Flow of human blood through fabricated replicas of microvascular bifurcations
by Bruce Miles Fenton

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in CHEMICAL ENGINEERING
Montana State University

Abstract:
In view of the extremely limited knowledge of the relationships between the macroscopic rheological properties of blood and the microscopic behavior of the constituents of blood during flow through the actual geometries present in the microcirculation (such as bifurcations, capillary networks, etc.) vascular replicas of sections of rabbit ear blood vessels were studied.

The main objective of this study was to determine the feasibility of using these replicas in the mathematical prediction and subsequent measurement of the pressure flow relationships of human blood crossing a bifurcation.

Using both Newtonian (distilled water, S-3 silicone oil, and S-60 silicone oil) and non-Newtonian (red blood cell suspensions in plasma) fluids, differential pressures could be measured across the system and the effect of the flow distribution could be studied. The applicability of various mathematical models to non-uniform tapered tubes was investigated and it was determined that a "series of cylinders" approach was a close approximation for calculating pressure gradients across the tube (in reference to the specific vessel geometries studied.) Based on experimental results presented, it was concluded that the prediction of a large pressure drop at a bifurcation is not supported by the experimental data, but is instead directly contradicted, with the measured pressure gradient being almost exactly equivalent to that predicted in the absence of branching.

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Date 9-9-75

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BRUCE MILES FENTON

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Head, Major Department

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ABSTRACT

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INTRODUCTION

In an attempt to quantitatively understand both the microscopic and macroscopic behavior of blood as it flows through the human body, much effort has been devoted to investigations of the distribution of the circulatory system. The question of blood flow distribution is thought to be an important one for several reasons. Most importantly perhaps is the fact that in organs where transfer of vital materials takes place, such as the lungs, alterations in blood flow distribution may affect the well-being of the entire organism.

Components of the Blood

Human blood is a suspension of formed elements, in a liquid medium known as plasma. The formed elements consist of red blood cells (RBC's), white blood cells, and platelets. The red blood cells, also known as erythrocytes, make up the major portion (about 97%) of the cell volume and have the undeformed shape of a biconcave disc. The cells average approximately eight microns in diameter and two microns in thickness at the widest point. Since the red blood cell is essentially a thin membrane filled with fluid, it is very flexible and easily deformed during flow.

The plasma is the liquid portion of the blood and represents approximately 55 per cent of the volume of whole blood in healthy

individuals. It is composed of 90 per cent water, with various chemical substances in solution. These include proteins, lipids, carbohydrates, non-protein-nitrogen compounds, and inorganic materials.

The white blood cells, or leukocytes are the least numerous but the largest of the cells, and the platelets are the smallest. Since the leukocytes have been shown to exert a negligible effect on flow properties when in normal physiological concentrations, these cells and the platelets are removed for the purpose of this study. This resulting absence of platelets also reduces the tendency for the blood to form aggregates.

The hematocrit (ht) is defined as the volume per cent red blood cells per volume whole blood. It can be determined by centrifuging a capillary tube full of blood and measuring the relative length of the packed red cells to total volume. This method is 99 per cent accurate, as approximately one per cent of the RBC volume is trapped plasma. After removal of the platelets and leukocytes, various hematocrits are obtained by mixing different suspensions of RBC's in plasma.

Plasma Skimming

It has been reported by many investigators that the percentage of red blood cells in the whole blood (hematocrit) is lower in the capillary bed than in the larger blood vessels of the central circulation,

although the extent to which the hematocrit is reduced varies from organ to organ (Lawson, 1967). This reduction in concentration, which occurs in the more slowly flowing branch of a bifurcation, has come to be known as plasma skimming and has been the object of a variety of *in vivo* and *in vitro* studies. Gelin (1963) perfused blood through branched capillary models to permit closer analysis of plasma skimming and the flow properties of the blood, while eliminating physiological influences. Bugliarello and Hsiao (1964) flowed a suspension of spheres through a bifurcation as a simplified macroscopic model of the flow of blood and found that the concentration in the side branch was generally lower than in the main branch. Johnson (1971) used *in vivo* studies to measure average blood opacities in capillaries of the cat mesentery. Using these opacities as a quantitative measure of the hematocrit, vessels within the capillary network showed sizable differences in opacity. It was found that the capillaries with the higher red cell velocity also had the higher opacities. The reader is cautioned at this point that these opacity readings are not necessarily a measure of plasma skimming exclusively. As was well documented by Barbee and Cokelet (1971) the presence of a reduced hematocrit inside vessels less than 300 microns is a normal phenomena (known as the Fahraeus effect) even in the absence of bifurcations.

For both the *in vivo* and *in vitro* studies, there are advantages and disadvantages. *In vivo* studies have the advantage of exact

simulation of the actual vessel characteristics and geometries, but the accompanying disadvantages of reduced control of specific parameters and possible experimental interference with the normal hormonal, local, and neurological controls of the vascular system. *In vitro* studies, on the other hand, have permitted greater control of a number of the parameters affecting plasma skimming but have neglected the effects of such variables as vessel wall permeability and elasticity, pulsatile flow, and inertial effects due to actual vessel geometries (including curvature and taper).

In an effort to obtain the best features of both *in vitro* and *in vivo* measurements, the present study utilizes hollow vascular replicas formed from polyester resin (see Cokelet and Meiselman (1975)).

> The advantages of this type of flow system over the comparable *in vivo* preparation are its stability, transparency, the constancy of the geometry of the network of vessels under varying flow conditions, the impermeability of the walls of the flow channels, and the realistic, real-size representation of the vascular system. Not only can experimental blood flow data obtained from such a replica be compared with theoretical models of vascular systems, but contributions to flow resistance from such *in vivo* factors as vessel distensibility and vessel wall permeability can be assessed by the comparison of data obtained with *in vivo* and replica systems.

In view of the extremely limited knowledge of the relationships between the macroscopic rheological properties of blood and the microscopic behavior of the constituents of blood during flow through the actual geometries present in the microcirculation (such as bifurcations, capillary networks, etc.) vascular replicas should prove especially useful in defining the parameters involved. This type of research could become particularly helpful in understanding pathological situations.

Research Objectives

The main objective of this study was to determine the feasibilty of using vascular replicas in the study of the pressure/flow relationships of human blood crossing a bifurcation.

Originally, the principle emphasis for this research was to have been on plasma skimming and the resultant erythrocyte distribution across a bifurcation. Attempts were to have been made to determine whether correlations could be found between some of the variables which could not be investigated using large scale models; in particular, the shape and flexibility of the red blood cells. As the work progressed however, it was seen that both of the models selected were in a size range too large for the occurrence of any signifigant plasma skimming. From this point on, the focus was on mathematically defining the

differential pressure across a bifurcation using both Newtonian and non-Newtonian fluids. From this work, it was hoped that the various parameters affecting this pressure gradient could be determined; in particular, the effect of the fractional flow through the side branch of the bifurcation.

EXPERIMENTAL APPARATUS AND PROCEDURE

The major portion of the research for this project was performed using two models for the study of pressure/flow relationships of various fluids (including human blood), and one for the measurement of the reduction in hematocrit present inside a small vessel, a phenomena known as the Fahraeus effect (see Barbee and Cokelet, 1971). Each of these phases of the research will be discussed separately, including details for the fabrication of the vascular replicas used.

Blood

Human blood was obtained in ACD anti-coagulant by normal blood bank procedures without preference to specific types. Suspensions of red blood cells (RBC's) in plasma were prepared by 1) centrifugation of whole blood (using a Sorvall RC2-B automatic refrigerated centrifuge) at 5000 rpm for 15 minutes, 2) removal of platelets and leukocytes using a large diameter hypodermic needle and syringe, and 3) similar separation of red blood cells from plasma. Various hematocrits were then obtained by mixing specific volumes of RBC's and plasma together.

Viscosity Measurements

The viscometer used was a Wells-Brookfield Micro Viscometer (cone and plate type), which was calibrated using the S-3 viscosity standard. Measurements were made on each blood sample at shear rates of 300, 750, and 1500 inverse seconds, at the recorded run temperature.

Viscosity measurements were also made using the GDM viscometer. This viscometer is a concentric cylinder type that is used to make viscosity measurements of RBC suspensions at shear rates from .06 to 60 inverse seconds. It was designed by Gilinson, Dauwalter, and Merrill, precisely for the measurement of non-Newtonian properties, and has a precision of nearly .1% for stress measurements. Torque/rotational speed data were reduced to shear stress-shear rate values by use of a computer program utilizing the Krieger-Elrod equation (see reference 9).

Calibration Techniques

Nominal flow rates were available for the Harvard Apparatus syringe pump, but for each specific syringe, more accurate rates were found by measuring mercury displacements at a number of different flow rates.

The pressure transducer was calibrated using a mercury manometer. For this model, actual induced pressures were recorded along with the accompanying digital voltmeter readings. A statistical least-squares

computer program was then used to correlate the experimental data and obtain straight line equations for each of the necessary attenuations of the amplifier. This calibration was checked periodically and readjusted as necessary.

Fabrication of Vascular Replicas

The method for fabricating the vascular replicas used in this study is thoroughly described elsewhere by Cokelet and Meiselman (1975), whose work can be briefly summarized as follows:

Ears obtained from male New Zealand rabbits are perfused, in sequence, with three liquids: (1) isotonic saline, to wash the blood out of the vascular system; (2) silicone oil, to provide a more favorable interface for the gallium; and (3) gallium, to form a replica of the vascular system.

Since gallium has a melting point of 29.8 C, it could be injected into the blood vessels in a liquid state and then allowed to solidify at normal room temperature. After solidification of the gallium, an enzymatic process was used for removal of the ear tissue. This process required from three to six weeks and utilized a protein denaturing solution of urea and $CaCl_2$ and a solution of pancreatin in a phosphate buffered saline. The tissue was subjected to a sequence of alternate solutions, repeated until all of the tissue

was removed from the gallium. The gallium network could then be separated into the desired bifurcations, which were then cast using a polyester resin (Silmar Polyester Resin S-40, Vistron Corp., with MEC catalyst, Norac Co.).

When the resin had hardened, holes were drilled into the vessels and the gallium was removed using first hot water and then a concentrated solution of hydrocholoric acid. This left only the hollowed out replication of the circulatory system, which was a transparent, impermeable, three-dimensional replica of the desired vessels. Reproducibility of the vascular sections was so keen, that actual endothelial cells could be seen in scanning electron micrographs of the model along with banding suggestive of vascular smooth muscle.

Model I Apparatus

For this portion of my research, an experimental setup was used as shown in Figure 1. This apparatus consisted of a syringe pump, connecting sections of polyethylene tubing, a polyester resin block containing a hollow replica of a section of rabbit-ear blood vessel, and a pressure transducer with accompanying amplifier and digital voltmeter readout.

Brief descriptions of the various components are as follows:

(1) The syringe pump was a Harvard Apparatus Co. Model 902

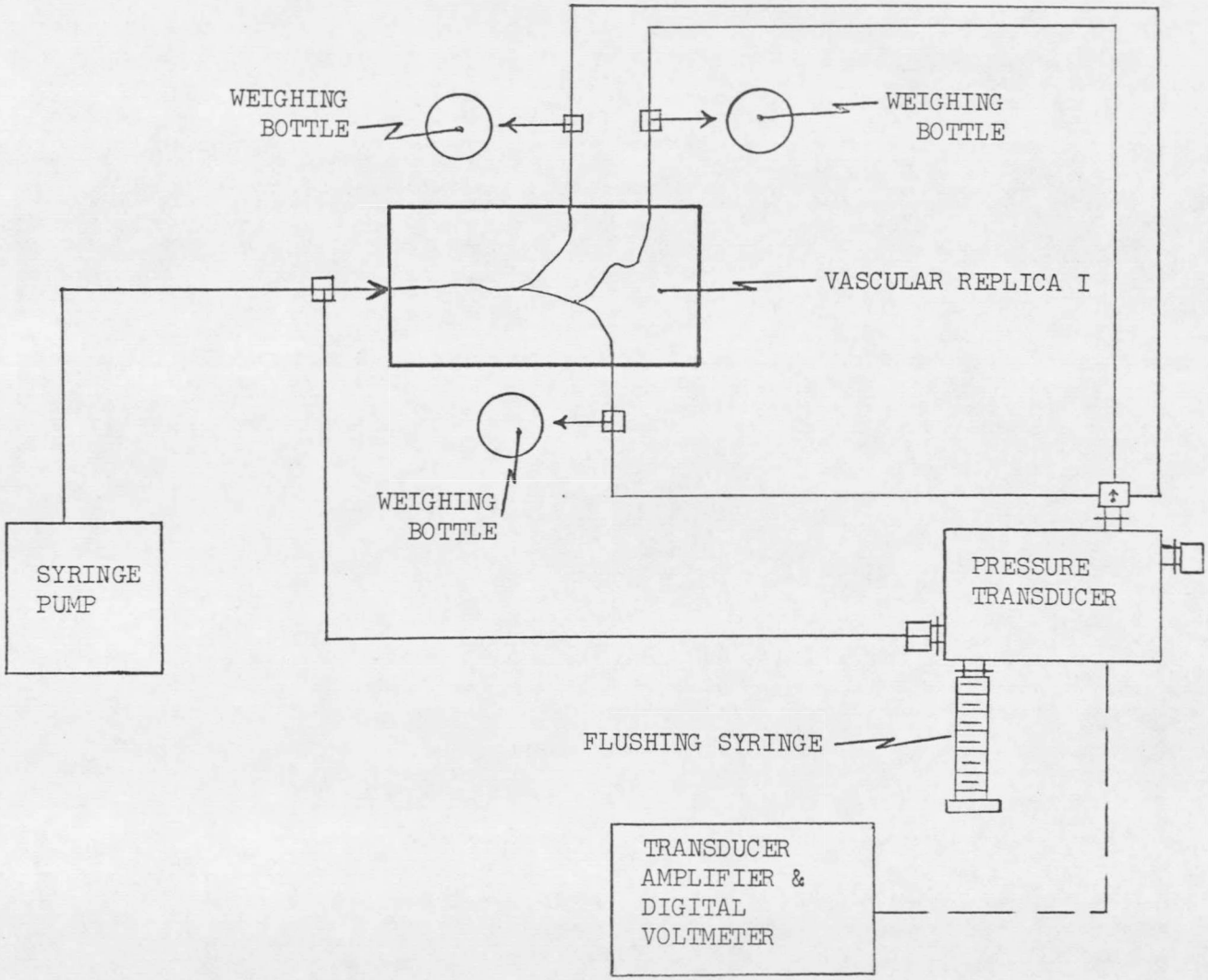

Figure 1. Schematic of Model I Apparatus

Infusion/withdrawal pump, which when fitted with 30 and 50 ml gas-tight syringes, could provide volumetric flow rates ranging from .0154 to 117.3 ml/minute.

(2) Connecting tubing for fluid flow was .105 inch medical grade polyethylene tubing (Becton, Dickinson, and Co.) with two-way and three-way valves and connecting fittings (Hamilton Co.).

(3) Connecting tubing for the pressure transducer leads was .023 inch I.D. polyethylene tubing. This particular size was chosen in order to eliminate air bubbles throughout the system and was filled with the fluid being tested (except in the case of red blood cell suspensions, in which case the leads were filled with plasma).

(4) The transducer used was a Sanborn Model 267BC differential pressure transducer (permitting both absolute and differential measurements) attached to a Sanborn Model 311A transducer amplifier. A Systron-Donner digital voltmeter was also added for instantaneous readout of the amplified transducer voltage.

(5) A 30 ml flushing syringe was utilized for the elimination of any air bubbles in the transducer and accompanying leads before commencement of a run.

(6) Mass flow rates through each of the branches were determined using three weighing bottles and a stop watch.

(7) Connections from the outside of the polyester resin model to the actual vessel inside were made using three 27 gauge blunt

hypodermic needles cemented in place using epoxy hardener.

Model II Apparatus

The apparatus for this model was similar to that used for Model I, although there was one major improvement to the system. In the preliminary experiments, all differential pressure measurements contained a certain amount of experimental uncertainty introduced by the drive fluctuations of the syringe pump. These fluctuations were caused by the slightly pulsatile flow of the pump, which in turn was produced by slight bows in the rotating screw mechanism which moved the syringe plunger. In order to minimize this uncertainty, a vessel was fabricated from glass tubing as illustrated in Figure 2. Fluids flowing from the syringe pump passed through this pressure damper, and then on to the vascular model. The sinusoidal pressure variations created by the pump were damped out by means of the air space above the blood reservoir, and after steady state had been reached, the transducer readout was seen to be essentially constant.

For measurements involving blood, it was also necessary to add a magnetic stirrer to the damper to prevent the settling out of RBC's when using low flow rates (as the experiment progressed, it was seen that a vessel of this type was required on the basis of its stirring capabilities alone, in order to provide a constant hematocrit feed

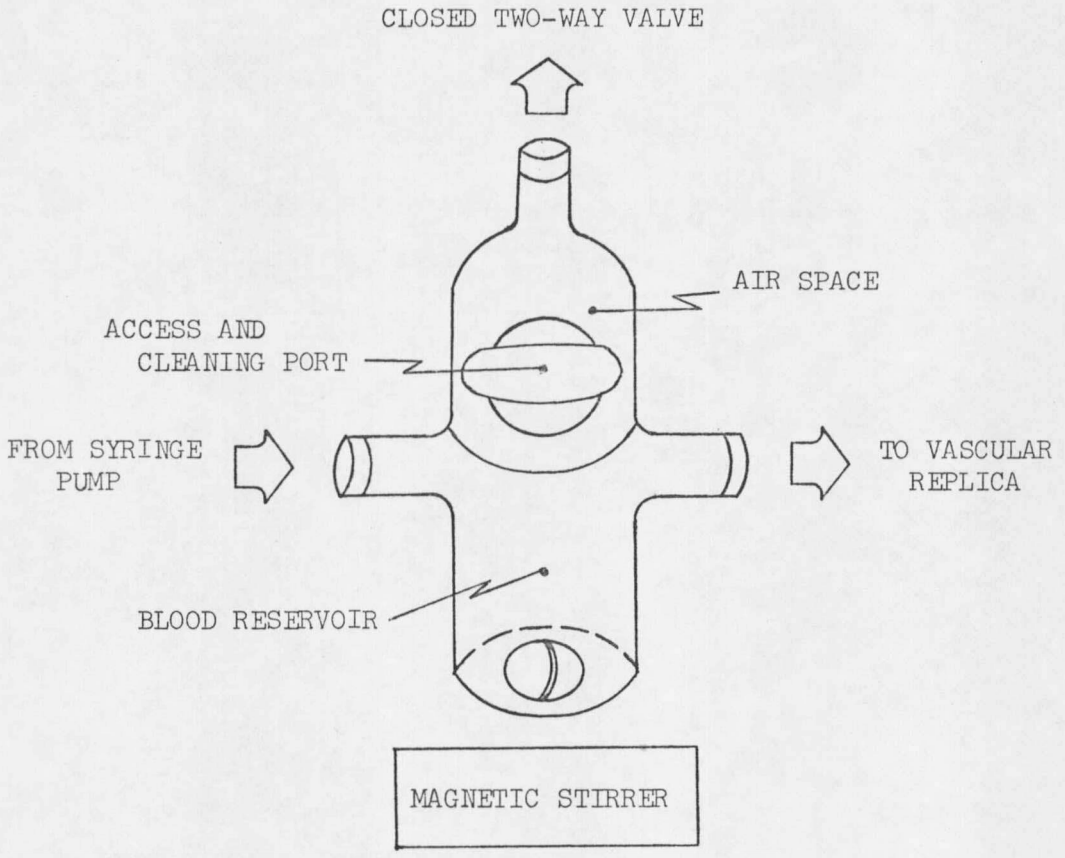

Figure 2. Pressure Damper and Stirrer