A Parametric Study of Blood Flow in the Microvessels

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Background

The circulatory system plays a vital role in the maintenance of a constant internal environment for the cells of all living tissues. Its organs, tissues, and cells supply all body cells with oxygen, essential nutrients, vitamins, and hormones. The circulatory system also removes metabolic waste products and excess heat from the cells.

The major components of the circulatory system include a network of channels through which flows blood, pumped by the heart. The blood vessels are classified according to their structures as arteries, arterioles, capillaries, venules, or veins. The arteries and arterioles transport oxygenated blood to the tissues of the body while the venous portion of the network returns blood to the heart and pulmonary circulation for reoxygenation. Bridging the arterioles and venules are the capillaries, the smallest of the blood vessels, ranging in size from 7-9um in inside diameter (Guyton, 1971). In contrast, the largest arteries are typically 1.0 cm in inner diameter (Cromwell, et al, 1973). It is primarily within the capillary beds that the materials exchange takes place.

Blood, the transport medium for the circulatory system, is a suspension of various cells (erythrocytes, white blood cells, and platelets) in a fluid medium called plasma. The plasma contains many complex protein molecules, fats, carbo-

hydrates, ions, and small amounts of dissolved gases such as oxygen. However, the red blood cells are the principle carriers of oxygen in the blood. These cells, also called erythrocytes, normally resemble biconcave disks in shape. (The chicken is a notable exception because its erythrocytes are ellipsoidal in appearance.) Erythrocytes contain molecules of a protein complex called hemoglobin, which binds and releases oxygen. White blood cells, far less numerous than the red blood cells, serve as the body's defense against foreign agents, toxins, and disease organisms. Platelets are important in the clotting of blood.

Since blood is the transport medium for the materials to be exchanged, the understanding of the flow of blood is essential to the understanding of the materials exchange. The nature of the blood flow varies considerably throughout the cardiovascular system. The flow is typically pulsatile in the large vessels and arterioles, but is quite irregular within the capillaries where the diameter of the erythrocytes corresponds to that of the vessels themselves.

The characteristics of blood flow in any given vessel are related to the pressure difference and the resistance involved. Two factors are largely responsible for the resistance to flow:

1. The vessel geometry (length, diameter)

2. The flow properties of blood (viscosity)

Especially at the level of the microcirculation, the vessel diameter is important.Because the diameters of the vessels

within the microcirculation are small compared with those of the arteries and veins, the microvessels offer the largest resistance to flow. The resistance to flow is roughly proportional to the reciprocal of the fourth power of the vessel diameter (Guyton, 1971). Thus, as the diameter of a vessel decreases, the resistance increases, but at a much faster rate.

The contribution of the viscosity of blood to the resistance is also significant. Blood viscosity is dependent upon three factors:

1. Hematocrit

2. Erythrocyte geometry

3. Types and concentrations of proteins in the plasma

Of these, the hematocrit, or the concentration of the red blood cells, is the most important. The hematocrit for a given volume of blood, defined as the ratio of the volume of the red blood cells to the volume of the whole blood, is most commonly determined clinically by centrifuging a column of whole blood for a standard length of time. During the centrifugation, the erythrocytes are packed at the bottom of the column (erythrocytes are more dense than plasma). The hematocrit is then expressed as the quotient of the red blood cell column height and the total column height. As shown in Figure 1, an increase in the hematocrit results in an exponential increase in the blood viscosity.

Normal hematocrits vary among species (goat, 33%;

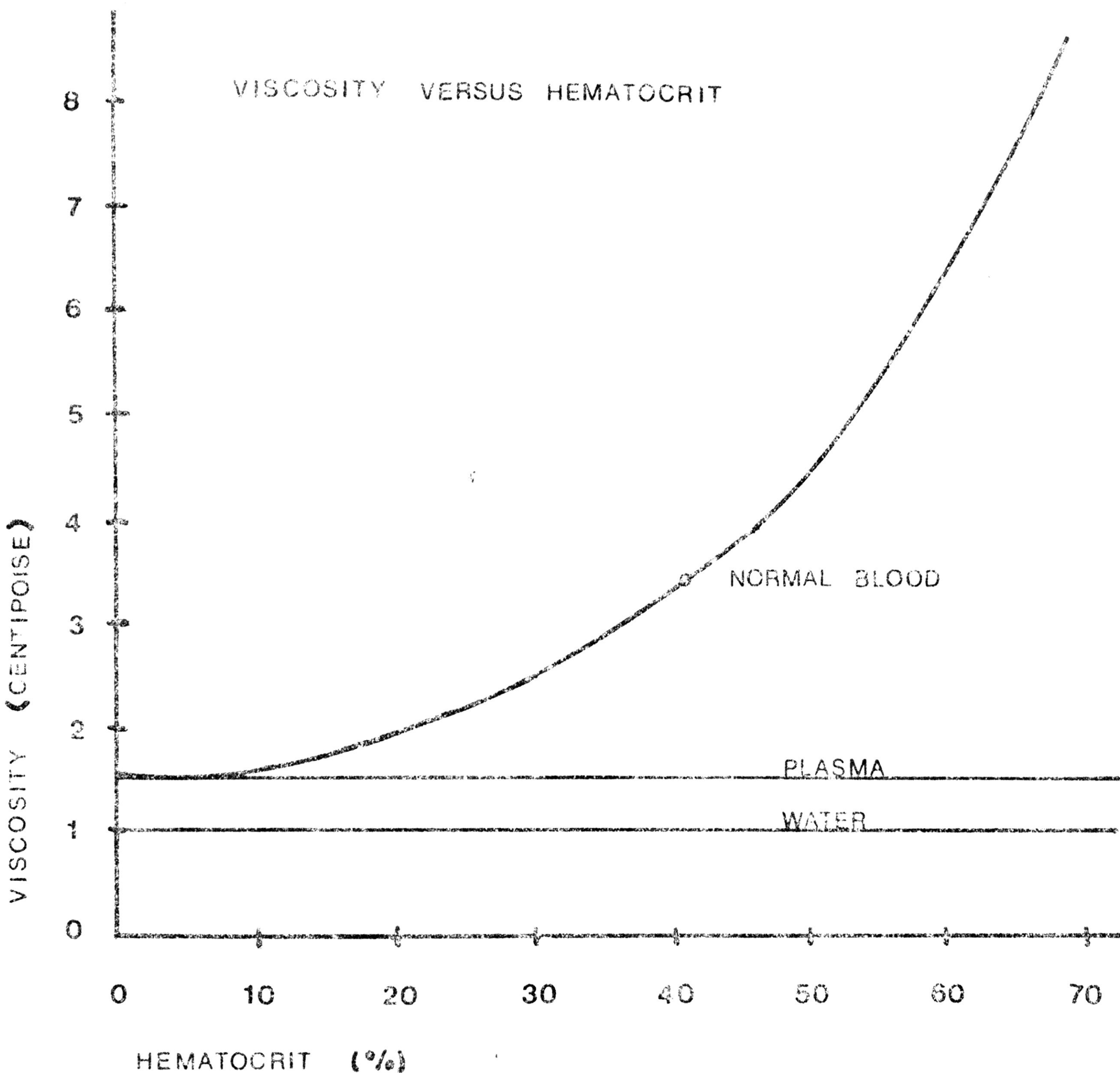

Figure 1

human, 35-50%) and among individuals within a given species. The state of health, activity, and altitude influence the relative volume of the erythrocytes of an individual. Hematocrits for a member of a single species are also organ-dependent (human spleen blood, 70%; human kidney blood, 20%; Selkurt (1966)).In addition, several *in vivo* studies (Johnson (1971), Johnson, *et al* (1971), Jendrucko and Lee (1974)) have provided evidence that hematocrit fluctuates with time in a given microvessel and among microvessels within the microcirculation.

In addition to the local variations in hematocrit within the microvessels, there is an apparent reduction in hematocrit in vessels smaller than about 0.3 mm (300um) in inner diameter. This phenomenon was first documented by Fahraeus in 1929 after a series of studies using small glass capillary tubes ranging from 47- 507 um in inside diameter. The magnitude of this reduction is important phisiologically because the hematocrit of a volume of blood affects flow properties,as indicated above, and this, in turn, affects erythrocyte distribution and thus oxygen distribution at branch points. The viscosity decrease which accompanies the hematocrit reduction, called the Fahraeus Effect, allows the heart to pump blood through the small vessels with less effort than it might otherwise have to exert.

Literature Survey

Three primary investigations concerning the magnitude

of the Fahraeus Effect have been conducted using in vitro models of the microcirculation because of difficulties encountered in attempting to quantify the magnitude of the hematocrit reductions in an in vivo system. Fahraeus (1929), Hochmuth and Davis (1968), and Barbee and Cokelet(1971) have used microbore glass tubes in their studies. Based on the model studies in glass tubes, it has been found that the magnitude of the hematocrit reduction increases with decreasing tube diameter. This is shown in Figure 2 where Hr is the quotient of the tube hematocrit and the feed reservoir hematocrit, and Dt is the tube diameter in microns. As indicated by the curves in Figure 2, the data of Hochmuth and Davis (1968) suggest that the magnitude of the Fahraeus Effect may not be as large as originally suggested by Fahraeus. It has been speculated by the author that the different experimental conditions involved in each of the three studies have contributed to the discrepancy. A table of some of the experimental conditions used in the investigations is presented in Figure 3.

It is of primary research interest to gain a quantitative understanding of the distribution of erythrocytes within the vessels of the microcirculation. The purpose of the proposed research was to investigate the parameters which influence the magnitude of the Fahraeus Effect as observed in an in vitro system. The parameters investigated include:

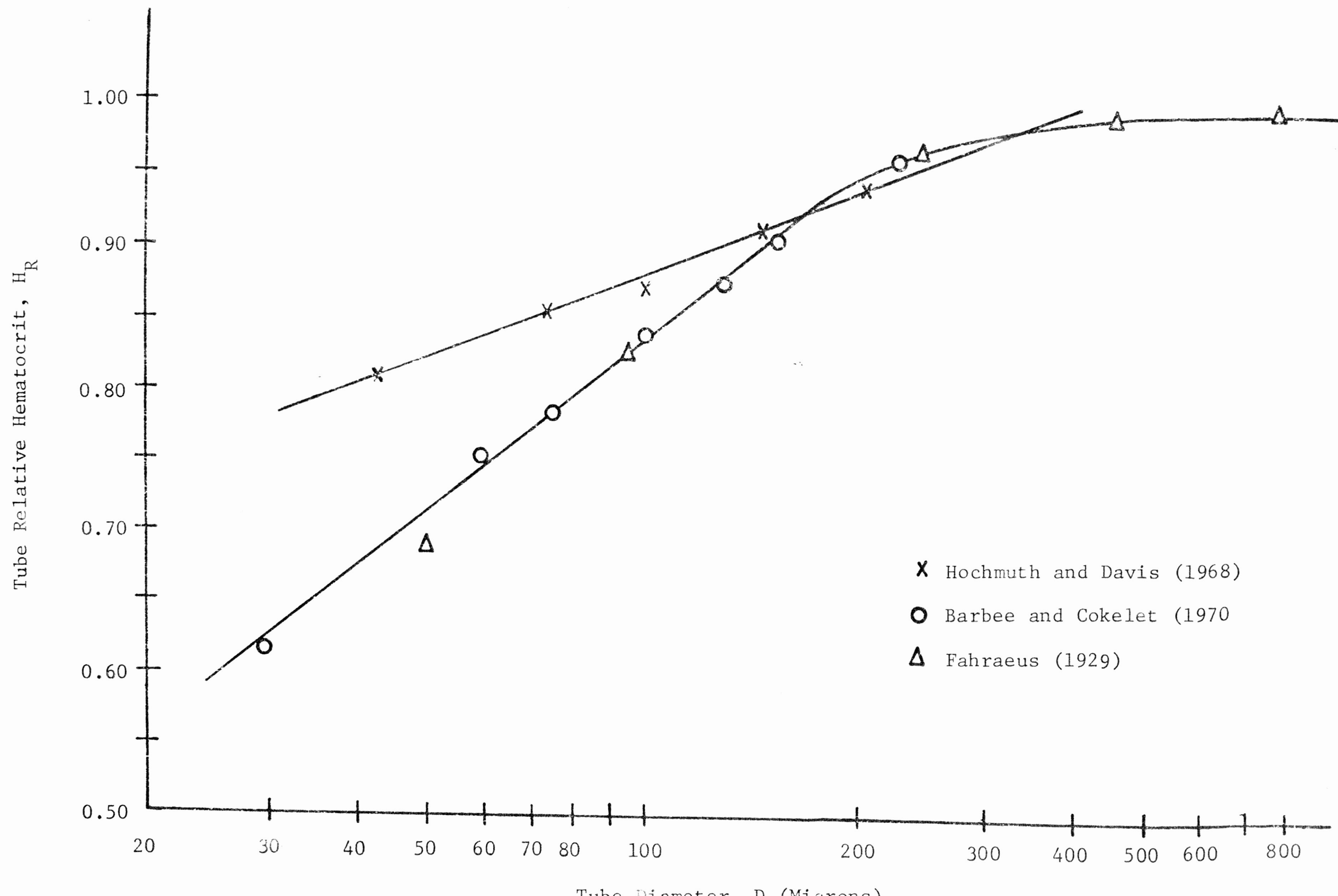

Figure 2

Parameter	Fahraeus (1929)	Hochmuth & Davis (1968)	Barbee & Cokelet (1970)	Mosley & Jendrucko (1975)
Blood anticoagulant	Sodium citrate	EDTA	ACD	EDTA
Nature of suspension	Whole human blood	Human blood; WBC removed?	Human blood WBC removed	Human blood WBC removed
Suspension stability	Tube rotated	no stirring or tube rotation	electromagnetically stirred	manually stirred
H_F	~40-45%	40%, 60%	~10-55%	2%-45%
D_T	50μ-1100μ	43μ-248μ	29μ-811μ	39μ-205μ
System temperature	38°C	room temperature ?	room temperature ?	room temperature 25°C
Tube entrance geometry				
Tube orientation	Horizontal	Horizontal	Horizontal	Vertical
Re	?	0.8-140	29μ: 0.001-0.025 811μ: 0.8-19.2	64μ: 0.12-0.95
H_T measurement	?	Centrifugation of tubes 2500 RPM for 1 hour	Spectrophotometry; plasma-trapping correction	Contrifugation of tubes; microhematocrit centrifuge-3min

Figure 3

1. Tube diameter
2. Blood temperature
3. Nature of the tube glass
4. Microbore tube length
5. Microbore tube orientation
6. Nature of the erythrocyte suspension
7. Anticoagulent
8. Refrigeration of the blood
9. Flow rate
10. Centrifugation technique
11. Donor species

Methods

The experiments, as suggested in the original proposal, were conducted in an *in vitro* system using straight lengths of smooth bore glass capillary tubes ranging in diameter from 39-204 um (Friedrick- Dimmock, Inc. Milville, N.J.).

First, the diameters of the tubes were measured by direct end-on viewing through a calibrated ocular grid in a light microscope. The measurements were verified by the collection of pressure-flow data for distilled water and the calculation of corresponding diameters from Poiseuille's Law (Guyton,1971). The method of obtaining the pressure-flow data was the same as that described below for blood.

The apparatus used is pictured in Figure 4.

The same basic experimental set-up was used in the in-

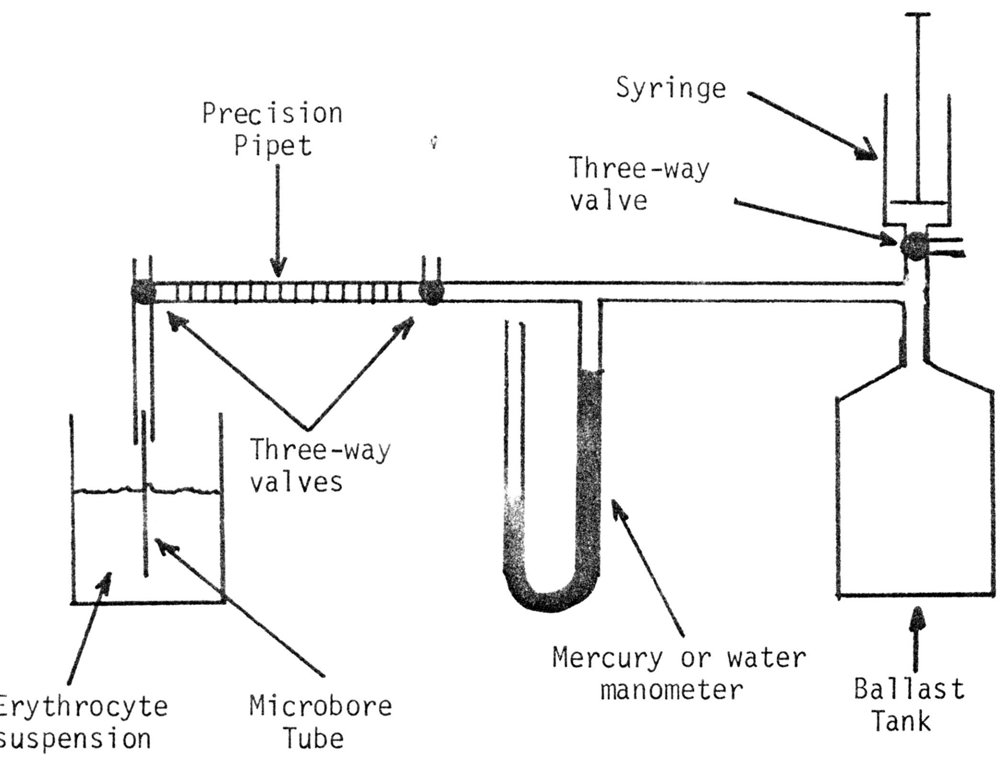

Figure 4

vestigation of each of the parameters of interest (discussed below).The equipment consisted of a microbore glass tubing connected to a 0.1 ml graduated pipette by means of polyethylene tubings and a three-way stopcock.

The pipette was connected in series to a ballast tank which served to reduce the effects of any gas leaks upon the pressure of the system and to minimize the effects of any change in volume or pressure due to the fluid movement in the pipette. The ballast tank was connected in series to an open-end, U-tube manometer and to a syringe used to draw a vacuum of variable magnitude.

Before each run with an erythrocyte suspension, the microbore tube, stopcock, and part of the pipette were filled with distilled water in order to allow measurement of the flow rate for that run, and to insure the abrupt cessation of flow at the end of each run.

During each run, the open end of the capillary tube was immersed in the erythrocyte suspension in the reservoir, and the vacuum was used to draw blood through the capillary tube. Steady state was assured if the flow rate was constant when measured at several volumetric intervals along the graduated pipette.(The measurement of the flow rate is described below.) The moveable meniscus of the fluid in the pipette was stopped by abruptly removing the vacuum by appropriate positioning of the stopcocks. Then, the open end of the tube was sealed

with clay and the tube was centrifuged for several minutes in either a microhematocrit centrifuge or a clinical centrifuge, depending upon the outer diameter of the mibrobore tube. In either case, the microbore tube was placed in a water-filled tube in the centrifuge to prevent any leakage of the suspension. Both the donor species and the centrifugation speed were considered in the selection of a time for centrifugation. The tube hematocrit was then calculated as the quotient of the erythrocyte column height and the total column height in the capillary tube.(The column heights were measured with a precision ruler.)

The reservoir or feed hematocrits were determined in a similar manner, but standard clinical hematocrit tubes(non-heparirized) were used in place of the microbore tubes. The tubes were filled by capillary action rather than by application of a vacuum.The tubes which were centrifuged in the microcentrifuge were not placed inside water-filled tubes.

In the pressure-flow studies, the meniscus movement between two of the volumetric graduations (0.01 ml) of the pipette was timed with a stopwatch. The flow rate was calculated as the change in volume per unit time.

The blood samples used in the studies were obtained from human, goat, and dog donors. The samples were collected in either disposable syringes or evacuated test tubes. Upon withdrawal, the whole blood was mixed with a standard amount

of one of several anticoagulents. The concentrations used appear in Figure 5. When removed from its normal body environment, blood will clot unless an anticoagulent is added.

In studies with saline suspensions, the whole blood was centrifuged, the white blood cells (the buffy coat) and plasma were removed, and the remaining erythrocytes were washed with isotonic saline (9.0 gm/l) and centrifuged twice. After the second washing and centrifugation, the cells were resuspended in isotonic saline to give the desired feed hematocrit. For whole blood suspensions, either plasma or red blood cells were removed to give the desired feed hematocrit. Centrifugation was required to separate the two components.

Results

Initially, tube hematocrits for glass tubes with diameters ranging from 39-204 um and for feed hematocrits of 40% were measured.The data obtained are plotted in Figure 6, which shows the dependence of the tube relative hematocrit, Hr, upon the tube diameter, Dt. The data agree with that reported by Hochmuth and Davis (1968), indicating that the magnitude of the Fahraeus Effect may not be as large as originally recorded by Fahraeus (1929) and later documented by Barbee and Cokelet (1971). It then became of primary research interest to identify those parameters affecting the magnitude of the Fahraeus Effect, and to then quantify the effects of those parameters.

The results of the parametric study are now described:

Anticoagulent	Concentration
EDTA	0.1 ml 10% solution/ 10.0 ml blood
Heparin	0.2 ml/ ml blood
ACD	15.0 ml solution/ 100.0 ml blood *
Sodium citrate	1.0 ml 0.11M solution/ 10.0 ml blood
Potassium oxalate	2.0 mg/ml blood

* Solution consists of the following in 1000 ml total volume:
  - 22.0 gm trisodium citrate
  - 8.0 gm citric acid
  - 24.5 gm dextrose

Figure 5

Figure 6

1. Blood temperature: The first parameter studied was blood temperature. It is known that the temperature of a fluid may affect its flow properties, and in particular, its viscosity. The data reported concerning the Fahraeus Effect were obtained using erythrocyte suspensions maintained at either room temperature, 24°C (Hochmuth and Davis,1968),or at body temperature,37°C (Barbee and Cokelet,1971).However, a comparison of the hematocrit reductions occurring in plasma suspensions maintained at 37°C and at 24°C indicates that the system temperature does not measureably affect the magnitude of the Fahraeus Effect over the range of temperature tested.

2. Nature of the tube glass:It seemed possible that if the wall of the the mibrobore tube either attracted or repelled the erythrocytes flowing through the tube, the tube hematocrit could be significantly altered. A very brief examination of the nature of the microbore tube glass as reflected by the charge of the glass surface indicated that the tube hematocrits are independent of the surface charge. However, more work is needed in this area.

3.Microbore tube length: Microbore tubes of different lengths were used to investigate the possible importance of tube length. Barbee and Cokelet (1971) used relatively long tubes (10 in), while those used by the author were typically 2.5 inches in length. The results of studies with different tube lengths ranging from 2-12 inches indicate that tube length does not

significantly alter the magnitude of the hematocrit reduction.

4. Microbore tube orientation: The contribution of the orientation of the microbore tube to the magnitude of the Fahraeus Effect for the suspension flowing through that tube was studied. The data of Fahraeus(1929), Hochmuth and Davis (1968), and Barbee and Cokelet (1971) were reported for systems using horizontal capillary tubes while that of the author were obtained using vertical microbore tubes. To test the importance of the orientation, hematocrit reductions for horizontal and vertical tubes, and for tubes at intermediate positions, were measured, and no significant differences were observed.

5. Nature of the erythrocyte suspension: In order to eliminate variations in the capillary tube hematocrits due to the nature of the continuous phase, saline suspensions rather than plasma suspensions were used in the author's experiments. An examination of the effect of the nature of the suspension, using both plasma and saline suspensions of equal hematocrits, indicated that hematocrit reductions occurring in a capillary tube with a given diameter are independent of the nature of the suspension. This data, in contrast to that of Hochmuth and Davis (1968), agrees with that of Barbee and Cokelet (1971).

6. Anticoagulent: The contribution of the addition of different anticoagulents to the magnitude of the Fahraeus

Effect was also investigated. It is conceivable that such chemicals could alter the mechanical properties of the erythrocytes, in particular, the flexibility of the cell membranes, and this could be important in determining the hematocrit reduction which takes place. To compare the effects of different anticoagulents, five samples of dog blood were obtained from a single donor animal. To each was added a different anticoagulent (in the amount required for the standard clinical concentration). The agents used were: ACD (acid citrate dextrose), heparin, EDTA (ethylene diamine tetraacetate), sodium citrate, and potassium oxalate. Based upon a set of preliminary experimental data, it seems that the anticoagulent does not affect the magnitude of the hematocrit reduction for a given feed hematocrit and a given tube diameter.

Similar experiments performed with human blood samples in ACD, sodium citrate, and EDTA produced similar results and conclusions.

7. Refrigeration of the blood: When it becomes necessary to store fresh blood, it is refrigerated at 4°C. Studies with fresh and stored blood have shown that the storage does not affect the hematocrit reductions occurring in the microbore tubes used.

8. Flow rate: The effect of flow rate upon the tube hematocrit was examined in order to establish a basis of comparison between the data of previous experiments and that of

the author. Steady-state pressure flow data and the corresponding tube relative hematocrit data were collected. It was found that, for a given tube size and a given feed hematocrit, the tube relative hematocrit is apparently independent of the pressure difference imposed, and thus the flow rate, at steady state.

It is possible that the flow rates measured are average values, and that the flow experienced by the suspension in the microbore tube fluctuates about the mean rate. It was not possible to investigate the potential importance of unsteady flow with the equipment used.

9. Centrifugation technique: The tube hematocrits measured were found to be dependent upon the centrifugation technique employed. Both time and acceleration are important in determining the column height of the erythrocytes. Maximum packing is achieved only after a certain centrifugation time. The time required for this to take place is species-dependent for a given acceleration. This is shown in Figure 7 by the curves for human and goat suspensions centrifuged in both clinical (macro) and microhematocrit (micro) centrifuges. In Figure 7, the ratio of the hematocrit at a given time, t, to the final hematocrit reached is plotted against the time, t. As shown in Figure 7, goat suspensions require extended centrifugation times as compared to human suspensions. Goat cells are smaller and less dense than human cells.

Figure 7

The final column height reached is related to the acceleration during the centrifugation. High-speed centrifuges pack cells more efficiently than do low-speed centrifuges. Packing factors, or correction factors, for clinical tube hematocrits have been tabulated considering the following factors(Chien, et. al., 1965):

1. Nature of the suspension (plasma or electrolyte)

2. Acceleration during centrifugation

10. Donor species: The dependence of blood flow upon the geometry was shown by a series of studies involving blood obtained from three different species: goat, human, and dog. Normal goat cells are 3.2 um in diameter; human, 8.0 um; dog, 7.0 um. Saline suspensions of the cells exhibited different flow behavior for a given tube diameter and a given feed hematocrit. The differences are shown in Figure 8. τ_w represents the shear wall stress in dynes per square centimeter and is proportional to pressure (Equation 1).

$$\tau_w \quad \frac{\text{(pressure drop) (tube diameter)}}{\text{4 (tube length)}} \qquad \text{Equation 1}$$

$\overline{U}$, proportional to the flow rate (Equation 2), is the reduced flow velocity in sec^{-1}.

$$\overline{U} \quad \frac{\text{4 (flow rate)}}{\pi\text{ (tube diameter)}^3} \qquad \text{Equation 2}$$

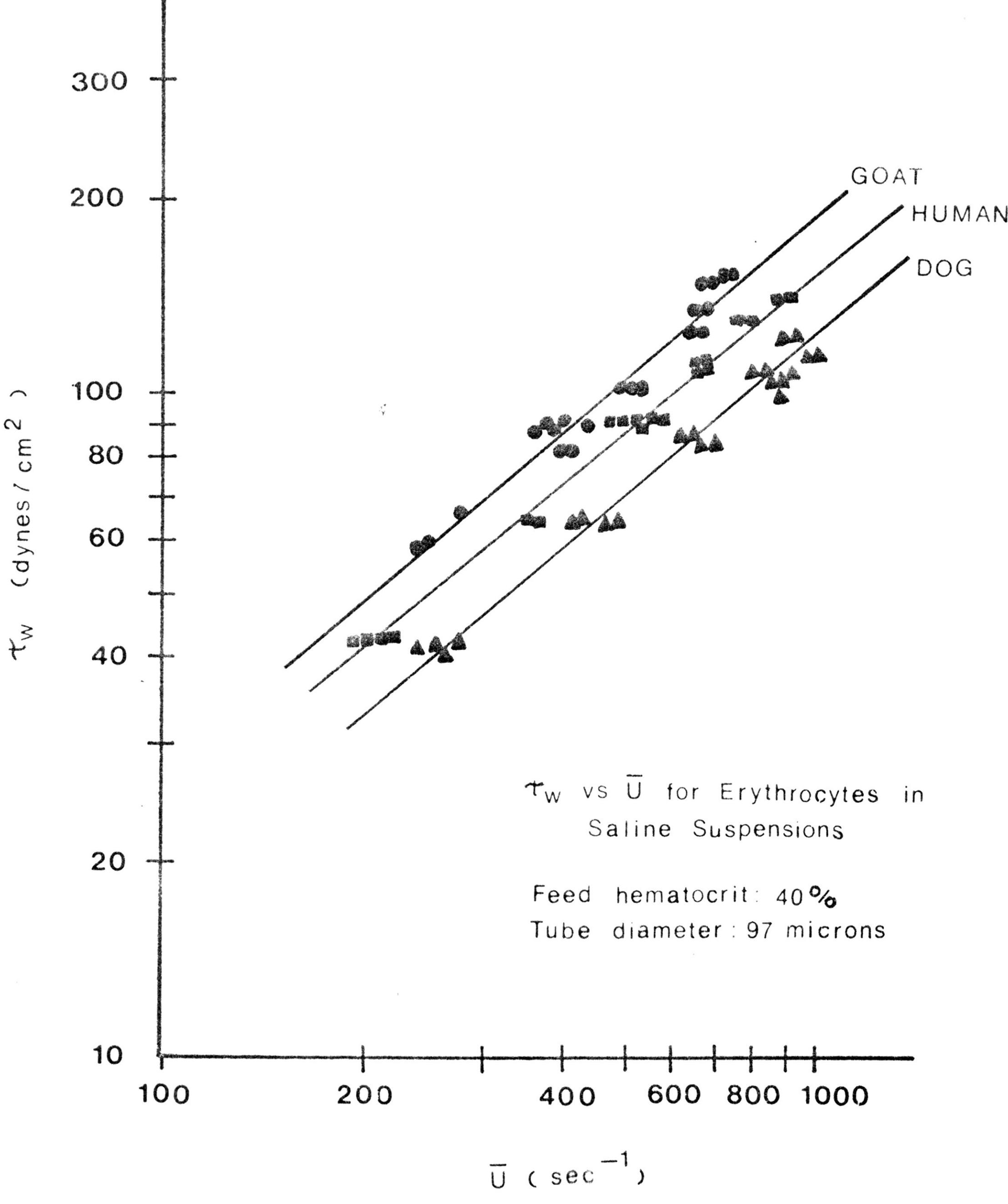

Figure 8

The data are plotted in this manner in order to show the relationship between pressure and flow. The resultant curves ($\ln \tau_w$ versus $\ln \bar{U}$) are nearly linear, and represent wide ranges in both τ_w and $\bar{U}$. The slope of each curve at a given point is directly proportional to the apparent viscosity. Nevertheless, the pressure-flow data are plotted in Figure 9.

Apparent viscosities (the viscosity for a non-Newtonian fluid is dependent upon the conditions of the measurement) were calculated for each species for a reduced bulk average flow velocity of 300 sec^{-1}: goat, 3.0 cp; human, 2.5 cp; dog, 2.0 cp.The data indicate that the flow behavior is not simply a function of either cell diameter or mean cell volume (Figure 10).It follows that the relative tube hematocrits are different for the three species. Preliminary data indicates that this is indeed the case. The tube hematocrits measured were highest for the goat suspensions and lowest for the dog suspensions, as would be expected.

Future Studies

The next step is to measure the absolute hematocrits in the microbore tubes. This will require the development of one or more reliable methods for determining the absolute hematocrits in tubes smaller than about 300um in diameter. Then, the species-dependence studies may be completed for the 97 um tube and the 40% feed hematocrit. The effects of other tube diameters and different feed hematocrits should also be

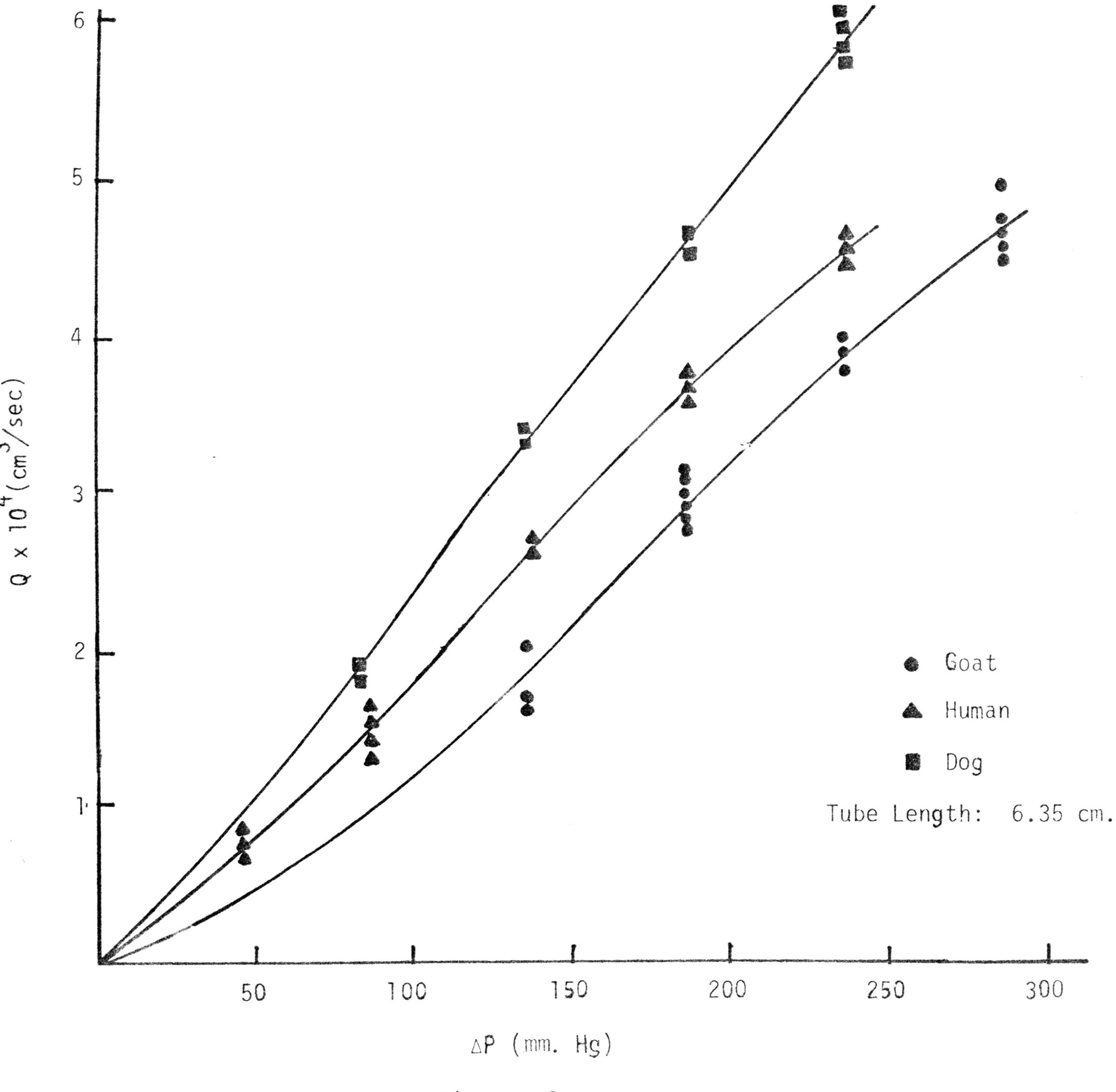

Figure 9

Species	Erythrocyte Diameter (microns)	Erythrocyte Mean Cell Volume (cubic microns)	Apparent viscosity* (centipoise)
Goat	3.2	25	3.0
Human	8.0	87	2.5
Dog	7.0	70	2.0

* Tube diameter - 97μ

Feed hematocrit - 40% (saline suspension)

$\bar{u}$ = 300 sec^{-1}

Figure 10

studied.

Other future investigations involve the collection of pressure-flow data, or τ_w versus $\bar{U}$ data, for plasma suspensions of human erythrocytes. Although the nature of the suspension does not seem to affect the hematocrit reduction, the flow properties of the suspending phase do affect the apparent viscosities observed. Thus, the τ_w versus $\bar{U}$ curves for saline suspensions and plasma suspensions would not coincide since plasma is more viscous than saline (Figure 11). If indeed the tube hematocrits observed by the author are the same as those measured by Fahraeus(1929) and Barbee and Cokelet (1971), the τ_w versus $\bar{U}$ curves should coincide with those of Barbee and Cokelet (1971). On the other hand, any discrepancies arising should be investigated. A possible source of discrepancy is the fluctuation in the flow rate experienced by the erythrocyte suspension. If it were pulsatile, even though not detectable using the present system, the tube hematocrits could be affected. Cell crenation may also play an important role in determining the magnitude of the Fahraeus Effect since such deterioration, usually irreversible, affects membrane properties.

Since the cross-sectional areas and shapes of the vessels in the microcirculation are highly irregular and rarely circular, it would be of interest to measure the hematocrit reductions occurring in glass microbore tubings with other than

circular cross-sections.

As the vessels of the microcirculation form a highly meshed network, studies of erythrocyte distributions at bifurcations would aid in understanding the oxygen transport to the cells.

Conclusions

In summary, the magnitude of the Fahraeus Effect seems to be independent of the following parameters:

1. Blood temperature
2. Nature of the tube glass
3. Microbore tube length
4. Microbore tube orientation
5. Nature of the erythrocyte suspension
6. Anticoagulent
7. Refrigeration of the blood
8. Flow rate

The magnitude of the hematocrit reductions measured in the microbore tubes seems to be dependent upon the following parameters:

1. Tube diameter
2. Centrifugation technique
3. Donor species

Why the magnitude of the Fahraeus Effect as measured by Hochmuth and Davis (1968) and by the author differs from that reported by Fahraeus (1929) and Barbee and Cokelet(1971) is still unknown.

Significance

The information obtained from the research already completed and the studies to be performed in the future is expected to contribute substantially to the body of know-legde on microcirculation biophysics. In particular, the results of the experiments will contribute to the understanding of the phenomena contributing to and controlling the magnitude of the Fahraeus Effect. Controlled variations in flow rates and feed he atocrits will aid in the understanding of the dynamic conditions affecting the erythrocyte concentration and distribution at microvascular branchings. Investigations of the effects of tube orientation, anticoagulent used, and donor species involved will help establish the relevance of the results previously documented by the other investivators.

Solutions to these problems are of critical importance if the exchange of oxygen, nutrients, and waste products occurring in the capillary beds is to be fully understood. An increased knowledge of the mechanisms of oxygen transport would be most valuable since the basic functions of all cells are oxygen-dependent.

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Bibliography

Barbee, J.H. and G.R. Cokelet, "The Fahraeus Effect", Microvasc. Res., 3:6-16, 1971.

Barbee, J.H. and G.R. Cokelet, "Prediction of Blood Flow in Tubes with Diameters as Small as 29 u", Microvasc. Res. 3:17-21.

Berne, R.M. and M.N. Levy, Cardiovascular Physiology, St. Louis, C.V. Mosby Co., 1967, pp. 52-57.

Chien, Shiu, R.J. Dellenback, S. Usami, and M.I. Gregersen, "Plasma Trapping in Hematocrit Determination. Differences Among Animal Species", Proc. Soc. Exp. Biol. and Med., 119:1155-1158, 1965.

Cromwell, Leslie, F.J. Weibell, E.A. Pfeiffer, and L.B. Usselman, Biomedical Instrumentation and Measurement, New Jersey, Prentice-Hall, Inc, 1973, pg. 75.

Fahraeus, R., "The Suspension Stability of Blood", Physiol. Rev., 9:241-274, 1929.

Fahraeus, R. and T. Lindqvist, "The Viscosity of the Blood in Narrow Capillary Tubes", Amer. J. Physiol., 96: 562-568, 1931.

Guyton, A.C., Textbook of Medical Physiology, Philadelphia, W. B. Saunders Co., 1971, pp. 204-206, 212, 382, 486.

Hochmuth, R.M. and D.O. Davis, "Change in Hematocrit for Blood Flow in Narrow Tubes", Bibl. Anat., 10:59-65, 1968.

Jendrucko, R.J. and J.S. Lee, " Hematocrit Measurement in Cat Arterioles by Microphotometry", Fed. Proc., 33(3): 313, 1974.

Johnson, P.C., "Red Cell Suspension in the Mesenteric Capillary Network", Amer. J. Physiol., 221(1):99-101. 1971.

Johnson, P.C., J. Blaschke. K.S. Burton, and J.H. Dial, " Influence of Flow Variations on Capillary Hematocrit in Mesentery", Amer. J. Physiol., 221(1):105-112, 1971.

Selkurt, E.E., ed., Physiology, London, Little, Brown, and Co., 1966.