Critical Focus

The Cardiovascular System of Antarctic Icefish Appears to Have Been Designed to Utilize Hemoglobinless Blood

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Abstract

Viscosity is inversely related to temperature. The circulatory system of Antarctic icefish may have been designed to prevent high blood viscosity at low temperatures by taking advantage of the increased solubility of oxygen at low temperatures, allowing use of hemoglobin-free blood. This necessitates a high-output, high-velocity, low-pressure, low-resistance circulation. High-velocity flow requires adequate viscosity to minimize loss of laminar flow and increased friction. This creates an interesting design problem: in other animals, hemoglobin determines blood viscosity via the hematocrit, whereas in icefish, blood viscosity is produced largely by antifreeze glycoproteins. The effect of inappropriate blood viscosity on maximal cardiac output is seen in experiments with a related fish, *Pagothenia borchgrevinki*. In this species, acclimation to a particular temperature involves tailoring blood viscosity to cardiac power, which varies with the availability of oxygen and temperature. The factorial scope for cardiac output—i.e., the ratio of maximal to basal cardiac output—is greater in acclimated than unacclimated fish despite the similar availability of oxygen. Experiments also suggest that blood viscosity determines the maximum tolerable temperature in Antarctic fish. Those experiments demonstrate that blood viscosity is actively controlled. It is part of what the physiologist Claude Bernard called the *milieu intérieur*. The hemoglobinless phenotype requires simultaneous customization of the heart, vasculature, and blood, including its viscosity. Simultaneous, coordinated acquisition of multiple unique features, as required by the absence of hemoglobin, is inconsistent with Darwinian evolution, which postulates that species develop by small, incremental changes over time.

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INTRODUCTION

Viscosity affects the work of pumping any fluid. By increasing resistance, an increase in blood viscosity causes a threefold decrease in blood flow [1]. Because the hematocrit (volume percent of blood composed of erythrocytes, i.e., red blood cells) is the strongest determinant of blood viscosity, one might imagine that Antarctic icefish, which lack erythrocytes, would be an opportunity to test the advantages of extremely low-viscosity blood. In fact, the viscosity of icefish blood at its native temperature, approximately 0°C, is very similar to that of human blood at 37°C. This is notable because viscosity is inversely related to temperature and suggests there must be a "floor" to the viscosity of physiologically useful blood.

This paper will explain why there is an optimal blood viscosity which is specific to every organism. I will argue that blood viscosity is one of the fundamental quantities that is maintained in what the eminent physiologist Claude Bernard called the "*milieu intérieur*." Finally, I will argue that the cardiovascular system of Antarctic icefish could not have evolved via a series of gradual steps. Instead, Antarctic icefish are evidence of intelligent design because, as previously observed, natural selection can only explain the survival of the fittest, not the arrival of the fittest [2].

HEMATOLOGY

The hematocrit, which is 0% in icefish and normally around 40% in humans, is very strongly correlated with the concentration of hemoglobin. Because blood viscosity is not often measured, the hemoglobin concentration and hematocrit are used in this paper to infer the viscosity of normal hemoglobin-containing blood. The erythrocyte count correlates less well with blood viscosity because of variation in the erythrocyte size, especially between species.

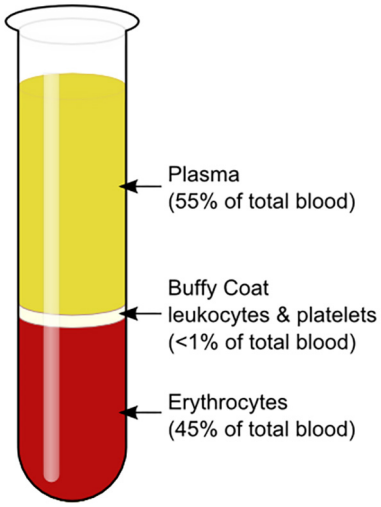

Figure 1: The hematocrit as determined in a tube after blood has been centrifuged. The white blood cells form a layer which can vanishingly thin in humans. Courtesy of Knute Knudsen at English Wikipedia (Creative Commons | Attribution 3.0 Unported). **doi:**10.5048/BIO-C.2022.2.f1

The hematocrit is reported in many papers because it is easily determined. A glass capillary tube is filled with blood, sealed at one end, and then centrifuged. Centrifugal force compacts the erythrocytes, explaining why the hematocrit is also called the "packed cell volume." The hematocrit is the percentage of the height of the column of erythrocytes compared to the height of the entire column of blood (Figure 1). On the other hand, the "leukocrit," or percentage of blood composed of leukocytes (white blood cells), is <1% in normal humans. Because icefish lack erythrocytes, what is often called the "hematocrit" in these fish is more accurately termed a leukocrit. The hemoglobin concentration is typically determined spectrophotometrically using an automated analyzer after erythrocytes are lysed because hemoglobin is exclusively carried within erythrocytes. The hematocrit is then calculated. Both the hematocrit and hemoglobin concentration convey identical information, i.e., the contribution of erythrocytes to the viscosity of whole blood.

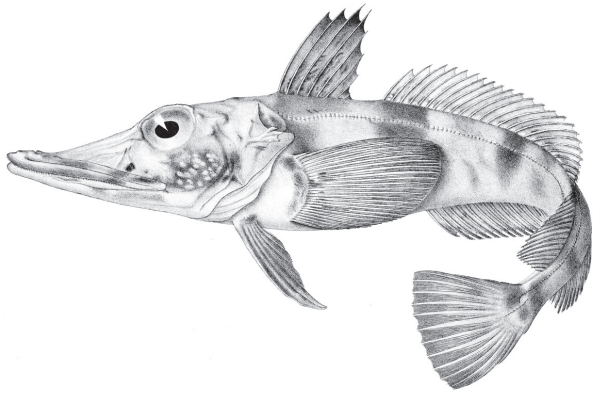

Figure 2: *Chaenocephalus aceratus*, a commonly studied channichthyid. Public domain. **doi:**10.5048/BIO-C.2022.2.f2

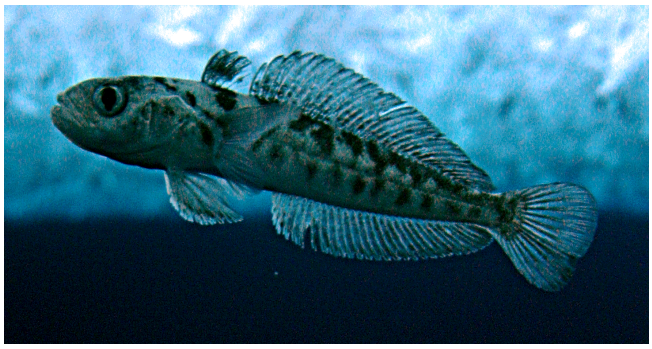

Figure 3: *Pagothenia borchgrevinki*, a red-blooded notothenioid. Courtesy of Endless Ocean Wiki, CC BY-SA. **doi:**10.5048/BIO-C.2022.2.f3

ANTARCTIC ICEFISH

The hemoglobinless icefish are members of the family Channichthyidae, which contains 11 genera and 25 species. They are restricted to the Southern Ocean surrounding Antarctica. Channichthyids generally have fusiform pike-like bodies with large heads and depressed, elongated snouts and measure up to 75 cm in length (Figure 2). Most combine pelagic (swimming in the open ocean) and benthic (living on the ocean bottom) lifestyles and migrate vertically to feed on pelagic prey, especially fish and krill [3].

Notothenioids comprise approximately 91% of fish biomass overlying the Antarctic continental shelf [3]. A second family of notothenioids, the Nototheniidae, is the dominant fish taxon of the Southern Ocean. Notothenioids express hemoglobin and are found in pelagic, benthic, and demersal (near the bottom) zones. They are the most morphologically and ecologically diverse notothenioid [3]. Although they are also sometimes referred to as "icefish," that term will be reserved for channichthyids in this paper. Because of the difficulty in maintaining channichthyids in captivity [4], many experiments involving Antarctic fish have used notothenioids such as *Pagothenia borchgrevinki* (Figure 3). It is an active cryopelagic (inhabiting the water underneath ice) fish which commonly measures 28 cm in length.

BLOOD VISCOSITY

The blood viscosity of the icefish *Chaenocephalus aceratus* is 3.27 centipoise (cP) at 0°C [5]. This is very similar to the viscosity of human blood, 3.26 ± 0.43 cP at 37°C at a high shear rate (flow velocity) [6]. The viscosity of human blood increases to 16.7 cP at 0°C [5], which is incompatible with survival. The deaths of elite cyclists who doped with synthetic erythropoietin demonstrate the harm that elevated blood viscosity can cause [7, 8]. Because the hematocrit is the strongest determinant of blood viscosity, the hemoglobinless phenotype is one solution to the problem posed by increased blood viscosity at low temperatures.

A CRITICAL REVIEW OF ORTHODOXY

The idea that the hemoglobinless phenotype is advantageous at 0°C has been rejected by many because of the high energy cost of the icefish circulation. An estimated 22% of their basic metabolic rate is devoted to cardiac work, compared to 0.5% to 5.0% in temperate-zone fish. The orthodox view is that the hemoglobinless phenotype results from a deleterious mutation and icefish survive only because of relaxed competition and increased activity of nitric oxide (NO) [9].

The idea that icefish face relaxed competition is debatable. This assertion is based on fossil evidence that a taxonomically diverse fauna in the Southern Ocean was replaced by a taxonomically restricted one during the Eocene epoch [3]. This created unoccupied niches which notothenioids successfully filled [9]. Commenting on this, the eminent ichthyologist Joseph T. Eastman wrote, "Although the Antarctic fauna is usually described as holding lower species diversity than the Arctic fauna, the ecological and morphological diversity in the family Nototheniidae far exceeds that of the entire high Arctic fauna" [10]. It seems, then, that notothenioids compete with other notothenioids in the Southern Ocean. There is no reason why competition between notothenioids should be less fierce than competition between members of different taxa. It does not necessarily follow that loss of taxonomic diversity suspends survival of the fittest. Indeed, the reverse is equally likely to be true: loss of taxonomic diversity may have occurred because only the fittest survived. The Southern Ocean is populated only by species which tolerate 0°C, resulting in a unique ecosystem with very short food chains in nutrient-rich water [11].

The assertion that the hemoglobinless phenotype can be rescued by increased NO activity is attractive because hemoglobin scavenges the molecule, limiting its activity. According to this hypothesis, increased concentrations of NO cause cardiac hypertrophy and increase tissue vascularization, providing adequate oxygenation [9, 12]. However, the hypothesis that otherwise supraphysiologic concentrations of NO are present in channichthyids and can rescue the hemoglobinless phenotype is speculative and not well supported by evidence.

The icefish *Chionodraco hamatus* and European eel *Anguilla anguilla* have similar concentrations of NO metabolites in plasma, hemosylate, and effluent from the isolated, perfused heart [13]. Another study found the blood concentrations of NO metabolites overlapped in channichthyids and red-blooded notothenioids. That study also reported no difference in the quantity of retinal mRNA for vascular endothelial growth factor and hypoxia-inducible factor-1 in species with and without hemoglobin. This suggests that hemoglobinless blood can deliver sufficient oxygen to the retina, which is amongst the most metabolically active tissue. The authors concluded that there is no evidence that adult icefish have tissue hypoxia but speculated that elevated levels of NO might still be important earlier in development [14].

Evidence from *Homo sapiens*, the most studied vertebrate, suggests that compensation for *de novo* loss of functional hemoglobin fails during development. Severe loss of functional hemoglobin occurs in humans in a genetic condition called hemoglobin Bart's hydrops fetalis. In this condition, 80% to 95% of hemoglobin is hemoglobin Bart's, a tetramer of gamma hemoglobin molecules that binds oxygen (O_2) too tightly to release it to tissue. This challenge to homeostasis is fatal in utero without drastic medical support [15].

Like adult icefish (see below), fetuses with hemoglobin Bart's hydrops fetalis have cardiomegaly, increased blood volume, and increased cardiac output ($\dot{Q}$) [16]. These compensations are adequate until late in gestation when anaerobic metabolism no longer produces sufficient energy. This reduces primary active transport of ions across all cell membranes resulting in hydropic change (swelling) of every cell in the fetus. Survival of fetuses with Hb Bart's requires intrauterine transfusion and either lifetime transfusion support or bone marrow transplantation. Many survivors have birth defects.

Another lesson from *H. sapiens* is that compensatory responses require time to develop [17]. A challenge to homeostasis must be sufficiently mild that an organism survives long enough to develop compensation. A human fetus can tolerate hypoxia because the lactic acid generated by anaerobic metabolism is eliminated by the maternal circulation via the placenta, maintaining fetal acid-base balance. This allows time for the fetus to develop cardiomegaly and hypervolemia.

Reactive cardiomegaly due to a mutation necessitates an increase in an organism's basal metabolic rate because of the requirements of the additional muscle mass. Cardiomegaly increases the organism's oxygen requirement and as a result, it tires more easily. These changes make reactive cardiomegaly a pathologic condition, distinguishing it from a large heart which is normal for an organism, as in channichthyids. For these reasons, cardiomegaly, like many other compensations, does not restore an organism to its original level of fitness, but simply allows it to survive. Physiologic reserve is decreased after many compensations [17], making the organism less able to tolerate additional challenges to homeostasis such as disease, starvation, temperature change, etc. Without established robust health, it is debatable whether a larval fish can survive a severe acute challenge to homeostasis.

INTELLIGENT DESIGN

The Southern Ocean is not a metaphorical "Island of Misfit Toys" where defective fish find refuge. Nor is a simple fix for the loss of hemoglobin likely. Therefore, the icefish cardiovascular system could have been designed to solve the problem of increased blood viscosity at cold temperatures. Contrary to orthodoxy, the success of channichthyids suggests that the high energy cost of the hemoglobinless cardiovascular system is "the cost of doing business" in Antarctic waters. This cost is more likely to be met by a customized cardiovascular system than a conventional one that was pressed into service when a mutation caused the loss of hemoglobin expression. Indeed, the icefish heart has been described as "a very specialized high volume pump, able to displace relatively high volumes of blood at high flow rates and low pressures [18]."

The channichthyid cardiovascular system, including hemoglobinless blood and antifreeze glycoprotein (AFGP) is here

reexamined in light of the principles of intelligent design. Anatomy and physiology are viewed as the means by which biological engineering problems are solved. Words such as "tailor" and "customize" will be used to convey that intelligence was involved in the origin of the channichthyids. Given the statistical unlikelihood that the interdependent changes required for a hemoglobinless cardiovascular system arose simultaneously by multiple random mutations, the author believes that such expressions are the most accurate terminology.

CARDIOVASCULAR CUSTOMIZATIONS

The hemoglobinless phenotype is possible because the solubility of O_2 increases as temperature decreases. Nevertheless, the O_2 concentration in channichthyid blood is 10% or less of that in red-blooded notothenioids. Thus, channichthyids possess several features which increase O_2 delivery. $\dot{Q}$ is much greater in channichthyids than in red-blooded notothenioids. The $\dot{Q}$ figures for the icefish *Pseudochaenichthys georgianus* and *C. aceratus* are 80.5 $ml \cdot kg^{-1} \cdot min^{-1}$ and 77.0 $ml \cdot kg^{-1} \cdot min^{-1}$, respectively. In contrast, $\dot{Q}$ in the red-blooded notothenioids *Trematomus bernacchii* and *P. borchgrevinki* is 17.6 $ml \cdot kg^{-1} \cdot min^{-1}$ and 29.6 $ml \cdot kg^{-1} \cdot min^{-1}$ [9]. The large $\dot{Q}$ in channichthyids is accomplished with a low heart rate, less than 20 beats per minute in *C. aceratus* [18].

The icefish vasculature is tailored for a high-output, low-resistance circulation. Their trunk skeletal muscle capillaries are two to three times greater in diameter than those of typical teleosts, reducing vascular resistance [19]. Icefish retinas are more densely vascularized than those of red-blooded notothenioids, increasing O_2 delivery to this metabolically active tissue. In the red-blooded notothenioids, hematocrit correlated inversely with retinal vascular density (r^2 = 0.934) and directly with intervascular distance (r^2 = 0.898) over a >2.3-fold range of hematocrits [20].

The channichthyids' large $\dot{Q}$ and customized vasculature require a blood volume two to four times greater than that of red-blooded fish [21]. This in turn requires a customized heart that is heavier and has a larger stroke volume than that of red-blooded notothenioids. In the latter, the ratio of heart weight to body weight is similar to the ratio in typical teleosts. The ratio in *C. aceratus*, in contrast, is similar to that in mammals and tuna, one of the few fish able to maintain a body temperature above ambient water. The stroke volume of the icefish heart is 6 to 15 times greater than in other teleosts [21].

Cardiomyocytes in the icefish heart are relatively large and contain a relatively large number of mitochondria. Forty-four percent of the cardiomyocyte volume is devoted to mitochondria in the icefish *C. hamatus*, the highest in any teleost and higher than in any vertebrate except for the Etruscan shrew, *Suncus etruscus*, in which 45% of myocyte volume contains mitochondria [22]. The volume in red-blooded notothenioids ranges from 20 to 25%. For the sake of comparison, the volume in the Atlantic bluefin tuna (*Thunnus thynnus)*, an active, pelagic temperate/tropical fish is 39%, and 17% in the shorthorn sculpin (*Myoxocephalus scorpius*), a benthic, sedentary subpolar fish [22]. The volume of mitochondria in billfish heater cells is 63%–70%; it is 43% in bumblebee (*Bombus* sp) flight muscle myocytes, 42% in laboratory rat cardiomyocytes, 29% in the pectoralis myocytes in the pigeon (*Columbia livia*), and 25% in the pectoral myocytes in the red-blooded notothenioid *Gobionotothen gibberifrons* [23]. Heater cells are specialized muscle cells in the base of the brain in billfish. Their high metabolic rate raises the temperature of the billfish brain and eyes above ambient temperature. These cells have the highest percent of volume devoted to mitochondria of any animal cell. The increased cell volume devoted to mitochondria in the icefish heart necessarily decreases the volume of contractile myofilaments [22], with the result that the icefish heart fails at modest afterloads [13].

The low-pressure circulation of notothenioids may explain why they have aglomerular kidneys. Glomerular filtration creates urine by using blood pressure to drive intravascular fluid from glomerular capillaries into Bowman's space. In an aglomerular kidney, waste molecules are actively secreted from a low-pressure vascular sinus into blind tubules. Water follows and creates urine. This customization is commonly rationalized by the need to prevent loss of low-molecular-weight AFGP in urine [24]. However, the mean arterial pressure in the notothenioid circulation may be too low to drive glomerular filtration. The mean ventral aortic pressure is 2.30 kPa in *C. aceratus*, 3.60 kPa in the red-blooded notothenioid *P. borchgrevinki* [9], about 5 kPa in the shark catfish *Pangasianodon hypophthalmus* [25], about 5 kPa in the male rainbow trout [26], and 10.4 kPa in the yellowfin tuna [27]. Pressure in branches of the ventral aorta is lower. Whether aglomerular kidneys were necessitated by the need to conserve AFGP or low blood pressure, this customization had to be adopted simultaneously with an Antarctic existence and low-pressure circulation.

Comparison with the icefish cardiovascular system shows why compensation for hemoglobin Bart's fails in humans. The ratio of cardiomyocyte volume devoted to mitochondria and contractile elements is wrong in the fetal human heart. The reduction of contractile elements in channichthyids requires a low-pressure, low-resistance circulation, which is provided by the large capillaries in the trunk skeletal musculature in icefish. Compensatory enlargement of capillaries in the skeletal musculature, the tissue which receives the bulk of $\dot{Q}$, has not been reported in humans with hemoglobin Bart's.

ICEFISH BLOOD

The absence of erythrocytes in icefish requires customizations to make hemoglobinless blood functional. For example, the carbonic anhydrase activity of fish erythrocytes must be replaced. This enzyme catalyzes the interconversion of carbon dioxide and water to carbonic acid. To provide this activity, channichthyid blood contains a special carbonic anhydrase–containing cell [28]. Its appearance is unlike the familiar inflammatory white cells—lymphocytes, granulocytes, and monocytes—in icefish and other organisms. It has no obvious role in inflammation. Given the close association of the term "white blood cell" with inflammation, this cell will be referred to as a "corpuscle."

When stained, this corpuscle has acidophilic cytoplasm like an erythrocyte, explaining why it is referred to as a "vestigial erythrocyte" [29] or "erythrocyte-like" [12]. All proteins are identical on routine histochemical stains because staining is due to a chemical reaction with the positively charged amino acids lysine and arginine, present in all proteins. To the author's knowledge, this corpuscle is present only in channichthyids. The apical cell membrane of the pillar cells of icefish gills also has carbonic anhydrase activity, which has not been reported in other teleosts. The paper reporting this is also notable for observing that loss of hemoglobin and replacement of erythrocyte carbonic anhydrase expression must occur simultaneously [30].

As a consequence of these corpuscles, the leukocrit of icefish is slightly higher than that of humans. Nevertheless, the major determinant of blood viscosity in icefish is plasma viscosity, which is determined by the concentration of plasma proteins. In *C. aceratus*, which has a leukocrit <1%, the viscosity of its whole blood is less than 3% higher than its plasma viscosity [5]. Data in humans suggests that the impact of leukocytes on blood viscosity is similar to the effect of erythrocytes when the leukocrit is less than 15% [31].

The blood viscosity, leukocrit, and hematocrit of various polar fish and two widely distributed nonpolar fish, the rainbow trout and yellowfin tuna, are shown in Table 1. The two red-blooded notothenioids, *Notothenia coriiceps* and *T. bernacchii,* and the rainbow trout show marked increases in their hematocrit with the stress of venipuncture. Specimens obtained from indwelling cannulas are associated with less trauma and have lower hematocrits.

A trend towards lower hematocrits is seen in Antarctic fish, culminating in the zero hematocrit of channichthyids. A hematocrit less than 20% is considered to indicate anemia in other teleosts [32], demonstrating how exceptional the notothenioid

Table 1: Blood viscosity, leukocrit, and hematocrit of select fish*

Species	Blood Viscosity (cP)	Hct or Lct (%)	Reference	Notes
Rainbow trout *Oncorhynchus mykiss* acute stress	4.8	25.1	58	Widely distributed, inhabiting water temperatures from 10°–24°C. Commonly 60 cm in length, benthopelagic. Stress of venipuncture increases the hematocrit.
Rainbow trout *O. mykiss*, cannulated	4.0	19.4	58	Cannulation is the least traumatic method of obtaining a specimen of blood.
Notothenia coriiceps. rest	5.3	7.5	5	Demersal, commonly 50 cm in length
Notothenia coriiceps, stressed	9.1	25	5	
Chaenocephalus aceratus	3.3	<1	5	Demersal, commonly 50 cm in length
Chionodraco hamatus	3.6	1.23	59	Demersal , 50 cm maximum length
Cryodraco antarcticus	3.8	1.25	59	Bathydemersal, 50 cm maximum length
Trematomus bernacchii, cannulated	4.4	7.6	59	
Trematomus bernacchii, acute stressed	6.8	15.4	59	
Arctic char *Salvelinus alpinus*	10	27	60	Circumpolar North distribution, neritopelagic, benthopelagic, 60 cm in length, lives in 4°–16°C water
Shorthorn sculpin *Myoxocephalus scorpius*	7	24	61	Benthic, subpolar distribution, 15–30 cm in length
South Georgia icefish *Pseudochaenichthys georgianus*	2.1	-	61	Demersal, 50 cm in length
New Zealand blue cod *Parapercis colias* acute sample	6.5	21.2	61	Demersal, carnivorous, inhabits temperate water, 45 cm in length
Trematomus bernacchii, acute sample	7.5	15.2	61	
Trematomus bernacchii, cannulated	4.2	7.6	61	
Yellowfin tuna *Thunnus albacares*	2.6	35	62	Worldwide distribution, tropical and subtropical, up to 239 cm in length
Homo sapiens	16.7	40	5	Viscosity at 0°C

* Blood viscosity was measured at a high shear rate at native temperature, except in the case of *Homo sapiens*. Different methods were used to determine blood viscosity in these studies. For details of measuring blood viscosity, see the Appendix.

cardiovascular system is. Several observers have noted the trend toward lower hematocrits in Antarctic fish and see this as a means of decreasing blood viscosity. In this view, the hemoglobinless phenotype is the culmination of this trend. In contrast, others see a fundamental difference between downregulation of hemoglobin expression, as in red-blooded notothenioids, and loss of hemoglobin due to a gene deletion, as in icefish [9]. Proponents of intelligent design see customizations to decrease blood viscosity as examples of teleology in biology.

The increase in hematocrit with stress or activity noted above is a customization. This phenomenon has been studied in detail in *P. borchgrevinki.* A substantial portion of its erythrocytes are stored in the spleen when inactive and released when the fish is active. In one study, its hematocrit at rest was 8.6%, increased to 20.6% in the resting, fed state and 26.7% with forced exercise. Thus, *P. borchgrevinki* tailors its hematocrit and blood viscosity to meet immediate metabolic demands [33].

THE NEED FOR BLOOD VISCOSITY

In addition to replacing carbonic anhydrase activity, the viscosity produced by hemoglobin-containing erythrocytes must also be replaced in channichthyids. In adult humans, insufficient vascular resistance due to low blood viscosity causes the high-output heart failure seen in severe anemia [34]. In severe anemia, the hematocrit, hemoglobin concentration, and erythrocyte count are all decreased. This creates an imbalance between cardiac power and vascular resistance, with cardiac power in excess. As a result, cardiac output is pathologically elevated, creating turbulent blood flow. Turbulence increases the dissipative forces which decrease the pressure head driving fluid flow, which is called "drag." Regardless of whether the decreased vascular resistance and excessive cardiac output are due to severe anemia or another cause, the result is the major symptom of cardiac failure, easy fatigability. Compensatory vasoconstriction does occur, but this is an imperfect response because it decreases tissue perfusion and oxygen delivery.

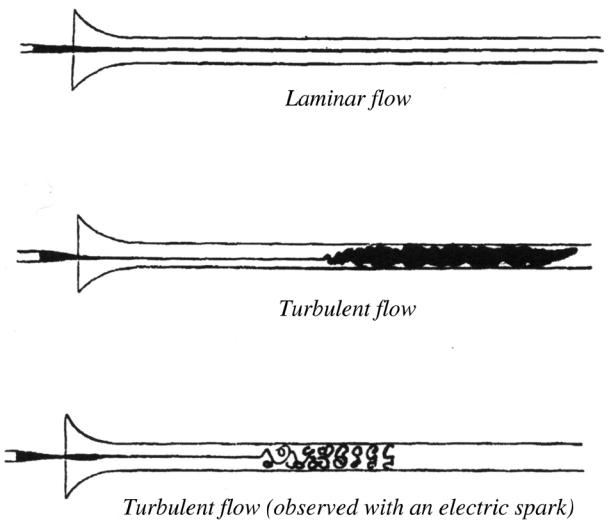

Figure 4: Water flow in a transparent tube as drawn by Osborne Reynolds. Water flows from left to right, and flow is visualized using a black dye in the center of the column of flowing water. Laminar flow occurs when the Reynolds number is low, which occurs when flow velocity is slow and viscosity is adequate. Turbulent flow occurs when the Reynolds number is high, which occurs with high velocity flow and inadequate viscosity. Areas of focal recirculation called vortices are seen in the turbulent flow when observed with an electric spark. These create drag and increase energy losses to friction. Reynolds published these drawings in his influential March 15, 1883 paper, "An Experimental Investigation of the Circumstances Which Determine Whether the Motion of Water in Parallel Channels Shall Be Direct or Sinuous and of the Law of Resistance in Parallel Channels," *Proceedings of the Royal Society*, Vol. 35 (1883):84–99, available at https://royalsocietypublishing.org/doi/epdf/10.1098/rspl.1883.0018. Images (from pages 91–92 of the paper) courtesy of Wikimedia Commons. Public domain. **doi:**10.5048/BIO-C.2022.2.f4

The obvious oxygen-transporting function of hemoglobin obscures its impact, via the hematocrit, on blood viscosity, and the need for viscosity is not widely recognized. Icefish provide an opportunity to observe the impact of blood viscosity on cardiovascular physiology not possible in animals that express hemoglobin.

Two variables, the Reynolds and Dean numbers, determine how much viscosity is needed to optimize laminar flow in the circulation. Laminar flow uses the least energy to transport solutes. Loss of laminar flow increases friction and energy loss, which is called *drag*. In channichthyids, blood viscosity is largely produced by AFGP. In red-blooded notothenioids, blood viscosity is produced by a combination of the hemoglobin concentration, via erythrocytes and the hematocrit, and AFGP. The plasma concentrations of a second antifreeze protein, antifreeze potentiating protein, are unknown but could contribute to blood viscosity.

BLOOD VISCOSITY AND FLOW

The likelihood of laminar conditions in a straight tube during nonpulsatile flow is described by Reynolds number, *Re*, which is determined by the equation $Re = \rho vL/\mu$, where ρ is the fluid density, v is its velocity, L is vessel diameter, and μ is dynamic viscosity. Laminar flow occurs at low *Re* and is lost when blood velocity and $\dot{Q}$ increase if viscosity is insufficient. It is likely that flow in the largest vessels, where velocity is highest, determines the normal blood viscosity in an organism. There is a spectrum of departures from laminar flow as *Re* increases, from focal areas of disturbed flow to full turbulence (Figure 4).

Because it creates high-velocity blood flow, a high-volume, high-output, low-resistance circulation, as present in icefish, is prone to develop nonlaminar flow. Adequate blood viscosity will maximize the amount of flow that is laminar, but excess blood viscosity will increase vascular resistance and cardiac work. The optimal blood viscosity will balance cardiac work and energy lost to friction. The result is that there is only a limited range of viable blood viscosities, from 2.1 to 10 cP, with most at the lower end of that range (Table 1). In contrast, the range of hematocrit in fish is 0% to 35%.

In a typical *C. aceratus* with a blood viscosity of 3.27 cP at 0°C [5], ventral aorta diameter of 4 mm [35], peak ventral aorta blood velocity of 100 cm/s, and blood density of 1.045 g/ml,

the peak *Re* in the ventral aorta is 128. This is far below the *Re* typically associated with turbulent flow, which is ~2500. If blood is replaced with seawater with a density of 1.027 g/ml and viscosity of 1.87 cP [36, 37], *Re* increases to 220. Thus, the blood concentration of AFGP—and, to a lesser extent, other proteins and osmolality—largely determine blood viscosity and *Re* in icefish.

The likelihood of losing laminar flow increases when vascular geometry deviates from a straight tube because momentum causes all bodies to resist a change in direction. The chance of losing laminar flow in a curved tube is described by the Dean number, $De = Re\sqrt{D/2Rc}$, where D is the vessel diameter and Rc is the radius of the curve formed by the blood vessel [38]. *De* has special significance in cardiovascular physiology because laminar flow is lost at *De* that are much lower than *Re*.

In a study that used a curved rectangular channel, flow was laminar at $De < 40$. For $De > 400$, flow was fully turbulent [39]. For $De < 40$ and $Re = 128$, Rc must be greater than 15.4 mm to prevent secondary flow in a 3 mm vessel (D = 3 mm), a reasonable size for the primary branches of the ventral aorta of *C. aceratus*. There is an energy cost if anatomy requires tighter curves or large angle branches. In humans, vascular pathology develops in these locations, such as the Circle of Willis at the base of the brain and the coronary arteries. *Re* and *De* demonstrate that practical vascular geometry is limited by blood viscosity. If blood viscosity is too low, nonlaminar blood flow develops too easily.

OPTIMAL BLOOD VISCOSITY

In Antarctic fish, cardiac power decreases in warmer water because of the decreased availability of O_2. Without a homeostatic response, there will be a mismatch between cardiac power and vascular resistance. This imbalance will worsen when *Re*, *De*, and $\dot{Q}$ increase with forced exercise. Maintaining $\dot{Q}$ in the face of decreased cardiac power requires decreasing vascular resistance. The decreased blood AFGP concentration in *P. borchgrevinki* after acclimation to warmer water is probably a homeostatic response to maintain the balance between cardiac power and vascular resistance by lowering blood viscosity. Acclimation to 4°C for 16 weeks causes a 50% decrease in AFGP concentration and a 15% decrease in serum osmolality in this fish [41]. A similar homeostatic response occurs in *H. sapiens*, which develop**s** anemia in response to heart failure [42].

This homeostatic response provides an experimental model of mismatch between cardiac power and vascular resistance. The limited cardiac power of *P. borchgrevinki* make it sensitive to the dissipative forces from nonlaminar blood flow. The cardiac power of *P. borchgrevinki* is 1.76 mW/kg [9], compared to 3.59 ± 0.76 W/kg produced by the heart of the Pacific bluefin tuna (*Thunnus orientalis*) at 20°C [40].

These experiments show that matching blood viscosity and vascular resistance to cardiac power increases the factorial scope (the ratio of maximal to normal) for $\dot{Q}$ despite the similar availability of O_2 to acclimated and unacclimated fish [43]. The factorial scope for $\dot{Q}$ at 4°C in fish acclimated to 4°C is 2.2, compared to 1.8 in fish acclimated to -1°C (Figure 5).

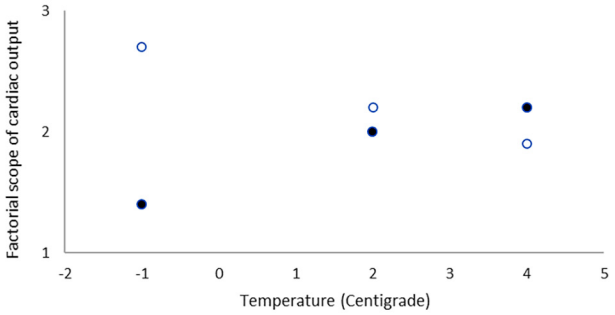

Figure 5: Factorial scope (the ratio of maximal to resting) for cardiac output of *Pagothenia borchgrevinki* acclimated to either -1°C (open circles, n=8) or 4°C (closed circles, n=8) determined after the fish was placed in water of different temperature. In fish acclimated to -1°C, blood viscosity is in excess relative to cardiac power, when power is decreased at higher temperatures because of the reduced availability of oxygen. In fish acclimated to 4°C, blood viscosity is insufficient relative to cardiac power at higher temperatures because of the greater availability of oxygen. Viscosity insufficiency creates a condition analogous to high-output heart failure in humans. Viscosity excess directly increases vascular resistance. Both decrease factorial scope for cardiac output. Data are from Franklin et al. [37]. **doi:**10.5048/BIO-C.2022.2.f5

Similarly, fish acclimated to -1°C have a greater factorial scope at -1°C than fish acclimated to 4°C (2.7 versus 1.4). When fish acclimated to 4°C are placed in 2°C water, the factorial scope decreases to 2.0 despite the greater availability of O_2. In -1°C water, their factorial scope decreases further to 1.4.

The factorial scope for $\dot{Q}$ decreases when fish acclimated to 4°C are placed in cooler water because their blood viscosity is too low to prevent the development of nonlaminar flow and friction when cardiac power and peak blood velocity increase in water with greater O_2 content. There is a mismatch between cardiac power and blood viscosity and vascular resistance, with cardiac power in excess. As the imbalance worsens, the amount of energy and power wasted progressively increases as nonlaminar flow develops in progressively smaller arteries. This creates a condition analogous to high-output cardiac failure in humans.

An imbalance between cardiac power and blood viscosity also occurs when *P. borchgrevinki* acclimated to -1°C are placed in warmer water. The factorial scope for $\dot{Q}$ in *P. borchgrevinki* acclimated to -1°C progressively decreases from 2.7 at -1°C to 1.8 at 4°C. At temperatures greater than -1°C, the decreasing O_2 content of warmer water reduces cardiac power. Decreased cardiac power reduces maximal $\dot{Q}$ in the face of the increased vascular resistance offered by the higher blood viscosity that is optimal for -1°C. Again, there is an imbalance between blood viscosity, vascular resistance, and cardiac power, with viscosity and resistance in excess.

OXYGEN TRANSPORT VERSUS VISCOSITY

Those experiments suggest that blood viscosity plays an important role in cardiovascular homeostasis. They differ from experiments performed to determine the maximum temperature

at which acutely warmed fish can maintain homeostasis, as determined by the onset of a visible physical impairment such as loss of the reflexive ability to remain upright. That temperature is called the critical thermal maximum (CT_{max}). These experiments show that the hematocrit is directly related to CT_{max} in Antarctic fish [44]. In experiments in which water temperature was increased 3.6°C per hour, two channichthyids, *Chionodraco ratrospinus* and *C. aceratus*, had the lowest CT_{max}, 13.3° ± 0.2°C and 13.9° ± 0.4°C, respectively. In red-blooded notothenioids, the CT_{max} of *Lepidonotothens squamifrons*, hematocrit 21%, was 14.2°C; the CT_{max} of *G. gibberifrons*, hematocrit 25%, was 15.5°C; and the CT_{max} of *N. coriiceps*, hematocrit 35%, was 17.1°C. Additionally, warming increased the hematocrit 7% in *L. squamifrons*, 24% in *G. gibberifrons*, and 16% in *N. coriiceps.* Given that the increase in hematocrit occurred within hours, it was probably caused by release of sequestered erythrocytes from the spleen. An increase in the hematocrit and hemoglobin concentration due to release of erythrocytes from the spleen in response to warming is documented in *T. bernacchii* [45].

Elevated temperature potentially affects numerous physiologic processes. The close phylogenetic relationship of these notothenioids and their origin from a similar environment suggest these species could have similar values of CT_{max} and adds weight to the argument that the differences in hematocrit are causal [44]. The association of hematocrit with CT_{max} raises the possibility that the ability to deliver O_2 limits $CT_{max.}$ This possibility was tested by measuring CT_{max} in *C. aceratus* and *N. coriiceps* in both normoxic and hyperoxic conditions. The dissolved O_2 concentration in the hyperoxic tank was consistently three- to fourfold higher than the concentration in the normoxic tank at the same temperature. The CT_{max} of both species was unchanged by the O_2 concentration, being 13.1°C and 16.8°C respectively [46].

With falsification of the hypothesis that CT_{max} is limited by O_2 availability, the possibility that blood viscosity limits CT_{max} should be considered. Acute warming causes an increase in heart rate and $\dot{Q}$ in fish [3]. Increasing the environmental temperature also directly decreases blood viscosity. Blood viscosity in *C. aceratus* increases from 3.4 cP at -1°C to 2.8 cP at 5°C, or 18% [5]. Without a homeostatic response to maintain blood viscosity, these changes will cause loss of laminar flow. Thus, the increase in hematocrit in notothenioids with warming could be viewed as homeostatic response to maintain blood viscosity and laminar blood flow. When the capacity to maintain blood viscosity is exceeded, hypoviscosity will cause terminal high-output cardiac failure unless cardiac power fails first. The experimental design cannot distinguish between these possibilities.

Figure 6: ***Trematomus bernacchii.*** Courtesy of Zureks and Wikimedia Commons. **doi:**10.5048/BIO-C.2022.2.f6

An experiment in which the O_2-carrying capacity of hemoglobin is greatly reduced but its viscogenic activity is unaffected provides more direct evidence that hemoglobin plays important in maintaining blood viscosity via the hematocrit. When 95% of its hemoglobin is converted to carboxyhemoglobin, which cannot transport O_2, *T. bernacchii* suffers no ill effects, even during forced exercise [47]. *T. bernacchii* is a benthic notothenioid which commonly measures 25 cm in length (Figure 6). Because hemoglobin is not necessary for O_2 transport in *T. bernacchii* in the cold water of the Southern Ocean, the question arises, "Why does this fish expend the resources to produce erythrocytes?" To Darwinists, unnecessary expenditure of limited resources is not unexpected because they see organisms as unperfected works in progress [48]. In contrast, proponents of intelligent design see organisms as the result of goal-oriented thought with few if any useless parts. In this paradigm, erythrocytes and hemoglobin provide *T. bernacchii* with essential viscosity as well as O_2-carrying capacity.

The hypothesis that hemoglobin and erythrocytes are necessary to maintain blood viscosity can be tested in *T. bernacchii* by replacing its erythrocytes with an equal volume of its plasma. This will reduce viscosity and perhaps even increase O_2 transport compared to blood containing preponderantly carboxyhemoglobin. The resulting abnormally low blood viscosity should decrease the factorial scope for $\dot{Q}$ which should not be reversible with hyperoxic conditions.

Data in mammals suggest that optimization of blood viscosity is prioritized over maximizing O_2 transportation. Kilbridge et al. showed in hypoxic mice that increasing the hematocrit without increasing O_2 transport inhibits erythropoiesis [49]. Mice were made hypoxic by incubation in a rarified atmosphere (0.4 atmospheres) for 4.5 hours. In one protocol, hypoxic mice were transfused with erythrocytes containing methemoglobin, which, like carboxyhemoglobin, cannot carry oxygen. Transfusion with both normal and methemoglobin-containing erythrocytes to a hematocrit of 60% decreased erythropoietin production to less than 15% of that in hypoxic nontransfused mice. In a second experimental protocol, the hematocrit was increased in hypoxic mice by inducing dehydration. Erythropoietin was reduced to 49% of that in hypoxic control mice.

Both protocols cause blood hyperviscosity. Given the strong inverse relationship of blood viscosity to blood flow [1], it is not surprising that optimizing blood viscosity is prioritized over maximizing O_2 transport. In tissues with the capacity for substantial anaerobic metabolism, delivery of glucose is more important than delivery of O_2 in the short term. These experiments provide more evidence that blood viscosity is under physiologic control.

THE *MILIEU INTÉRIEUR*

The optimal value of blood viscosity in an organism depends on a host of variables, including arterial geometry and diameter, peak blood velocity, and cardiac power. These variables, as well as construction materials (collagen, elastin, proteoglycans, etc.) and a means of controlling blood viscosity must be specified in the design of a cardiovascular system. Like the compression strength of bone, yield strength of tendons, and elasticity of arteries, viscosity is a critical property of blood. It is the precise value of these essential properties in these tissues that makes them useful in fulfilling their biological role, which, in the case of blood, is transporting solutes.

Blood viscosity should be considered to be an element of what the great nineteenth-century physiologist Claude Bernard called the *milieu intérieur*. He introduced that term to describe the key physiological variables such as body temperature, pH, and transmembrane electrical potential that are maintained within narrow limits. "Homoeostasis" describes the state when these elements are within design specifications. Death occurs when the *milieu intérieur* can no longer be maintained. The noted cardiologist Gheorghe Pop was the first to argue that blood viscosity should be considered to be an element of the *milieu intérieur*.

CONTROL OF BLOOD VISCOSITY

Given its critical importance in cardiovascular physiology, a means of controlling blood viscosity is necessary. In mammals, stretch receptors in the left ventricle control the systemic vascular resistance response (SVRR) [50]. In this response, stretching of cardiomyocytes, as in heart failure or increased systemic vascular resistance, causes release of B-type natriuretic factor (BNP) and decreases erythrocyte mass via several mechanisms, thereby reducing vascular resistance and normalizing $\dot{Q}$. Stretch receptors are present in the icefish heart [12]. The gene for BNP is highly conserved in teleosts, including *C. hamatus* [51]. Thus, an SVRR-like response may control AFGP concentrations in channichthyids.

AFGPs are a family of polymers of a glycosylated tripeptide, Ala(Pro)-Ala-Thr, which differ in length and molecular weight. They are the major plasma protein fraction in most notothenioids, with concentrations ranging from 0.5 to 14.4 g/dl in channichthyids and 28.1 to 35.1 in red-blooded notothenioids [41]. In humans, albumin is present in higher concentrations than any other protein except for hemoglobin. Like albumin and hemoglobin in humans, AFGPs fill multiple roles in icefish. AFGPs provides oncotic pressure in icefish just as albumin does in humans [52]. Decreased AFGP concentrations with thermal acclimation require loss of sodium cations to maintain electrical neutrality, reducing osmolality [41]. Hypoalbuminemia causes hyponatremia and reduces oncotic pressure in humans [53].

Both albumin and AFGP bind exogenous compounds. In the case of AFGP, that compound is ice. In channichthyids, AFGPs also have the viscogenic function of that hemoglobin has in humans. Combining these roles into AFGPs allows the blood viscosity of *C. aceratus* at 0°C to be almost identical to that of *Homo sapiens* at 37°C. The higher AFGP concentration in red-blooded notothenioids compared to channichthyids is reflected in their higher blood viscosity (Table 1). Determining why this is the case will require additional knowledge of hemodynamics in red-blooded notothenioids.

The persistence of 50% of the original blood AFGP concentration after acclimation to 4°C in *P. borchgrevinki* suggests that they have a function in addition to binding ice crystals. The antifreeze protein (not AFGP) of an unrelated fish, the winter flounder (*Pseudopleuronectes americanus*), disappears after three weeks of acclimation to 12°C [41]. Further, the red-blooded notothenioid *T. loennbergii* has a high concentration of AFGP, 2.8 ± 0.5 g/dl, [41] despite inhabiting an ice-free environment in the deep Southern Ocean. It is a bathydemersal (living close to the bottom of deep water) fish which commonly measures 20 cm in length.

The antifreeze activity of an AFGP molecule is generally proportional to its molecular weight [54]. Large molecules increase plasma viscosity more than smaller ones [55]. Every AFGP is encoded by a different gene, with *C. aceratus* having 11 genes [56]. This array of AFGP genes could provide the flexibility to meet both the need for blood viscosity and preventing growth of ice crystals. In all species examined except *T. loennbergii*, the majority of AFGPs are low in molecular weight [41], which favors lower blood viscosity over maximal prevention of ice crystallization.

HALLMARKS OF INTELLIGENT DESIGN IN ICEFISH

The customized icefish heart, vasculature, and blood form a system with mutually dependent parts. These cannot be exchanged with those from red-blooded fish. A non-customized heart would have the incorrect ratio of mitochondria to contractile elements in cardiomyocytes. As a result, ATP insufficiency would develop. If the icefish vasculature were replaced with one from a red-blooded fish, the increased vascular resistance would cause the heart to fail. If an icefish's blood were replaced with red blood, the icefish would die because of hyperviscosity.

The cardiovascular systems of other teleosts do not show the same degree of customization. If the heart of one teleost were replaced with one from another teleost of similar size and lifestyle, the teleost would probably survive long enough to reproduce. In general, it is probably fair to say that replacing the circulatory system and blood of one teleost with that of another would be tolerated.

The hemoglobinless phenotype requires simultaneous, coordinated acquisition of multiple unique features. This is difficult to explain with Darwinian evolution, which postulates that species develop by small, incremental changes over long periods. It is unlikely that the changes required by the hemoglobinless phenotype can be accomplished with only one or two mutations. A small number of random but bold, lucky strokes is the most change that can be accomplished by Darwinian evolution within a short period of time. This is simply not enough to develop the hemoglobinless phenotype. It is likely that the

creation of a large heart with abundant mitochondria requires one developmental program, of a carbonic anhydrase–containing corpuscle another program, of large-diameter capillaries a third program, and gill epithelial cells that express carbonic anhydrase yet another program. Thus, an origin of icefish via Darwinian processes from red-blooded notothenioids, or from the phylogenetic notothenioid sister lineage, *Percophis brasiliensis* [57], is unlikely.

SUMMARY

The cardiovascular system of fish of the family Channichthyidae appears to have been designed to utilize hemoglobinless blood to solve the problem of increased viscosity at low temperatures. A design for any cardiovascular system must specify each component, including blood viscosity and vascular geometry, in order to maximize laminar flow and minimize vascular resistance. It also must include a mechanism to maintain blood viscosity within specifications. The appropriate value of blood viscosity allows vascular resistance to match cardiac power. This value can be estimated by Reynolds and Dean numbers. Because blood viscosity is an important determinant of blood flow, it should be considered part of the *milieu intérieur*. Multiple customized components are necessary to utilize hemoglobinless blood. Actualizing the design for the icefish cardiovascular system requires each customized component to be in place simultaneously. This is more innovation than can be accomplished by random mutation as postulated in Darwinian evolution.

APPENDIX

When thinking of fluids, shear and flow are synonyms. The unit of shear rate is inverse seconds (1/s). Blood flow can be envisioned as occurring at either high shear and high velocity, ≥100/s, or low shear and low velocity, ≤1/s. High shear conditions occur in arteries in systole, especially long straight arteries. Low shear conditions occur in capillaries, veins, and in association with changing arterial geometry, particularly in diastole.

Blood containing erythrocytes shows strong non-Newtonian properties, meaning that its viscosity varies with the rate of shear or flow velocity (Figure A1).

Blood viscosity increases as shear rate or flow velocity decreases. This is because erythrocytes reversibly aggregate, which increases the mass and inertia of the bodies in suspension. At high shear rates, blood viscosity decreases because erythrocytes reversibly deform. This decreases the surface area of their leading edge and resistance to flow.

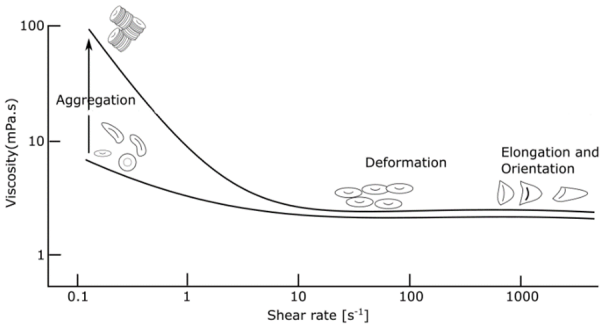

Figure A1. The relationship of blood viscosity to shear rate. Courtesy of MagMedics, LLC. **doi:**10.5048/BIO-C.2022.2.fA1

Channichthyid blood exhibits less pronounced non-Newtonian properties than blood with erythrocytes. Plasma exhibits little to no non-Newtonian properties.

Blood viscosity is measured with two methods: rotational viscometry and capillary viscometry. In rotational viscometry, blood is placed between two closely apposed surface, one of which is rotated. The distance between the two surfaces is less than 1 mm. This allows approximation of a single shear rate. The viscosity of the specimen is determined by the torque needed to cause rotation and velocity of rotation caused by that torque. The precision of the method is limited chiefly by the ability to deliver a precise quantity of torque. Rotational viscometry underestimates low-shear viscosity because the growth of erythrocyte aggregates is limited by the small distance between the two surfaces. Erythrocyte aggregates can reach a size >1 mm in diameter. Also, shearing prevents erythrocyte aggregation.

In capillary viscometry, the elapsed time for the meniscus of a column of blood to pass between two hash marks is measured. This is compared to the time for distilled water. There are an infinite number of shear rates in the column of flowing blood. The highest shear rate is at the capillary wall and is typically >100/s. Capillary viscometers are available with different diameters to measure fluids of different viscosity. Erythrocyte aggregates form in the low shear conditions in the center of the column.

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1. Dormandy JA (1971) Influence of blood viscosity on blood flow and the effect of low molecular weight Dextran. Br Med J 18:716-9. **doi:**10.1136/bmj.4.5789.716
2. Axe DD, Gauger AK (2015) Model and laboratory demonstrations that evolutionary optimization works well only if preceded by invention—Selection itself is not inventive. BIO-Complexity (2):1–13. **doi:**10.5048/BIO-C.2015.2
3. Eastman JT (2005) The nature of the diversity of Antarctic fishes. Polar Biol 28:93–107. **doi:**10.1007/s00300-004-0667-4
4. O'Brien KM, Joyce W, Crockett EL, Axelsson M, Egginton S, et. al (2021) Resilience of cardiac performance in Antarctic notothenioid fishes in a warming climate. J Exp Biol 15;224(10):jeb220129. **doi:**10.1242/jeb.220129
5. Egginton S (1996) Blood rheology of Antarctic fishes: Viscosity adaptations at very low temperatures. J Fish Biol 48:513–521. **doi:**10.1111/j.1095-8649.1996.tb01444.x
6. Rosenson RS, McCormick A, Uret, EF (1996) Distribution of blood viscosity values and biochemical correlates in healthy adults. Clin Chem 42:1189–95. **doi:**10.1093/clinchem/42.8.1189
7. Piloto N, Teixeira HM, Teixeira-Lemos E, Parada B, Garrido P, et al. (2010) Erythropoietin Doping as a Cause of Sudden Death in Athletes—An Experimental Study. In: Vieira DN, Busuttil A, Cusack D, Beth P, eds. Acta Medicinae Legalis et Socialis Academia Internationalis Medicinae Legalis (Coimbra) pp 267-74. **doi:**10.14195/978-989-26-0173-1_47
8. Heuberger JAAC, Tervaert JMC, Femke ML, Schepers FML, Vliegenthart ADB, et al. (2013) Erythropoietin doping in cycling: Lack of evidence for efficacy and a negative risk-benefit. Br J Clin Pharmacol 75:1406–21. **doi:**10.1111/bcp.12034
9. Sidell BD, O'Brien KM (2006) When bad things happen to good fish: The loss of hemoglobin and myoglobin expression in Antarctic icefishes. J Exp Biol 209:1791–1802. **doi:**10.1242/jeb.02091
10. Eastman JT (1997) Comparison of the Antarctic and Arctic fish faunas. Cybium 21:335–352.
11. Science Learning Hub Pokapū Akoranga Pūtaiao. Antarctic Marine Ecosystem. Published 4 September 2008. https://www.sciencelearn.org.nz/resources/949-antarctic-marine-ecosystem
12. Garofalo F, Pellegrino D, Amelio D, Tota B (2009) The Antarctic hemoglobinless icefish, fifty-five years later: A unique cardiocirculatory interplay of disaptation and phenotypic plasticity. Comp Biochem Physiol Part A 154:10–28. **doi:**10.1016/j.cbpa.2009.04.621
13. Pellegrino D, Palmerini CA, Tota B (2004) No hemoglobin but NO: The icefish (*Chionodraco hamatus*) heart as a paradigm. J Exp Biol. 207:3855–64. **doi:**10.1242/jeb.01180
14. Beers JM, Borley KA, Sidell BD (2010) Relationship among circulating hemoglobin, nitric oxide synthase activities and angiogenic poise in red- and white-blooded Antarctic notothenioid fishes. Comp Biochem Physiol A Mol Integr Physiol 156(4):422–29. **doi:**10.1016/j.cbpa.2010.03.027
15. Jandl JH (1996) Blood Textbook of Hematology. Little, Brown and Company (Boston).
16. Thammavong K, Luewan S, Jatavan P, Tongsong T (2020) Foetal haemodynamic response to anaemia. ESC Heart Fail 7(6):3473–3482. **doi:**10.1002/ehf2.12969
17. Tomanek RJ (1990) Response of the coronary vasculature to myocardial hypertrophy. J Am Coll Cardiol 15:528–33. **doi:**10.18356/333473a2-es
18. Tota B, Acierno R, Agnisoloa S (1991) Mechanical performance of the isolated and perfused heart of the haemoglobinless Antarctic icefish *Chionodraco hamatus* (Lonnberg): Effects of loading conditions and temperature. Philos Trans R Soc. Lond. B 332:191–98. **doi:**10.1098/rstb.1991.0049
19. Fitch NA, Johnston IA, Wood RE (1984) Skeletal muscle capillary supply in a fish that lacks respiratory pigments. Respir Physiol 57:201–11. **doi:**10.1016/0034-5687(84)90093-8
20. Wujcik JM, Wang G, Eastman JT, Sidell BD (2007) Morphometry of retinal vasculature in Antarctic fishes is dependent upon the level of hemoglobin in circulation. J Exp Biol 210(Pt 5):815–24. **doi:**10.1242/jeb.001867
21. Tota B, Cerra MC, Mazza R, Pellegrino D Icardo J (1997) The heart of the Antarctic icefish as paradigm of cold adaptation. J Therm Biol 22:409–417. **doi:**10.1016/S0306-4565(97)00060-0
22. Tota B, Agnisola C, Schioppa M, Acierno R, Harrison P, et al. (1991) Structural and mechanical characteristics of the heart of the icefish *Chionodraco hamatus*. In: Di Priso G, Maresca B, Tota B, eds. Biology of Antarctic Fish. Springer-Verlag (Berlin) pp. 204–19.
23. Tullis A, Block BA, Sidell BD (1991) Activities of key metabolic enzymes in heater organs of scombrid fishes. J Exp Biol 161:383–403. **doi:**10.1242/jeb.161.1.383
24. Eastman JT, Devries AL (1986) Renal glomerular evolution in Antarctic notothenioid fishes. J Fish Biol 29:649–62. **doi:**10.1111/j.1095-8649.1986.tb04981.x
25. Armelin VA, Thomsen MT, Teixeira MT, Florindo LH, Bayley M, et al. (2019) Cardiovascular and ventilatory interactions in the facultative air-breathing teleost *Pangasianodon hypophthalmus*. J Comp Physiol B. 189(3–4):425–40. **doi:**10.1007/s00360-019-01225-9
26. Clark RJ, Rodnick KJ (1999) Pressure and volume overloads are associated with ventricular hypertrophy in male rainbow trout. Am J Physiol 277(4):R938–46. **doi:**10.1152/ajpregu.1999.277.4.R938
27. Jones DR, Brill RW, Bushnell PG (1993) Ventricular and arterial dynamics of anaesthetized and swimming tuna. J Exp Biol 182:97–112. **doi:**10.1242/jeb.182kjj.1.97
28. Maffia M, Rizzello A, Acierno R, Rollo M, Chiloiro R, et al. (2001) Carbonic anhydrase activity in tissues of the icefish *Chionodraco hamatus* and of the red-blooded teleosts *Trematomus bernacchii* and *Anguilla anguilla*. J Exp Biol 204:3983–92. **doi:**10.1242/jeb.204.22.3983
29. Barber DL, Mills Westermann JE, White MG (1981) The blood cells of the Antarctic icefish *Chaenocephalus aceratus* Lönnberg: Light and electron microscopic observations. J Fish Biol 19(1):11–28.
30. Harter TS, Sackville MA, Wilson JM, Metzger DCH, Egginton S, et al. (2018) A solution to Nature's haemoglobin knockout: A plasma accessible carbonic anhydrase catalyses CO_2 excretion in Antarctic icefish gills. J Exp Biol 221:jeb190918. **doi:**10.1242/jeb.190918
31. Lichtman MA, Rowe JM (1982) Hyperleukocytic leukemias: Rheological, clinical, and therapeutic considerations. Blood 60:279–83.
32. Clauss TM, Dove ADM, Arnold JE (2008) Hematologic Disorders of Fish. Vet Clin Exot Anim 11:445–462.
33. Brijs J, Axelsson M, Rosengren M, Jutfelt F, Gräns A (2020) Extreme blood-boosting capacity of an Antarctic fish represents an adaptation to life in a sub-zero environment. J Exp Biol 223(Pt 2):jeb218164. **doi:**10.1242/jeb.218164
34. Mehta PA, Dubrey SW (2009) High output heart failure. Q J Med 2009:102:235–41. **doi:**10.1093/qjmed/hcn147
35. Joyce W, Egginton S, Farrell AP, Crockett EL, O'Brien KM, et al. (2018) Exploring nature's natural knockouts: *In vivo* cardiorespiratory performance of Antarctic fishes during acute warming. J Exp Biol 221:jeb183160. **doi:**10.1242/jeb.183160
36. Nayar KG, Sharqawy MH, Banchik LD, Lienhard VJH (2016) Thermophysical properties of seawater: A review and new correlations that include pressure dependence. Desalination 390:1–15. **doi:**10.1016/j.desal.2016.02.024
37. Sharqawy MH, Lienhard V JH, Zubair SM (2010) Thermophysical properties of seawater: A review of existing correlations and data. Desalination and Water Treatment 16:354–80. **doi:**10.5004/dwt.2010.1079
38. LaDisa JF Jr, Hettrick DA, Olson LE, Guler I, Gross ER, et al. (2002) Stent implantation alters coronary artery hemodynamics and wall shear stress during maximal vasodilation. J Appl Physiol 93(6):1939–46. **doi:**10.1152/japplphysiol.00544.2002

39. Lignani PM (1994) A study of Dean vortex development and structure in a curved rectangular channel with aspect ratio of 40 at Dean numbers up to 430. U.S. Army Research Laboratory Contractor Report ARL-CR-l44. U.S. Army Research Laboratory, p. 233.
40. Blank JM, Morrissette JM, Landeira-Fernandez AM, Blackwell SB, Williams TD, et al. (2004) In situ cardiac performance of Pacific bluefin tuna hearts in response to acute temperature change. J Exp Biol 207(Pt 5):881–90. **doi:**10.1242/jeb.00820
41. Jin Y, DeVries AL (2006) Antifreeze glycoprotein levels in Antarctic notothenioid fishes inhabiting different thermal environments and the effect of warm acclimation. Comp Biochem Physiol Part B Biochem Mol Biol 144:290–300. **doi:**10.1016/j.cbpb.2006.03.006
42. Pop GAM, de Boer M-J, Stalenhoef AF (2016) Reducing cardiac after-load by lowering blood viscosity in patients with familial hypercholesterolemia—A pilot study. Possible mechanism for the occurrence of anemia in chronic heart failure patients? Cor et Vasa 58:e374–e378. **doi:**10.1016/j.crvasa.2015.10.001
43. Franklin CE, Davison W, Seebacher F (2007) Antarctic fish can compensate for rising temperatures: Thermal acclimation of cardiac performance in *Pagothenia borchgrevinki*. J Exp Biol 210:3068–74. **doi:**10.1242/jeb.003137
44. Beers JM, Sidell BD (2011) Thermal tolerance of Antarctic notothenioid fishes correlates with level of circulating hemoglobin. Physiol Biochem Zool 84:353–62. **doi:**10.1086/660191
45. Davison W, Franklin DE, McKenzie JC (1994) Haematological changes in an Antarctic teleost, *Trematomus bernacchii*, following stress. Polar Biol 14:463–466.
46. Devor DP, Kuhn DE, O'Brien KM, Crockett E. Hyperoxia does not extend critical thermal maxima (CTmax) in white- or red-blooded antarctic notothenioid fishes (2016) Physiol Biochem Zool 89:1–9. **doi:**10.1086/684812
47. Verde C, Giordano D, di Prisco G (2008) The adaptation of polar fishes to climatic changes: Structure, function and phylogeny of haemoglobin. IUBMB Life 60:29–40.
48. Snoke D (2014) Systems biology as a research program for intelligent design. BIO-Complexity 2014 (3):1–11. **doi:**10.5048/BIO-C.2014.3
49. Kilbridge TM, Fried W, Heller P (1969) The mechanism by which plethora suppresses erythropoiesis. Blood 33:104–13.
50. Sloop GD, Weidman JJ, St Cyr JA (2015) The systemic vascular resistance response: A cardiovascular response modulating blood viscosity with implications for primary hypertension and certain anemias. Ther Adv Cardiovasc Dis 9:403–11. **doi:**10.1177/1753944715591450
51. Inoue K, Sakamoto T, Yuge S, Iwatani H, Yamagami S, et al. (2005) Structural and functional evolution of three cardiac natriuretic peptides. Mol Biol Evol 22:2428–34. **doi:**10.1093/molbev/msi243
52. Kragh-Hansen U, Minchiotti L, Galliano M, Peters Jr. T (2013) Human serum albumin isoforms: Genetic and molecular aspects and functional consequences. Biochim Biophys Acta 1830(12):5405–17. **doi:**10.1016/j.bbagen.2013.03.026
53. Flear CT, Gill GV, Burn J (1981) Hyponatraemia: Mechanisms and management. Lancet 2(8236):26–31. **doi:**10.1016/s0140-6736(81)90261-0
54. Kim HJ, Lee JH, Hur YB, Lee CW, Park S-H et al. (2017) Marine antifreeze proteins: Structure, function, and application to cryopreservation as a potential cryoprotectant. Mar Drugs 15:27. **doi:**10.3390/md15020027
55. Késmárky G, Kenyeres P, Rábai M, Tóth K (2008) Plasma viscosity: A forgotten variable. Clin Hemorheol Microcirc 39:243–46. **doi:**10.3233/CH-2008-1088
56. Kim B-M, Amores A, Kang S, Ahn D-H, Kim J-H, et al. (2019) Antarctic blackfin icefish genome reveals adaptations to extreme environments. Nat Ecol Evol 3:469–78. **doi:**10.1038/s41559-019-0812-7
57. Near TJ, Dornburg A, Harrington RC, Oliveira C, Pietsch TW, et al. (2015) Identification of the notothenioid sister lineage illuminates the biogeographic history of an Antarctic adaptive radiation. BMC Evol Biol 15:109. **doi:**10.1186/s12862-015-0362-9
58. Wells, RMG, Weber RE (1991) Is there an optimal haematocrit for rainbow trout, *Oncorhynchus mykiss* (Walbaum)? An interpretation of recent data based on blood viscosity measurements. J Fish Biol 38:53–65.
59. Wells RNG, Macdonald JA, DiPrisco G (1990) Thin-blooded Antarctic fishes: A rheological comparison of the haemoglobin-free icefishes *Chionodraco kathleenae* and *Cryodraco antarcticus* with a red-blooded notothenid, *Pagothenia bernacchii*. J Fish Biol 36:595–609. **doi:**10.1111/j.1095-8649.1990.tb03560.x
60. Graham MS, Fletcher GL, Haedrich RL (1985) Blood viscosity in Arctic fish. J Exp Zool 234:157–60.
61. Macdonald JA, Wells RMG (1991) Viscosity of Body Fluids from Antarctic Notothenioid Fish. In: Di Priso G, Maresca B, Tota B, eds. Biology of Antarctic Fish. Springer-Verlag (Berlin) pp. 163–78.
62. Brill RW, Jones DR (1994) The influence of hematocrit, temperature, temperature, and shear rate on the viscosity of blood from a high-energy-demand teleost, the yellowfin tuna *Thunnus albacares*. J Exp Biol 189:199–212.