theory is applicable for maximal blood flow rates in vivo, and that systemic oxygen transport is the primary limitation to aerial \dot{V}_{O_2} max in amphibians.

transport (Hillman 1976, 1980, 1982) and neither alveolar ventilation (Withers and Hillman 1983) nor pulmonary diffusion (Hillman and Withers 1979) that limits \dot{V}_{O_2} max. Maximal systemic O₂ transport capacity (ml O₂/min) is the product of blood oxygen capacity (ml O₂/ml blood) and blood flow rate (ml blood/min). Direct measurements of maximal systemic O₂ transport capacity have never been made in amphibians, though such data would provide a conclusive test of the hypothesis that aerial \dot{V}_{O_2} max in amphibians is limited by systemic O₂ transport. If variation in \dot{V}_{O_2} max was linearly related to maximal systemic O₂ transport capacity, it would indicate that \dot{V}_{O_2} max is limited by systemic O₂ transport.

Varying hematocrit is a potential means of experimentally manipulating systemic O₂ transport capacity. Systemic O₂ transport is influenced in two ways by variation in hematocrit, as formulated by optimal hematocrit theory (Richardson and Guyton 1959; Murray et al. 1962; Crowell and Smith 1967). Erythrocythemia (increased hematocrit) increases blood oxygen capacity by increasing the potential amount of hemoglobin-bound oxygen, but increases blood viscosity and thus potentially decreases blood flow rate. Anemia, on the other hand, decreases blood oxygen capacity but potentially increases blood flow rate since blood viscosity is lowered. An intermediate hematocrit would maximize systemic O₂ transport in amphibians; this theoretical optimal hematocrit generally shows a close correspondence to the in vivo hematocrit (Weathers 1976a). These conclusions concerning optimal hematocrit are drawn from in vitro results and may not relate to the in vivo conditions where vasomotor tone and blood pressure also influence blood flow rates and optimal hematocrit.

The specific aims of this study were to measure (1) the in vitro effects of erythrocythemia on blood

Introduction

The rate-limiting process to maximal rates of aerial oxygen consumption (\dot{V}_{O_2} max) in anuran amphibians is not completely understood. Oxygen consumption is a series of fluxes representing convective processes (alveolar ventilation, systemic O₂ transport) and diffusive processes (pulmonary and tissue diffusion). Any of these transport steps could be limiting to total O₂ flux or all could be equally limiting. We have argued that it is systemic O₂

viscosity, (2) the in vivo effects of erythrocythemia on maximal blood flow rates, and (3) the relationship between maximal systemic O₂ transport capacity and \dot{V}_{O_2} max. These data will allow us to test whether optimal hematocrit theory is applicable to in vivo conditions during maximal cardiac output and establish whether \dot{V}_{O_2} max is normally limited by systemic O₂ transport. Maximal blood flow rates should vary inversely with blood viscosity if optimal hematocrit theory is applicable to in vivo conditions and \dot{V}_{O_2} max should be linearly related to maximal systemic O₂ transport capacity if the cardiovascular convective process limits aerial aerobic capacity in amphibians.

Materials and methods

Animals. *Bufo marinus* (150–250 g, \bar{x} = 208 g) were purchased from commercial suppliers. They were maintained at 20 °C with access to water at all times. No attempt was made to control photoperiod.

Blood flow rates. Toads were anesthetized by immersion in a solution of tricaine methane sulfonate (MS-222). An occluding cannula (PE 90) was inserted posteriorly in the ventral abdominal vein. A 2 or 2.5 mm magnetic blood flow probe (in Vivo Metrics) was then implanted around the dorsal aorta posterior to the union of systemic arches and anterior to the renal arteries. The probe was securely anchored to the dorsal body wall and the midlateral body wall musculature and skin incisions (1.5 cm) separately sutured closed. The toads were allowed 1.5 to 2.5 days to recover from the surgery and for the blood flow probe signal to stabilize. The dc-output of the probe (1–40 μ V) was amplified 1000 \times with a high-stability low-level dc preamplifier and recorded with a Narco-Bio Systems physiograph. Zero flow was always assumed to occur at the end of aortic diastolic period. This was validated in preliminary experiments utilizing total occlusion of flow in open chest preparations, and is consistent with the results of Shelton (1970), Langille and Jones (1977) and Weathers (1976b). Probes were calibrated by infusing saline at known flow rates through isolated vessels while flow probe output was recorded. The effect of hematocrit on probe signal output was determined by pulsing known volumes of plasma and blood (hematocrits of 20, 35, 44, and 55) through an isolated vessel. There was no significant difference between the probe output with saline, plasma or varying hematocrit. Mean blood flow rates were calculated by integration of recorded traces. A representative trace is presented in Fig. 1. All recordings were made subsequent to a 3 min bout of activity, a procedure previously described to elicit \dot{V}_{O_2} max in amphibians (Seymour 1973; Hillman et al. 1979; Miller and Hutchison 1980). Maximal systemic oxygen transport capacity was calculated as aortic blood flow rate \times blood oxygen capacity. Blood oxygen capacity is the product of hemoglobin concentration (vide infra) and the O₂ carrying capacity of amphibian blood, 1.3 ml O₂/g hemoglobin (Gahlenbeck and Bartels 1968; Hillman 1976).

Erythrocyte loading. Heparinized blood was collected from pithed donor toads by cardiac puncture of the exposed ventricle. The plasma was removed after centrifugation and packed cells separated for loading. Measured volumes (1–2 ml) of



Fig. 1. A representative trace of blood flow recorded from the dorsal aorta of *B. marinus*

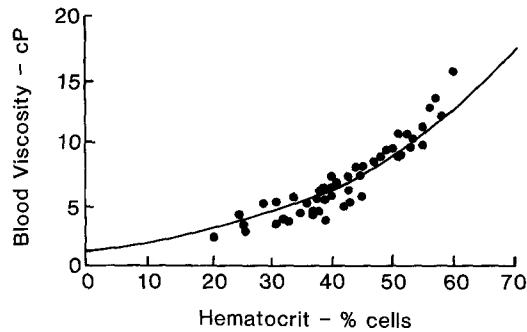


Fig. 2. The relationship between blood viscosity and hematocrit during the erythrocyte loading for 6 *B. marinus*

blood were removed from the experimental animal via the ventral abdominal vein cannula, and subsequently used for hematocrit, hemoglobin and viscosity determinations. An equal volume of packed red blood cells was then infused via the same cannula. Consequently blood volume remained the same. The cannula was then flushed with a small volume of heparinized saline. Equilibration times of 30–50 min, subsequent to packed cell loading, were allowed before metabolic rate and blood flow rate determinations. This procedure was repeated 6–8 times, so that hematocrit increased in increments of 1–5% from an average of 31% to an average of 57%.

\dot{V}_{O_2} max. The method for determining \dot{V}_{O_2} max is that described by Seymour (1973) and Hillman (1976). The procedure consists of placing the animal in a sealed metabolic chamber at 20 °C and then manually rotating the chamber to keep the animal constantly righting itself after being flipped on its back. After an activity bout of 3 min, an air sample is removed from the chamber, H₂O and CO₂ removed and the oxygen content determined with a Beckman OM-14 oxygen analyzer. From previous studies the measured \dot{V}_{O_2} max has been found to vary less than 10% in 5 separate determinations on the same individual from this procedure. Subsequent to the determination of \dot{V}_{O_2} max (within 30 sec), the animal was removed from the metabolic chamber and the blood flow probe attached to the recorder. The animal was then exercised for one min, and then blood flow rate was recorded for 30 s.

Blood viscosity and hematology. The blood sample removed from toads prior to each red cell loading was subdivided into aliquots for hematocrit, hemoglobin and viscosity determinations. Hematocrits were determined following 5 min of centrifugation at 13,000 g. Hemoglobin concentration was determined as methemoglobin using Drabkin's Reagent, with bovine hemoglobin (Sigma) as a standard. Viscosity of a single 0.2 ml sample was determined at a shear rate of 450 s⁻¹ with a Wells-Brookfield cone plate viscometer (Model LVT DCP, CP-50 cone) after the method of Wells et al. (1961). The standard error of this procedure for five analyses of the same blood sample was

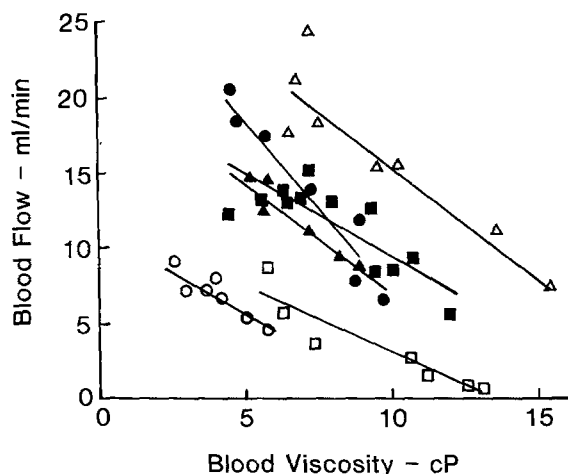


Fig. 3. The relationship between maximal blood flow rate in the dorsal aorta and blood viscosity. The different symbols represent individual animals, the lines the least squares linear regression for an individual animal

0.1 centipoise. There is good agreement between viscosity measured in vitro, our protocol, and viscosity measured in vivo at equivalent hematocrits under most flow conditions (Lipowsky et al. 1980).

Statistics. Values are presented as mean \pm standard error, with the number of observations in parentheses. Standard least squares linear regression analyses were used. Curvilinear regression analyses were accomplished using linear regression techniques with the y-values transformed to their natural logarithm i.e. $\ln y = \ln a + bx$ or $y = ae^{bx}$. Consequently, statistics for curvilinear relationships are for the semilogarithmic transformed data. Analysis of covariance was used to test the significance of slope and intercept differences for two linear regressions.

Results

The relationship between blood viscosity and hematocrit in erythrocyte loaded toads is curvilinear (Fig. 2). The relationship can be described as: $\ln \eta = 0.43 + 0.035 \text{ Hct}$ ($r^2 = 0.93$; $n = 51$; $P < 0.005$). This relationship was not significantly different from the viscosity-hematocrit relationship where erythrocytes and plasma from individual toads were mixed in vitro in varying proportions ($\ln \eta = 0.45 + 0.033 \text{ Hct}$; $r^2 = 0.90$, $n = 21$).

Maximal blood flow rates of the six toads at the initiation of the experiments averaged 62 ± 3 ml/kg min ($n = 6$). Maximal blood flow rates varied inversely with viscosity ($P < 0.05$; Fig. 3). Since the maximal blood flow rate varied interindividually, flow rates were normalized to blood flow rates at the mean viscosity for each toad for comparison of how well maximal blood flow rates correlated to predictions of the Poiseuille-Hagen flow formula. Normalized maximal blood flow rates were significantly related to the inverse of viscosi-

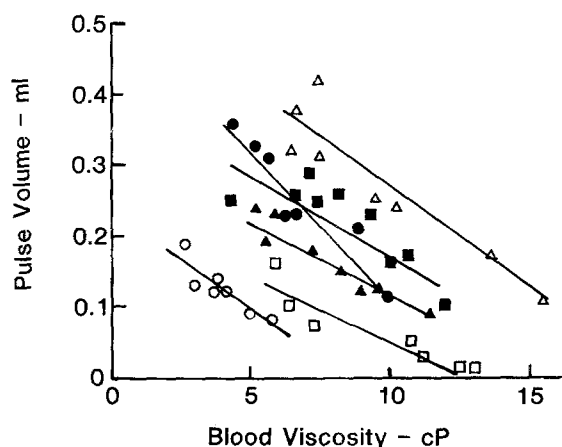


Fig. 4. The relationship between aortic pulse volume and blood viscosity. The different symbols represent individual animals, the lines are the least squares linear regression for an individual animal

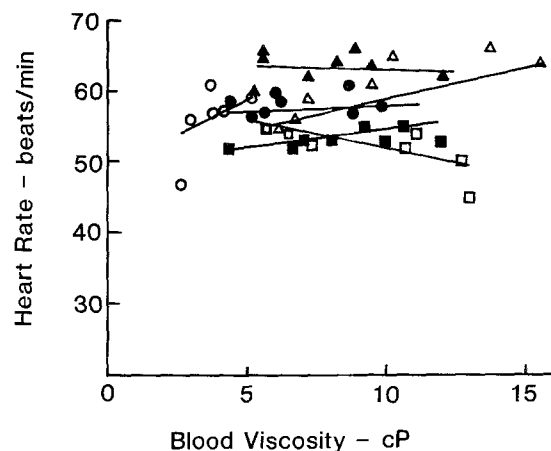


Fig. 5. The relationship between maximal heart rate and blood viscosity. Different symbols represent individual animals, the lines are least squares linear regressions for each individual

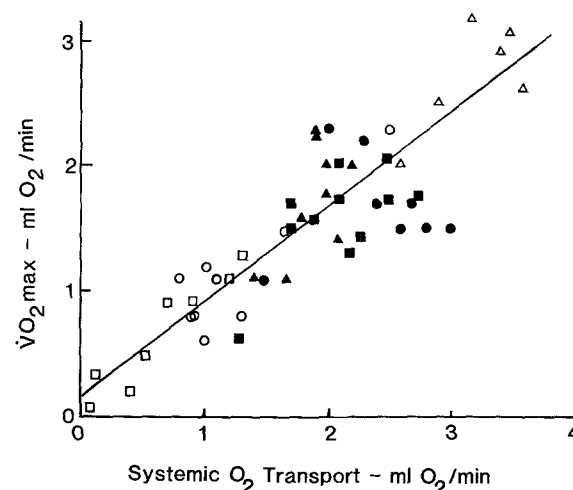


Fig. 6. The relationship between \dot{V}_{O_2} max and systemic oxygen transport capacity. Different symbols represent individual animals, the line is the least squares linear regression for all data

ty; fraction of normalized aximal blood flow = $(0.95/\eta) - 0.19$ ($r^2 = 0.80$, $P < 0.005$, $n = 48$). The slope of the relationship (0.95) was not significantly different ($P > 0.05$, $t = 0.77$) from the slope of 1 predicted from the Poiseuille-Hagen relationship. The relationship between blood flow and viscosity reflects an inverse relationship of aortic pulse volume and viscosity (Fig. 4); heart rate was independent of blood viscosity (Fig. 5).

\dot{V}_{O_2} max was directly proportional to maximal systemic oxygen transport capacity, measured in the dorsal aorta (Fig. 6); \dot{V}_{O_2} max = $0.75 \text{ SOT} + 0.18$ ($r^2 = 0.71$; $n = 51$; $P < 0.005$).

Discussion

Maximal aortic blood flow rates decreased inversely with blood viscosity as hematocrit was increased via erythrocyte loading. There are at least three obvious potential mechanisms for explaining these results. The first is that the animals could be undergoing a transfusion reaction resulting from the infusion of erythrocytes from donor toads. This transfusion could lead to anaphylactic shock. A second mechanism could be a preferential and proportional shunting of blood to the pulmonary circuit in response to viscosity changes. The third mechanism, that we consider the most likely, is that the animals had no further compensatory capacity for increasing systolic pressure or decreasing peripheral resistance and so aortic blood flow decreases in proportion to the increase in blood viscosity.

Transfusion reactions are characterized by two stages. The first stage is agglutination of foreign cells, the second stage is hemolysis of those agglutinated cells. We do not feel either occurred for the following reasons. Agglutination would result in higher blood viscosities compared to nonagglutinated blood at equivalent hematocrits. We found erythrocyte loaded individuals had equivalent blood viscosities to in vitro hematocrit-viscosity determinations where blood was not transfused or mixed interindividually. We also detected no significant hemolysis during our experiments, though in pilot studies, there was obvious and rapid (30 min) hemolysis when human erythrocytes or erythrocytes from another genus of amphibian (*Rana*) were used to load. Finally anaphylactic shock is a precipitous, non proportional depression of cardiac output. This is very different from the graded depression we observed.

A potential explanation for the graded decline in aortic flow is preferential shunting of blood to the pulmonary circuit that is proportional to

blood viscosity, since we are measuring only a fraction of cardiac output. We consider this a possible, though tenuous, interpretation of these results. Not only would the significance of such a shunt be obscure but its operation would require a unique physiologic control system to maintain a proportionality of pulmonary flow and blood viscosity.

We find the most straight-forward interpretation of the decreased blood flow rate with erythrocyte loading to be a direct effect of viscosity. The slope of the relationship between blood flow and the inverse of viscosity was predicted by the Poiseuille-Hagen flow formula. These data represent the first direct in vivo validation of viscosity effects on maximal systemic blood flow rates in vertebrates and strongly argue for an in vivo applicability of optimal hematocrit theory. The data also suggest that there is no possible compensation for the increased blood viscosity either by increased systolic blood pressure or decreased peripheral resistance, since the animals are unable to maintain systemic blood flow with the added viscosity load. This is corroborative evidence that our exercise regime is eliciting maximal systemic blood flow rates.

The adaptive significance of minimizing blood viscosity in ectotherms has consistently been interpreted as conserving cardiac energy expenditure (Snyder 1971; Weathers 1976a). The potential significance of optimal hematocrit theory to ectotherms has been clouded by two concerns. The first is the rather broad range of "optimal hematocrits" that essentially provide the same O₂ transport capacity. Second, there is the obvious in vivo potential for changes in vasomotor tone or blood pressure to compensate for elevated hematocrits in resting animals (Pavek and Carey 1974). This study points to the potential importance and application of optimal hematocrit theory at the extreme limits of cardiovascular performance i.e. during maximal activity or \dot{V}_{O_2} max. The data support the concept that blood doping beyond optimal hematocrit will limit rather than enhance aerobic capacity.

The effect of increasing blood viscosity was to decrease aortic pulse volume but not cardiac frequency. The mechanism responsible for the decrease in aortic pulse volume and lack of heart rate effect is not clear from these studies. The decreased aortic pulse volume could result from either diminished ventricular filling rate (i.e. limited venous return or preload) or ventricular emptying rate (i.e. limited flow into the systemic circulation or afterload).

The linear relationship between \dot{V}_{O_2} max and maximal systemic oxygen transport capacity rein-

forces the growing body of evidence that aerial \dot{V}_{O_2} max in amphibians is ultimately limited by the cardiovascular transport of oxygen (Hillman 1976, 1980, 1982). Previous studies on pulmonary convective limits to \dot{V}_{O_2} max (Withers and Hillman 1983) and pulmonary diffusive limits (Hillman and Withers 1979; Withers and Hillman 1983) have indicated that neither of these processes are normally limiting factors to \dot{V}_{O_2} max for amphibians exercising in air.

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