Rheology of embryonic avian blood

Sarah Al-Roubaie,* Espen D. Jahnsen,* Masud Mohammed, Caitlin Henderson-Toth, and Elizabeth A. V. Jones

Department of Chemical Engineering, McGill University, Montreal, Quebec, Canada

Submitted 11 May 2011; accepted in final form 12 September 2011

Al-Roubaie S, Jahnsen ED, Mohammed M, Henderson-Toth C, Jones EA. Rheology of embryonic avian blood. Am J Physiol Heart Circ Physiol 301: H2473–H2481, 2011. First published September 30, 2011; doi:10.1152/ajpheart.00475.2011.—Shear stress, a mechanical force created by blood flow, is known to affect the developing cardiovascular system. Shear stress is a function of both shear rate and viscosity. While established techniques for measuring shear rate in embryos have been developed, the viscosity of embryonic blood has never been known but always assumed to be like adult blood. Blood is a non-Newtonian fluid, where the relationship between shear rate and shear stress is nonlinear. In this work, we analyzed the non-Newtonian behavior of embryonic chicken blood using a microviscometer and present the apparent viscosity at different hematocrits, different shear rates, and at different stages during development from 4 days (Hamburger-Hamilton stage 22) to 8 days (about Hamburger-Hamilton stage 34) of incubation. We chose the chicken embryo since it has become a common animal model for studying hemodynamics in the developing cardiovascular system. We found that the hematocrit increases with the stage of development. The viscosity of embryonic avian blood in all developmental stages studied was shear rate dependent and behaved in a non-Newtonian manner similar to that of adult blood. The range of shear rates and hematocrits at which non-Newtonian behavior was observed is, however, outside the physiological range for the larger vessels of the embryo. Under low shear stress conditions, the spherical nucleated blood cells that make up embryonic blood formed into small aggregates of cells. We found that the apparent blood viscosity decreases at a given hematocrit during embryonic development, not due to changes in protein composition of the plasma but possibly due to the changes in cellular composition of embryonic blood. This decrease in apparent viscosity was only visible at high hematocrit. At physiological values of hematocrit, embryonic blood viscosity did not change significantly with the stage of development.

microelectromechanical systems; hematocrit; shear rate; shear stress; hemodynamic; rouleaux; vascular development

HEMODYNAMICS, or blood fluid dynamics, are important not only for cardiovascular function but also for the development of the cardiovascular system. Blood flow creates a force called shear stress. Chronic changes in shear stress levels lead to a remodeling of the vasculature that normalizes the level of shear stress in the adult (20). Shear stress has also been found to be important during cardiovascular development, affecting heart formation (17), vascular remodeling (25, 36), arterial-venous differentiation (21), and hematopoiesis by the vascular endothelium (1, 29). For these reasons, there has been a significant effort in recent years to measure the shear stress levels during early embryonic development and link specific flow patterns or levels of shear stress to events in vascular development.

Shear stress is a function of the shear rate and the viscosity of the fluid. The development of flow visualization techniques with micrometer-scale resolution, such as Doppler optical coherence tomography (12) and microparticle image velocimetry (32), has allowed flow patterns and shear rates to be measured in embryonic blood vessels. Although shear rates during development are known, the viscosity has never been measured, and, therefore, the only available published values of shear stress assume that embryonic blood viscosity behaves similarly to that of adult blood (19, 31, 32). The shear rate itself is not believed to have an effect on endothelial cells. When using fluids of different viscosity, flow-induced changes in endothelial cells such as intracellular adhesion molecule-1 expression or intracellular Ca2+ levels (2, 33) are proportional to the level of shear stress and not to the shear rate. Therefore, the knowledge of the shear rate in itself is not useful unless it is translated into a value of shear stress. For this reason, measuring embryonic blood viscosity is as important as measuring the shear rate.

Blood is a non-Newtonian fluid where the relationship between shear rate and shear stress is nonlinear. At very high shear rates, red blood cells streamline and deform within the flow to reduce the relative resistance of the fluid to flow (3), resulting in a lower viscosity. When blood is moving slowly, erythrocytes interact with one another and form linear aggregates of cells that are called rouleaux (16). This increases the apparent viscosity of the blood in a nonlinear fashion with decreasing shear rate. These effects can be quite significant, resulting in 10-fold changes in the apparent viscosity of blood for very low shear rates (9).

During cardiovascular development, multiple changes occur that could alter the viscous behavior of embryonic blood. The hematopoietic system is not fully developed when blood flow is initiated, and the concentrations and types of cells that are circulating vary with the stage of development. Adult mammalian red blood cells are biconcave in shape, but embryonic blood cells from all species are spherical. For birds, blood cells remain spherical after birth, and previous work with adult avian blood has shown that the spherical red blood cells are less deformable in flow (15). This decreased deformability should cause a reduction in shear-thinning behavior. At low shear rates, spherical erythrocytes would also be expected to show a lower capacity for rouleaux formation, since less surface area for cell-cell interaction is present. Plasma proteins affect cell-cell interaction during rouleaux formation (8), and to our knowledge, no one has studied variations in plasma protein composition during early cardiovascular development. All of these changes have the potential to affect the viscous behavior of embryonic blood, but it is not known whether blood viscosity changes significantly as the embryo develops. It is known that the total peripheral resistance of the cardiovascular system decreases 40-fold between the onset of cardiac function (Ham-
burger Hamilton stage HH12) and embryonic day 6 (HH29) in chick embryos (18). Most of this reduction is likely due to changes in the structure of the vascular network and not the blood itself, but the possibility remains that viscous properties of blood vary as the cardiovascular system develops.

The main limitation to the measurement of viscosity of embryonic blood is the difficulty in collecting significant quantities of blood from an embryo. Traditional viscometers require several milliliters of fluid for measurements. In the early stages of cardiovascular development, this represents more blood than is present in the whole embryo. Recent developments in microfluidic technology have allowed small-volume viscometers to be developed that reduce the volume required for viscosity measurements. In these small-volume viscometers, the viscosity of a fluid is measured by flowing the liquid through a very small rectangular channel (3 mm wide × 50 μm high) into which four pressure sensors have been integrated. The apparent viscosity of the fluid can be calculated from the pressure drop along the flow channel for a known flow rate.

In this work, we present the values for embryonic chicken blood viscosity at different hematocrits (Hcts), different shear rates, and different stages in development. We found that embryonic blood displays shear-thinning behavior, similar to the adult. This is accompanied by the aggregation of spherical red blood cells at low shear rates. We also measured embryonic blood Hct and found an increase in Hct as the embryo gets older, but we found that the blood viscosity at a given Hct decreases as the embryo develops. Both the non-Newtonian effects and stage-dependent effects were limited to measurements at very high Hct. For shear rates above 50 s⁻¹ and at physiological levels of Hct, only Hct significantly affected the measured viscosity. We therefore developed a mathematical relationship between the Hct and apparent viscosity with respect to developmental stage.

Fig. 1. Methodology for the measurement of embryonic blood viscosity and validation. Blood was collected from vessels of either the yolk sac or chorioallantoic membrane of chicken embryos at stages between 4 and 8 days of incubation. A needle with a tip diameter of ~50 μm (arrow) was inserted at a point where two veins merged, and blood was collected from the vessel (A). Viscosity was measured in the microviscometer, which operates by flowing solutions at a known flow rate through a channel and measuring the pressure at four locations along the channel (B). From the pressure drop, it is possible to calculate the apparent viscosity of the fluid (C). To validate measurements, the viscosity of blood from adult chickens was measured with both the microviscometer and with a cone-plate viscometer at 23°C. No significant differences were observed between the two sets of measurements (D). The image of the channel reprinted with permission from Rheosense. Scale bar = 500 μm.

MATERIALS AND METHODS

Eggs. Fertilized chicken eggs (Gallus gallus) were obtained from the couvoir Simetin and incubated at 37°C and ~60% humidity for 4–8 days as noted. For 4 days of incubation, only embryos at HH22 were used. The chorioallantoic membrane (CAM) obscured visualization of the embryos before blood collection for embryos incubated for 6 or 8 days. For this reason, embryos were staged by the extent of CAM growth such that the CAM was between 2.5 and 3.5 cm in diameter at 6 days and between 4.5 and 6 cm in diameter at 8 days. We verified the embryonic stage after blood collection, and this largely corresponded to HH28 at 6 days of incubation and HH34 at 8 days of incubation. With the exception of the embryos incubated for 4 days, ~4 ml of albumin were removed from the eggs after 3 days using a syringe, and the puncture site was sealed with a small piece of tape. This prevented the CAM of the embryo from attaching to the upper surface of the eggs.

Blood collection. On the 4th, 6th, or 8th day, a small window was cut in the top of the shell to expose the embryo and associated extraembryonic membranes. Blood was collected using a small pulled glass needle inserted into a large vein of the extraembryonic circulation (Fig. 1A). The needle was pulled using a Sutter P97 needle puller such that the needle tip was ~50 μm in diameter. The amount of blood collected per embryo depended on the stage. It was possible to collect ~20 μl of blood from embryos incubated for 4 days and ~80–100 μl from the oldest embryos. Because of the small volume collected, 4 dozen eggs were required per sample for embryos at 4 days of incubation, 3 dozen eggs per sample for the embryos at 6 days of incubation, and 2 dozen eggs per sample at 8 days of incubation. Blood from the youngest embryos (4 and 6 days of incubation) did not clot during collection and could in fact be kept at 4°C for 1–2 wk without any clotting occurring. By 8 days of incubation, the embryonic blood had gained the capacity to clot within minutes when exposed to air. For consistency, an anticoagulant was used for all collection regardless of the embryonic stage. Before collection, 1 M EDTA was aspirated into the collection needle and completely expelled, leaving a trace amount on the capillary tube wall of the pulled...
needle. Blood from multiple embryos was pooled in an microcentrifuge tube on ice during collection.

After collection, blood was centrifuged for 10 min at 1,000 rpm and 4°C to separate the blood plasma from the blood cells. The plasma was centrifuged a second time at 14,000 rpm to remove any remaining blood cells. Blood cells and plasma were then mixed at varying concentrations to give the appropriate Hcts. Viscosities were always measured on the same day as blood collection. Samples were loaded into the 100-μl Hamilton syringe of the syringe pump of the viscometer. Pipetting a very viscous suspension, such as the red blood cells, is not highly accurate. For this reason, the final volume in the Hamilton syringe was used to calculate the Hct. The Hct was equal to the total volume in the syringe, minus the plasma volume we had added, divided by the total volume in the syringe. Hcts were valid within 1% of the reported average values.

**Viscosity measurements.** Viscosity was measured using a RheoSense mVROC microviscometer capable of measuring microliter-sized liquid samples. The liquid is fed into the sensor using a syringe pump, allowing for measurement of the viscosity at different flow rates and therefore at different shear rates. All measurements were made at 37°C. To ensure accuracy, the viscosity of water was measured three to five times before the blood viscosity was measured. The microchip was washed once with PBS (500 μl) and once with embryonic blood plasma (80 μl) before a sample was measured. The syringe with embryonic blood at the appropriate Hct was loaded into the syringe pump of the microviscometer. Samples were allowed to equilibrate in the viscometer for 30 min before being analyzed not only to allow the blood to assume the correct temperature but also to allow the blood to degas as the presence of small air bubbles affects the measurement capacity of the microviscometer. The presence of bubbles causes the viscosity measurements to temporarily increase 5- to 10-fold and create a nonlinear pressure drop across the sensor. Once the blood had rested in the syringe pump for 30 min, the syringe was detached from the pump and tapped to force the bubbles to the top, and a small amount of sample was expunged. The blood was remixed by gyrating and rotating the syringe and then replaced in the syringe pump. After every two to three measurements, the blood in the syringe would be remixed to prevent the red blood cells from settling.

Each sample was measured at a range of shear rates. The order in which shear rates were measured was randomized. In most cases, it was only possible to run one Hct measurement per day. In cases where two different Hct levels were measured in 1 day, the lower Hct was always run first. Wetted surfaces of the viscometer are made of borosilicate glass and silicon. The viscometer measures the pressure drop at four locations and plots the pressure with respect to distance during the measurement (Fig. 1B). A total of four pressure sensors are located along the flow channel: the first at 800 μm from the entrance and then at 3,700, 7,500, and 10,000 μm. Only measurements that gave a linear pressure drop based on the r² value were used. A minimum r² value of 0.9 was accepted, although the vast majority of measurements had r² values above 0.95.

The required entrance length for channel flow can be calculated from the following equation:

\[
\frac{L}{D_h} = \left[ \left(0.631 \right)^{1.6} + \left(0.0442 \times Re \right)^{1.6} \right]^{1/1.6}
\]

where the L is the entrance length, D_h is the hydraulic diameter (4 times the area of the channel divided by the circumference), and Re is

<table>
<thead>
<tr>
<th>Incubation Period</th>
<th>Hct, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 days</td>
<td>19.4 ± 1.0</td>
</tr>
<tr>
<td>6 days</td>
<td>23.5 ± 0.7</td>
</tr>
<tr>
<td>8 days</td>
<td>28.6 ± 1.4</td>
</tr>
</tbody>
</table>

Values are means ± SE. Hct, hematocrit.

---

**Table 1.** Average Hct values from blood collected at different stages of development

---

**Fig. 2.** Apparent viscosity of blood at different stages of embryonic development. The effect on both hematocrit and shear rate on blood apparent viscosity was measured at stages of 4 days of incubation (Hamburger-Hamilton stage HH22; A), 6 days of incubation (−HH28; B) and 8 days of incubation (−HH34; C). After collection, the blood was centrifuged to separate the plasma from the blood cells. The blood cells were then resuspended in blood plasma at the appropriate hematocrit (vol/vol). At each stage of development and for every hematocrit measured, 70–80 μl of blood were run per sample while the shear rate was varied. Error bars represent SEs for between 4 and 7 samples. The exact values for each point are shown in Tables 2–4.
Measurements of embryonic day 6 blood viscosity at different Hcts and shear rates

\[
\text{Re} = \frac{\rho Q D_h}{wd \mu}
\]

where \(\rho\) is the density, \(Q\) is the volumetric flow rate, \(w\) is the channel width, \(d\) is the channel depth, and \(\mu\) is the viscosity of the blood. For all shear rates and measured viscosities, \(\text{Re}\) values ranged between \(\text{Re} = 0.001\) (shear rate of 20 s\(^{-1}\) and viscosity of 13.75 cP) and 0.85 (shear rate of 1,000 s\(^{-1}\) and viscosity of 0.91 cP). This resulted in a required entrance length between 62.1 and 62.5 \(\mu\)m. The first pressure sensor is located 800 \(\mu\)m from the viscometer entrance. Therefore, entrance effects should not be present in the acquired measurements.

The calculation of apparent viscosity (\(\mu_{\text{app}}\)) assumes two-dimensional flow between two parallel plates such that:

\[
\gamma_{\text{app}} = \frac{6Q}{wh^2}
\]

\[
\tau = -\text{slope}\left(\frac{wh}{2w + 2h}\right)
\]

\[
\mu = \frac{6Q\tau}{\gamma_{\text{app}}}
\]

where \(h\) is the height of the channel, \(\gamma_{\text{app}}\) is the calculated shear rate, and \(\tau\) is the calculated shear stress. Only measurements that gave a steady viscosity within 1 s were accepted (Fig. 1C). For a given sample, the viscosity was measured twice at all shear rates and averaged. At each stage of development and for each Hct, between three and seven different blood samples were analyzed. After each set of measurements, the viscometer was cleaned by running blood plasma and then PBS-Tween until the outlet ran clear. Bleach was then run through the system at a very low flow rate for 20 min. The system was rinsed several times with water, and the viscosity of water was measured to ensure that the flow channel was properly cleaned.

Validation of the measurements were performed by measuring adult chicken blood viscosity in the microviscometer and in a Bohlin CVO120 HRNF cone and plate viscometer with a 4\(^\circ\)20-mm cone. Chicken blood collected with EDTA was purchased from Lampire Biological Laboratories (cat. no. 7201408) and shipped overnight. The viscosity of the adult chicken blood was measured at 23°C and at shear rates of 100, 200, 500, and 1,000 s\(^{-1}\).

RESULTS

Blood was collected from embryos by drawing blood from veins of the embryonic yolk sac and CAM at different stages of development. The blood was centrifuged, and the average Hct for each embryonic stage was measured (Table 1). We found that the Hct increased linearly as the embryo developed from a value of Hct = 19.4% after 4 days of incubation (HH22) to a value of Hct = 28.6% after 8 days of incubation (−HH34) (n = 4–6 depending on stage). For comparison, the Hct of adult chicken blood is 35% (14).

We first sought to validate our measurements with the microviscometer. When blood flows through cylindrical channels with diameters of <300 \(\mu\)m, a decrease in the apparent Hct and a decrease in the apparent viscosity of blood are observed compared with the feed Hct. This is known as the Fahreus-Lindqvist effect (13). Although the channel of the microviscometer channel is 3 mm wide, its depth is only 50 \(\mu\)m. We therefore machined a clear flow channel of the same dimensions as the viscometer and flowed blood through the

<table>
<thead>
<tr>
<th>Hct</th>
<th>20 s(^{-1})</th>
<th>50 s(^{-1})</th>
<th>100 s(^{-1})</th>
<th>200 s(^{-1})</th>
<th>500 s(^{-1})</th>
<th>1,000 s(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>0.99 ± 0.15</td>
<td>0.97 ± 0.13</td>
<td>1.01 ± 0.18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5%</td>
<td>1.44 ± 0.09</td>
<td>1.35 ± 0.14</td>
<td>1.17 ± 0.11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7%</td>
<td>1.35 ± 0.15</td>
<td>1.18 ± 0.14</td>
<td>1.21 ± 0.07</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11%</td>
<td>1.43 ± 0.21</td>
<td>1.48 ± 0.02</td>
<td>1.45 ± 0.11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17%</td>
<td>1.36 ± 0.18</td>
<td>1.54 ± 0.19</td>
<td>1.59 ± 0.19</td>
<td>1.39 ± 0.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25%</td>
<td>2.28 ± 0.37</td>
<td>1.90 ± 0.17</td>
<td>1.62 ± 0.13</td>
<td>1.50 ± 0.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29%</td>
<td>2.14 ± 0.02</td>
<td>1.89 ± 0.18</td>
<td>1.65 ± 0.10</td>
<td>1.60 ± 0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>36%</td>
<td>2.17 ± 0.14</td>
<td>2.30 ± 0.34</td>
<td>2.56 ± 0.29</td>
<td>2.55 ± 0.44</td>
<td>2.49 ± 0.42</td>
<td></td>
</tr>
<tr>
<td>52%</td>
<td>4.56 ± 0.61</td>
<td>4.24 ± 0.34</td>
<td>3.64 ± 0.44</td>
<td>3.34 ± 0.50</td>
<td>3.10 ± 0.30</td>
<td>2.99 ± 0.36</td>
</tr>
<tr>
<td>78%</td>
<td>12.16 ± 1.73</td>
<td>10.42 ± 1.26</td>
<td>9.79 ± 1.49</td>
<td>8.57 ± 1.21</td>
<td>7.31 ± 1.05</td>
<td>7.21 ± 1.18</td>
</tr>
</tbody>
</table>

Values are means ± SE (in cP).
channel to observe the channel Hct. Collected blood was stained with DiI, a lipophilic membrane dye, before being flowed through the “mock” viscometer. For a feed of Hct /H11005 20%, we found that the measured channel Hct was 22/H11006 2%. For a feed of Hct /H11005 40%, we found the measured channel Hct was 39/H11006 2%. Therefore, no differences between the feed and channel Hcts was found for the geometry of the microviscometer. The Fahreus-Lindqvist effect is not the only concern in a microfluidic viscometer. The calculation of viscosity by the microviscometer assumes a no-slip condition at the wall. Fluid slip at the wall has been observed in microfluidic devices (27). We therefore further validated the measurements by the microfluidic viscometer by testing the same samples of adult chicken blood in both the microviscometer and a cone-and-plate viscometer (Fig. 1D). Both instruments measured similar values for the viscosity of the samples at all shear rates measured.

We found that the apparent viscosity of embryonic blood was shear rate dependent (Fig. 2). At all values of Hct tested and for all stages of development, the apparent blood viscosity

![Fig. 3. Trends in apparent viscosity with developmental stage. When the data were plotted with respect to the stage of the embryo, a decrease in apparent viscosity was observed at the higher values of hematocrit (1,000 s⁻¹; A). To study the effect of plasma proteins on this decrease, red blood cells were washed in PBS and resuspended in PBS at a hematocrit of 40%. The viscosity of the suspension decreased with embryonic stage (B). Viscosity values from shear rates above 200 s⁻¹ were averaged and plotted with respect to hematocrit (C). Regression of these curves gave an exponential term that decreased linearly with respect to days of incubation (D). Blood samples from embryos incubated for 4 days showed that a fairly homogenous cell population was present (E). By 8 days of incubation, the cellular components of blood varied in size and shape, indicating that a mixed population of cells was present (F). Scale bar = 30 μm.](http://ajpheart.physiology.org/)

Values are means ± SE (in cP).
remained fairly constant for shear rates above 200 s$^{-1}$. When the blood with higher Hct (52% and 78%) was examined, an increase in the apparent viscosity was present with decreasing shear rates between 20 and 200 s$^{-1}$. This non-Newtonian behavior was present at all stages of embryonic development that were studied. The exact values and associated SEs at each shear rate, Hct, and developmental stage are shown in Tables 2–4. The microviscometer requires a minimum pressure drop for accurate measurements. Since the pressure drop is related to both the shear rate and viscosity of the solution, we were unable to analyze the viscosity of blood at both low shear rate and low Hct. From work using adult human blood, non-Newtonian behavior is observed at shear rates below 1 s$^{-1}$ at a Hct of 36% (5). This is not within the measurement range of the instrument.

We observed changes in whole blood viscosity with respect to the stage of embryonic development. When the viscosity was plotted with respect to developmental stage at any of the shear rates tested, we consistently observed a decrease in the apparent viscosity in older embryos compared with younger embryos (Fig. 3A). By 8 days of incubation, the values from the embryonic blood were similar to those measured using adult chicken blood. We found no statistical difference in the viscosity of the blood plasma at the different stages of development examined (Tables 2–4). The average viscosity for blood plasma was found to be 0.97 cP at 37°C. The blood collected from older embryos (HH34, 8 days of incubation) would clot without the addition of EDTA, whereas the blood from younger embryos did not (4 and 6 days of incubation, HH22 and HH28). We therefore investigated whether plasma proteins were responsible for this decrease in apparent viscosity. We washed and resuspended red blood cells from each stage in PBS at a Hct of 40% and tested the apparent viscosity with shear rate. We found that the decrease in apparent viscosity with developmental stage persisted even in the absence of plasma proteins (Fig. 3B).

We next considered the relationship between Hct and the apparent viscosity of blood. We restricted the analysis to the region where apparent viscosity was independent of shear rate (above 200 s$^{-1}$). We averaged viscosity values in this range and plotted this with respect to Hct (Fig. 3C). The data were then regressed to fit an exponential equation for each stage of development. When the exponential term from the regression was analyzed, we observed that the term decreased linearly with the period of incubation (Fig. 3D). Therefore, all of the data (from all stages of incubation) could be modeled by the following equation:

$$\mu_{\text{app}} = 0.9664 e^{\text{Hct}(0.0333 - 0.0016D)}$$

where Hct was measured as percentage, D is the number of days of incubation, and $r^2 = 0.943$. The data could also be modeled by the following equation:

$$\mu_{\text{app}} = 0.9664 e^{\text{Hct}(0.0386 - 0.0005 HH)}$$

where HH is the Hamburger-Hamilton stage and $r^2 = 0.958$. Blood smears at 4 and 8 days showed differences in cellular composition of the blood at different stages (Fig. 3, E and F), highlighting the developing complexity to the nature of blood cells.

Several models exist to describe the shear-dependent behavior of blood, such as the power law, the Casson model, and the Carreau model. For low Hcts, we could not obtain data for the

The power law model is as follows: $K\gamma^{n-1}$; the Casson model is as follows: $\tau_\gamma + \eta + 2\sqrt{\eta\gamma}$; and the Carreau model is as follows: $\mu_s + \left(\mu_0 - \mu_s\right) \times \left[1 + (\lambda\gamma)^2\right]^{-1/2}$.

---

**Table 5. Modeling of the non-Newtonian behavior of embryonic blood**

<table>
<thead>
<tr>
<th></th>
<th>4 Days of Incubation</th>
<th>6 Days of Incubation</th>
<th>8 Days of Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hct = 52%</td>
<td>Hct = 78%</td>
<td>Hct = 52%</td>
</tr>
<tr>
<td><strong>Power law model</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K$, Pa$^{n}$</td>
<td>0.010</td>
<td>0.022</td>
<td>0.006</td>
</tr>
<tr>
<td>$n$</td>
<td>0.853</td>
<td>0.847</td>
<td>0.885</td>
</tr>
<tr>
<td>$\tau^2$</td>
<td>0.933</td>
<td>0.997</td>
<td>0.962</td>
</tr>
<tr>
<td><strong>Casson model</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\tau_s$, N</td>
<td>0.011</td>
<td>0.040</td>
<td>0.006</td>
</tr>
<tr>
<td>$\eta$, Pa$^{-1}$s</td>
<td>0.0035</td>
<td>0.0066</td>
<td>0.0027</td>
</tr>
<tr>
<td>$\tau^2$</td>
<td>0.980</td>
<td>0.811</td>
<td>0.922</td>
</tr>
<tr>
<td><strong>Carreau model</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\mu_0$, Pa$^{-1}$s</td>
<td>0.0074</td>
<td>0.0154</td>
<td>0.0047</td>
</tr>
<tr>
<td>$\mu_s$, Pa$^{-1}$s</td>
<td>0.0037</td>
<td>0.0037</td>
<td>0.0029</td>
</tr>
<tr>
<td>$\lambda$, s</td>
<td>0.033</td>
<td>0.076</td>
<td>0.020</td>
</tr>
<tr>
<td>$\kappa_c$, s</td>
<td>0.75</td>
<td>0.75</td>
<td>0.70</td>
</tr>
<tr>
<td>$\tau^2$</td>
<td>0.994</td>
<td>0.998</td>
<td>0.995</td>
</tr>
</tbody>
</table>

The power law model is as follows: $K\gamma^{n-1}$; the Casson model is as follows: $\tau_\gamma + \eta + 2\sqrt{\eta\gamma}$; and the Carreau model is as follows: $\mu_s + \left(\mu_0 - \mu_s\right) \times \left[1 + (\lambda\gamma)^2\right]^{-1/2}$. 

---

*Source: AJP-Heart Circ Physiol* • VOL 301 • DECEMBER 2011 • www.ajpheart.org
low shear values where non-Newtonian behaviors occur. We therefore modeled the data from Hcts of 52% and 78% using the three most common models for blood viscosity. Figure 4 shows data from 4-day-old embryos at a Hct of 52% fitted with three models, and Table 5 shows the parameters for all three models at all three stages tested. The Carreau model consistently provided the best fit to the data.

In adult human blood, the increase in viscosity observed at low shear rates is associated with rouleaux formation. Rouleaux are believed to arise either from protein interactions between adjacent cells (7) and/or by osmotic water exclusion between adjacent cells (28). Water exclusion is largely enhanced by the biconcave shape of adult human blood. Since avian embryonic blood is spherical, we investigated whether these cells could also aggregate under low shear. We placed a dilute suspension of blood cells in plasma on a slide and induced low shear conditions by slowly swirling the suspension on the slide for 2 min. At all stages of development that we tested, we observed the interaction and aggregation of the blood cells (Fig. 5). These aggregates did not form as linear rouleaux but rather formed large nonuniform clusters of cells. This, however, may be due to our method of inducing low shear conditions. If we did not swirl the samples, aggregates did not form even after 20 min.

**DISCUSSION**

Hemodynamic stimuli are essential for proper vascular development, but the study of hemodynamics during development has been hampered by a basic lack of knowledge of the level of shear stress present in the early embryo. Basic assumptions regarding the viscosity of embryonic blood have been made because the measurement of embryonic blood viscosity has not been possible in the past. The advent of micromachining that has allowed the creation of “lab-on-a-chip” technologies is breaking these barriers. In this work, we have presented the first measurements of chicken embryonic blood viscosity using a microviscometer. We found that embryonic blood displays non-Newtonian behavior similar to that of adult blood.

Published shear rates for the chick embryonic vasculature have indicated that for the heart and for most large blood vessels, embryonic blood can be treated as a Newtonian fluid (Table 6). Shear rates in some regions of the heart of 3-day-old chicken embryos have been reported to be as high as 800 s\(^{-1}\) (31). In the extraembryonic vasculature, shear rates vary between 10 and 75 s\(^{-1}\) during systole and diastole (12, 32). The Hct of embryonic blood is initially much lower than observed in the adult chicken. The Hct increases as the embryo develops, but even at 8 days of development, the embryonic blood does not have as high a concentration of red blood cells as the adult. Adult human blood does not display shear-dependent changes in viscosity until the Hct is above 30% at a shear rate of 15 s\(^{-1}\) (5). We measured viscosity at shear rates as low as 50 s\(^{-1}\) for a Hct of 36% and did not observe non-Newtonian behavior. We conclude that, at least for larger vessels, embryonic blood viscosity can be calculated based on Hct alone without introducing significant errors. Although we observed a decrease in

Table 6. **Summary of published shear rates in chick embryonic blood vessels**

<table>
<thead>
<tr>
<th>Embryonic Stage</th>
<th>Location</th>
<th>Shear Rate and Velocity Measurements</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HH15</td>
<td>Heart (ventricle)</td>
<td>Peak: 26 mm/s and (-1,000) s(^{-1})</td>
<td>35</td>
</tr>
<tr>
<td>HH18–HH23</td>
<td>Heart (ventricle)</td>
<td>Peak: 30 (HH18) to 63 (HH23) mm/s</td>
<td>30</td>
</tr>
<tr>
<td>HH18</td>
<td>Outflow tract</td>
<td>Peak: 40 mm/s and 400–800 s(^{-1})</td>
<td>31</td>
</tr>
<tr>
<td>HH18</td>
<td>Outflow tract</td>
<td>Peak: 35–50 mm/s</td>
<td>26</td>
</tr>
<tr>
<td>HH18–HH23</td>
<td>Outflow tract</td>
<td>Peak: 60 (HH18) to 90 (HH23) mm/s</td>
<td>30</td>
</tr>
<tr>
<td>HH12–HH29</td>
<td>Dorsal aorta</td>
<td>Mean: 0.9 (HH12) to 15 (HH29) mm/s</td>
<td>18</td>
</tr>
<tr>
<td>HH16–HH28</td>
<td>Dorsal aorta</td>
<td>Peak: 18 (HH16) to 57 (HH28) mm/s</td>
<td>34</td>
</tr>
<tr>
<td>HH18–HH27</td>
<td>Dorsal aorta</td>
<td>Mean: 3 (HH18) to 11 (HH27) mm/s</td>
<td>10</td>
</tr>
<tr>
<td>HH18–HH29</td>
<td>Dorsal aorta</td>
<td>Mean: 3 (HH18) to 15 (HH29) mm/s</td>
<td>11</td>
</tr>
<tr>
<td>HH20–HH35</td>
<td>Dorsal aorta</td>
<td>Mean: 4 (HH20) to 20 (HH35) mm/s</td>
<td>4</td>
</tr>
<tr>
<td>HH17</td>
<td>Extraembryonic</td>
<td>Peak: 25–75 s(^{-1})</td>
<td>12</td>
</tr>
<tr>
<td>HH17</td>
<td>Extraembryonic</td>
<td>Mean: 1.5–20 s(^{-1}) and 0.4–4 mm/s</td>
<td>6</td>
</tr>
<tr>
<td>HH18</td>
<td>Vitelline artery</td>
<td>Peak: 10–70 s(^{-1}) and 0.75 mm/s</td>
<td>32</td>
</tr>
<tr>
<td>HH18</td>
<td>Vitelline artery</td>
<td>Peak: 1.5 mm/s</td>
<td>23</td>
</tr>
<tr>
<td>HH18</td>
<td>Extraembryonic</td>
<td>Peak: 2 and 3.7 mm/s</td>
<td>24</td>
</tr>
</tbody>
</table>

Peak values represent the centerline velocity during the fastest flow of the cardiac cycle (i.e., peak systole for blood vessels). Mean velocities were averaged over the cardiac cycle. HH, Hamburger-Hamilton stage.
the apparent viscosity with developmental stage, this is only significant at high nonphysiological Hct values.

Our results indicate that the decrease in apparent viscosity with developmental stage is due to change in cellular composition and not plasma protein since this decrease is present even when blood cells are resuspended in PBS. The cellular composition of embryonic blood does change significantly during development. When we regressed the data within the Newtonian range to an exponential equation, the exponential term decreased linearly with respect to embryonic stage. The $r^2$ value for this relationship was astonishingly good (0.943 or 0.958). It should be noted, however, that if only data from the physiological range (Hcts of 0–40%) was used, no such trend was present. Therefore, the regression is heavily influenced by the high Hct, nonphysiological data. Furthermore, the value for the slope is so small that the correlation should be viewed with some scepticism. Interestingly, if our data from blood of adult chicken is regressed to fit an exponential equation (not shown), the exponent term equates to a stage of 9 days of incubation or to embryonic stage HH39, depending on the equation used.

The measurements of blood viscosity that we report here will allow the accurate assessment of wall shear stress in developing avian embryos. Shear stress is a very important signal for the developing cardiovascular system. Genes that are known to be induced by flow in the adult, such as as Krüppel-like factor 2, have been shown to be essential for proper vascular development and expressed in a pattern associated with flow disturbances during vascular development (22). A complete understanding of the interaction of mechanical forces and gene activation during vascular development is only possible if we can measure these quantities in situ. By combining our present results of the shear-dependent and stage-dependent behavior of blood viscosity with the measurement of flow dynamics produced by other groups, the measurement of wall shear stress is now possible. Knowledge of these values can bridge in vitro and in vivo systems used to study shear stress-induced gene regulation and thereby accelerate our understanding of the molecular mechanisms involved in flow-induced remodeling during development.

ACKNOWLEDGEMENTS

The authors thank Parnaz Tabrizian, Rozoebaf Safavieh, and David Juncker for the micromachining of the transparent flow channel. The authors also thank Seong-gi Baek and Ying-Chih Wang at the Rhoesense Corporation for the exceptional technical support.

GRANTS

This work was supported by grants from Natural Science and Engineering Research Council, the Foundation des Maladies du Coeur du Québec, the Fonds Québécois de la Recherche sur la Nature et les Technologies, and the Canada Research Chairs program. The microviscometer was purchased through the Research Tools and Innovation program of the Natural Science and Engineering Research Council.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


