



Flow of human blood through capillary tubes and red cell concentration
by Ann Elizabeth Berg

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE in Chemical Engineering
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Abstract:

The flow of human "blood through capillary tubes ranging from 15.1 to 36.9 microns in diameter was studied.

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Various feed concentrations were used, along with various flow rates. These did not change the red cell concentration of the discharge with respect to the feed.

High molecular weight Dextrans were added to change the properties of the blood. These did not change the results either; the discharge red cell concentration was virtually the same as the feed concentration.

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Date

September 4, 1973

FLOW OF HUMAN BLOOD THROUGH CAPILLARY
TUBES AND RED CELL CONCENTRATION

by

ANN ELIZABETH BERG

A thesis submitted to the Graduate Faculty in partial
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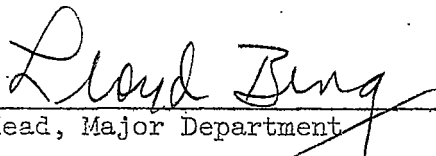
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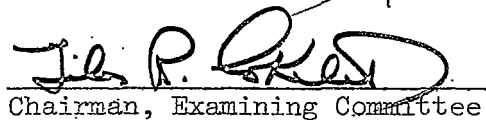
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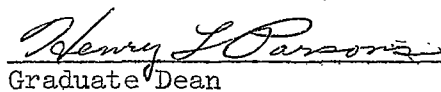
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Head, Major Department


Chairman, Examining Committee


Graduate Dean

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ABSTRACT

The flow of human blood through capillary tubes ranging from 15.1 to 36.9 microns in diameter was studied.

It has been previously noted (2) that at small tube diameters, the red cell concentration (hematocrit) at the exit of a capillary tube may be less than the concentration at the entrance. It was hoped to find exactly where this decrease occurs, if at all. The red cell concentrations of the feed and discharge blood were measured, and were found to be almost equal. Thus there is no screening effect causing a reduction in red cell concentration with flow through small tubes.

Various feed concentrations were used, along with various flow rates. These did not change the red cell concentration of the discharge with respect to the feed.

High molecular weight Dextrans were added to change the properties of the blood. These did not change the results either; the discharge red cell concentration was virtually the same as the feed concentration.

BACKGROUND

Blood Characteristics

Understanding of the flow properties of blood has applications in many medical and pharmaceutical areas. By understanding both the macroscopic and microscopic behavior of blood, predictions may possibly be made about the flow in the circulatory system. Also, this information may be used in designing such medical equipment as heart-lung machines and artificial organs. Knowledge of the mechanisms of blood flow in glass capillary tubes is the first step in learning about flow in the small capillary networks of the body.

Blood is a suspension of cells in a solution called plasma. The plasma is an aqueous solution of organic and inorganic salts, other small organic molecules, such as urea and glucose, and proteins. The proteins, which make up about 7.0% of the plasma weight, are macromolecules of molecular weights ranging from 40,000 to over 1,000,000. These proteins consist of albumin, fibrinogen, and globulins. Albumin, with a molecular weight of 69,000, serves as a super transport molecule and helps regulate plasma volume. Fibrinogen is much larger, with a molecular weight of 170,000. It plays an important role in the clotting mechanism. Thrombin converts it to fibrin, which is the monomer used in building up a clot.

The cells consist of red blood cells (RBC's), white blood cells, and platelets. The red cells, also known as erythrocytes, make up about 97% of the cell volume. These cells have a biconcave disc shape,

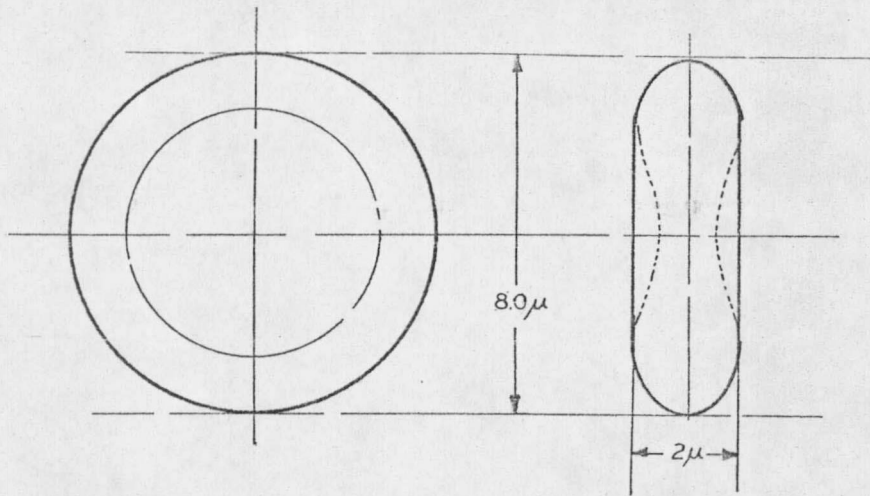


Fig. 1 Dimensions of a Red Blood Cell

averaging 8.0 microns in diameter and 2.0 microns in thickness. Various other values are also reported for the dimensions, however. A diagram of a red blood cell appears in Fig. 1.

The red cell is made up of a flexible membrane which is essentially unstretchable. The interior of this non-nucleated cell is a complex liquid, containing the hemoglobin. The membrane serves as a barrier to complex macromolecules, but allows small molecules to pass through rapidly. The membrane is the site of many chemical functions for the metabolism of the cell. The red cells serve to transport oxygen and carbon dioxide to and from the body.

The hematocrit is defined as the volume percent of the blood that is red blood cells. This is found by centrifuging a small volume of blood and comparing the relative volume of packed red cells to total volume. This gives accuracy to within 99%, as 1% of the packed cell

volume is trapped plasma.

The hematocrit can range from 37 to 54% in healthy humans. Changes in hematocrit for purposes of this study were made by centrifuging the sample and removing some of the plasma or red cell volume.

The white cells, also called leukocytes, are smaller in number and more varied in function than the red cells. There are five different kinds of white cells: basophils, eosinophils, segmented neutrophils, monocytes, and lymphocytes. Their sizes range from 8 to 22 microns in diameter. One of their main functions is fighting disease. These are nucleated cells and are more complicated in morphology than the red cells.

The platelets (thrombocytes) are small non-nucleated cells about 2-3 microns in diameter. Their main function is in blood coagulation, and thus they are closely involved in the clotting mechanism, or hemostasis. Hemostasis is activated in vivo by a complex chain reaction, initiated often by the rough edges of a cut in a vessel wall. This is of interest in the present study, as the rough ends of glass capillaries may also induce the clotting mechanism.

In studying the flow of blood, as well as in blood banking for medicinal transfusion or in clinical testing of blood, clotting presents a serious problem. Various anti-coagulants are used, including an acidified-citrate-dextrose (ACD) solution in the storage bags, and

heparin, a mucopolysaccharide obtained from dogs and cattle, used in hematocrit tubes to prevent coagulation. These compounds interfere with the clotting mechanism, usually by binding some protein in the reaction pathway, or by chelating calcium ions, which are essential for the clotting process.

When centrifuging the samples, the red cells are found in the bottom of the tube with a thin white layer on the surface of the red cell pack containing the white cells and platelets. It can be removed for investigations involving only red cells and plasma. This decreases the possibilities of clotting, due to the lack of platelets.

In normal, non-flowing blood, the red cells can aggregate face-to-face into a cluster known as a rouleau. At sufficiently high shear rates these rouleaux break up and the red cells exist individually. The addition of high molecular weight Dextran increases the formation of the rouleaux.

Many of blood's properties vary greatly from donor to donor. Hematocrit and protein content are among the characteristics that have large normal ranges. But for the enzyme specificity of the body's metabolic functions, temperature, pH and ionic strength are virtually the same level in all humans. Thus, when working with blood, one has to consider both the similarities and the differences encountered from sample to sample.

Blood Rheology

Rheology is the study of the flow properties of a fluid, dealing especially with viscoelastic and non-Newtonian fluids. Blood rheology is concerned with understanding the effects various changes in the blood will have on its measurable properties.

Shear stress for unidirectional flow between two parallel plates is the force required per unit area to move a top layer of liquid over a lower layer. This can be expressed as

$$\tau = \frac{F}{A} \quad (\text{dynes/cm}^2)$$

The strain rate is the velocity gradient and is given by

$$\dot{\gamma} = \frac{\Delta x}{\Delta t \Delta y} \quad (\text{sec}^{-1})$$

for a layer of liquid Δy cm. thick moving a maximum relative distance Δx . The viscosity coefficient is the shear stress divided by the strain rate

$$\eta = \frac{\tau}{\dot{\gamma}} \quad (\text{dynes/cm}^2, \text{ or poise})$$

and is a measure of the force required to move one layer of fluid over another at a given shear rate.

If one measured the viscosity of a sample of water at different shear rates, he would come out with a constant viscosity. But with blood, the viscosity varies over the range of shear rates, being higher at low shear rates, and lower at high shear rates.. The values obtained

are termed apparent viscosity, as they are the values the blood would exhibit at all points only if the blood were Newtonian. For some purposes, a more significant number is the relative viscosity, which is the ratio of the apparent viscosity of the blood to that of the suspending medium, in this case the plasma.

Poiseuille was one of the first to study the flow of blood. His famous pressure drop-flow rate relationship for steady flow through a tube was originally developed for blood flow; however, it only applies to Newtonian fluids such as water, alcohol, and mercury--the fluids he worked with.

$$Q = \pi \frac{\Delta P}{L} \frac{R^4}{8\eta}$$

where Q = flow rate, ΔP = pressure drop, R = tube diameter, L = axial length of the tube, and η = viscosity coefficient. This applies to blood only when the flow has moderate to high shear stresses.

Non-Newtonian flow occurs for blood in a tube less than 300 microns in diameter when the flows are physiologically significant. This was investigated by Fahraeus and Lindqvist in 1929 (7). They used a capillary viscometer to study the relationship between tube diameter and viscosity at such high shear rates that the blood acted as a Newtonian fluid. They found that the relative viscosity, that is, the viscosity found by taking the ratio of the apparent viscosity of blood to the

viscosity of water, decreases with tube diameter. This lowering of the viscosity is of great significance to the body, as it allows the heart to pump a given volume of blood through the circulatory system with a smaller pressure drop than if the blood had a constant viscosity. The pressure drop across the human circulatory system has been found to be about 120 mm Hg.

Another factor affecting the viscosity of blood is the hematocrit. The results of an investigation by Chien, et. al. (6) are shown in Fig. 2. The viscosity of suspensions of cells hardened with glutaraldehyde or acetaldehyde and of normal cells was measured as a function of hematocrit. These cells were all suspended in Ringer's solution. The hardened cells show little shear dependence, but the normal cells show dependence on both hematocrit and shear rate.

This decrease in viscosity with increasing shear rate is further illustrated in Fig. 3. The deformability of the normal cells causes them to slip past one another much more easily, thus lowering the viscosity. This is not the case for hardened cells. The marked difference in viscosity, especially at high shear rates, is easily seen.

Addition of Dextran-40 further decreases the relative viscosity at high shear rates. This is due to an increased plasma viscosity, which acts to deform the red cells even more. This is shown in Fig. 4, where various concentrations of Dextran-40 were added to the suspending media,

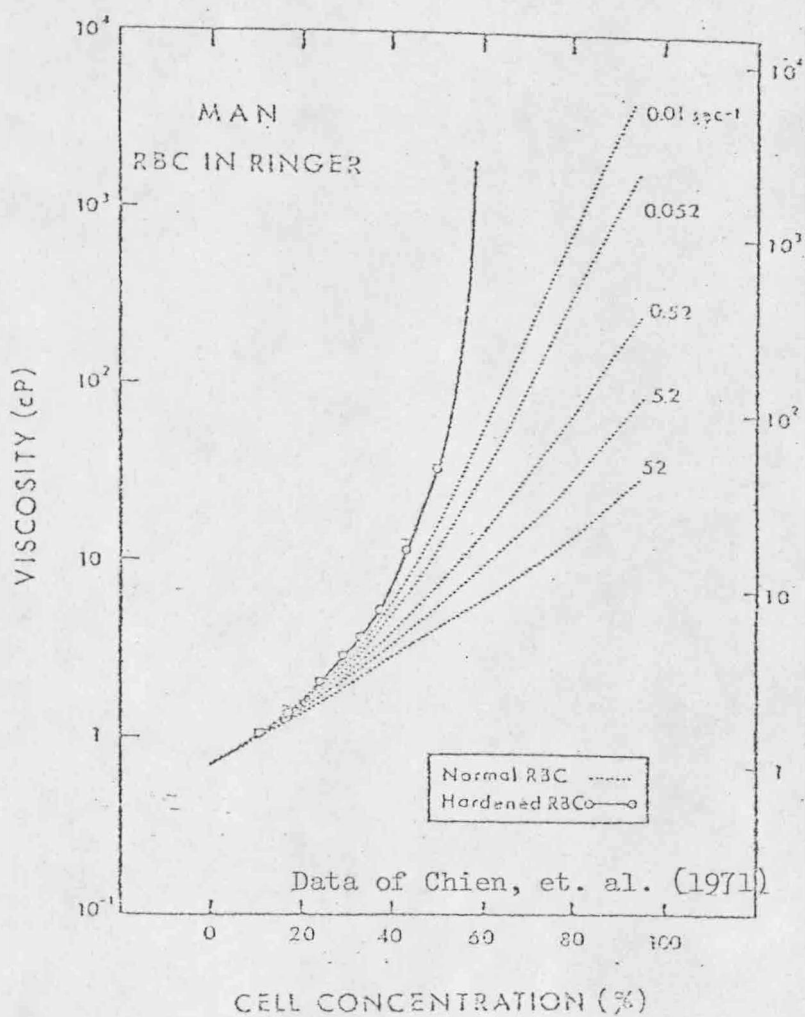


Fig. 2 Viscosity Dependence on Hematocrit

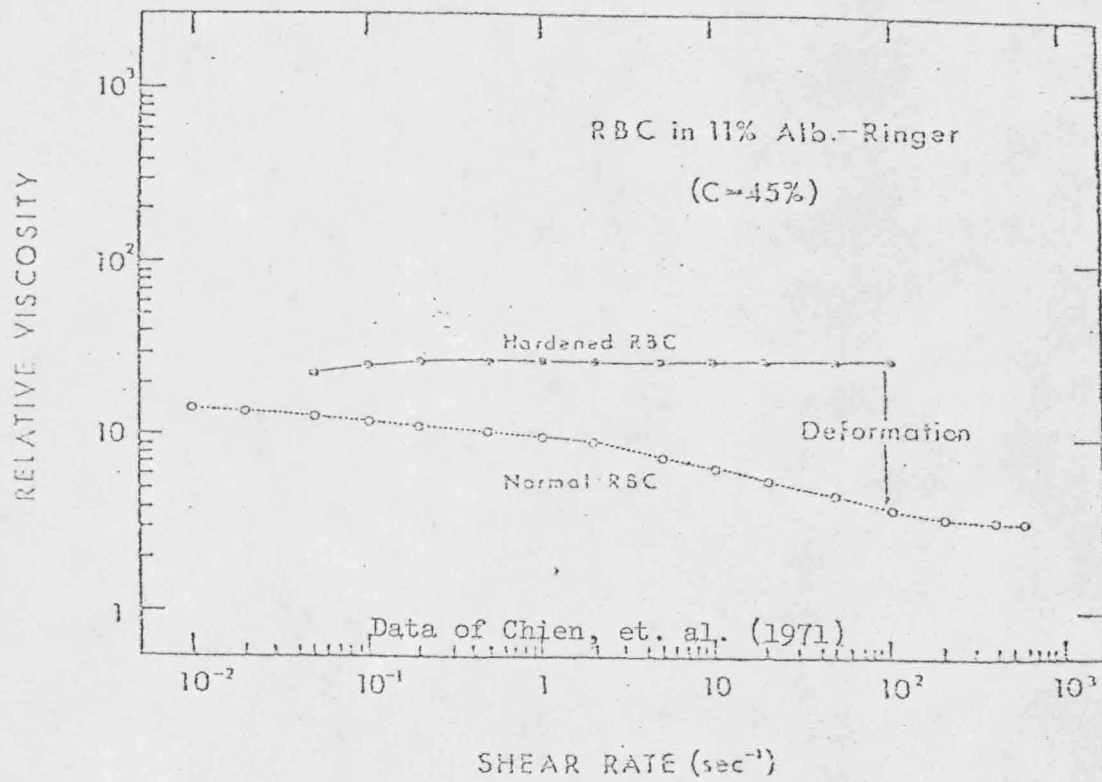


Fig. 3 Changes in Viscosity Due to RBC Deformation

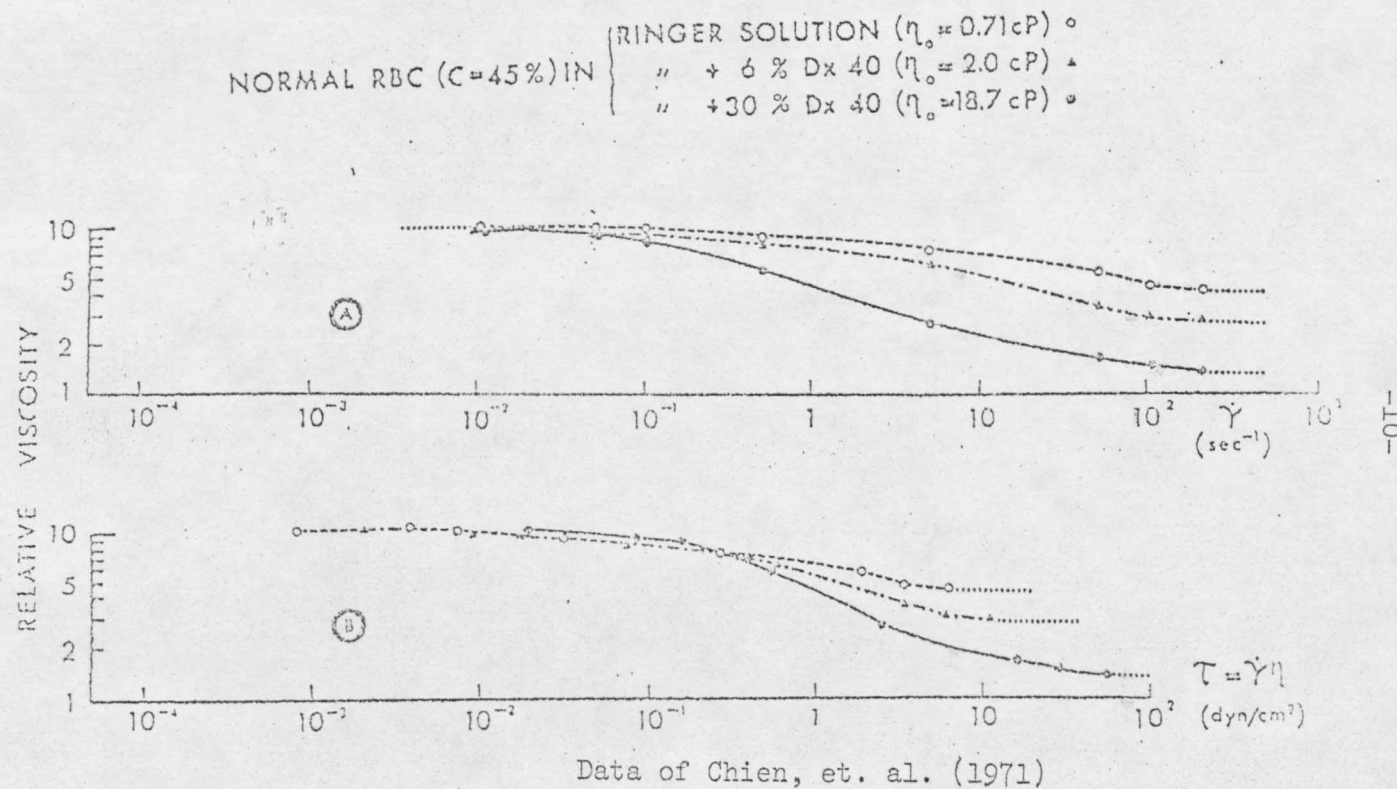


Fig. 4 Increase in Red Cell Deformability Due to Addition of Dextran-40 to Plasma

- A) Shear Rate Vs. Relative Viscosity
 B) Shear Stress Vs. Relative Viscosity

in this case Ringer solution. The abscissa is shear rate in A and shear stress in B.

The formation of rouleaux by red cells also has an effect on the viscosity. These rouleaux are broken up at high shear rates, but at low shears they serve to increase the viscosity. Fig. 5 shows the dependence of normal red cells suspended in plasma and in a Ringer solution containing 11% albumin. This latter solution does not allow the red cells to aggregate. Addition of high molecular weight Dextrans, such

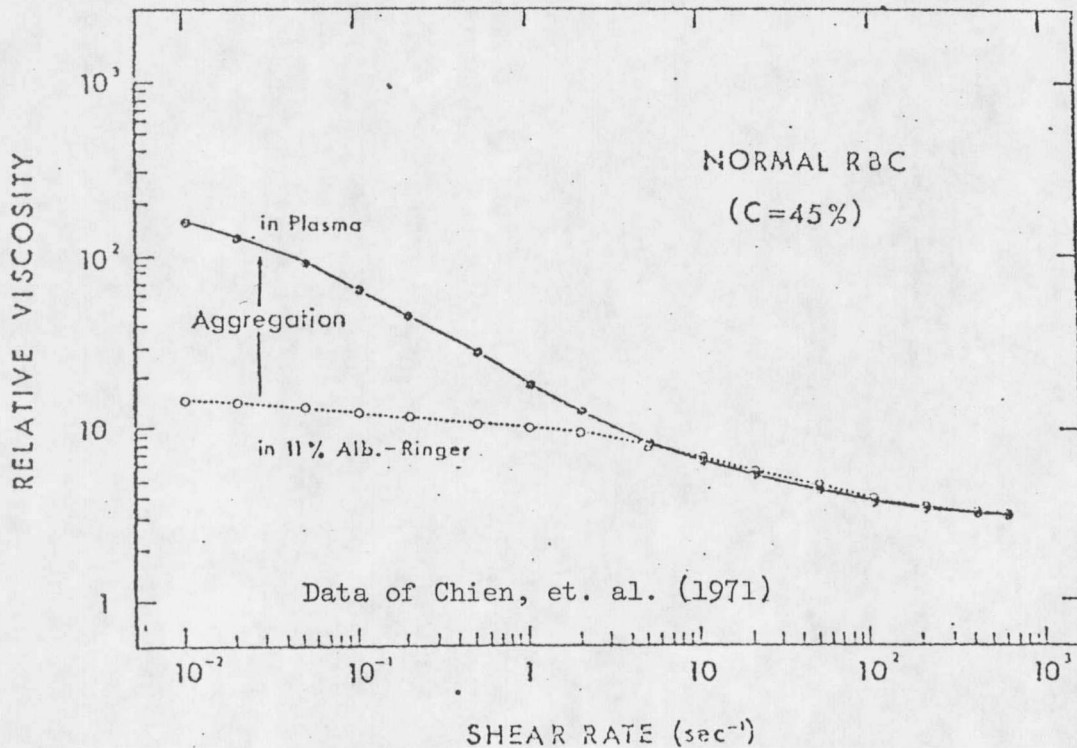


Fig. 5 Changes in Viscosity Due to Red Cell Aggregation

as Dextran-250, increases the aggregation of the cells, however, and a reverse effect would occur. The Dextran-250 line would be above the line for normal cells in this case.

Further investigation into the relationship between viscosity and tube diameter has led to the discovery that for tubes less than 300 microns in diameter, the hematocrit is actually less in the tube than in the feed reservoir, and yet the hematocrit of the blood in a discharge reservoir was virtually the same as the feed. Mass balance considerations require, therefore, that the average red cell velocity must exceed that of the bulk velocity of the whole blood. A number of mechanisms have been proposed to account for this effect. The Vand effect allows for a layer of plasma next to the wall of the tube in which the local concentration of red cells is lower than in the center of the tube because the centers of the particles cannot possibly be AT the tube wall (11). This would give a higher concentration of red cells in the fast moving center portion, and a low concentration, in fact, almost zero concentration, in the slow portion next to the wall. Fig. 6 shows a schematic comparison of the velocity profile of the whole blood compared to that of red cells only.

The tube hematocrit was found, by Barbee and Cokelet (1), to decrease with decreasing tube diameter, as is shown in Fig. 7. However, the hematocrit in the discharge reservoir remained the same until tubes

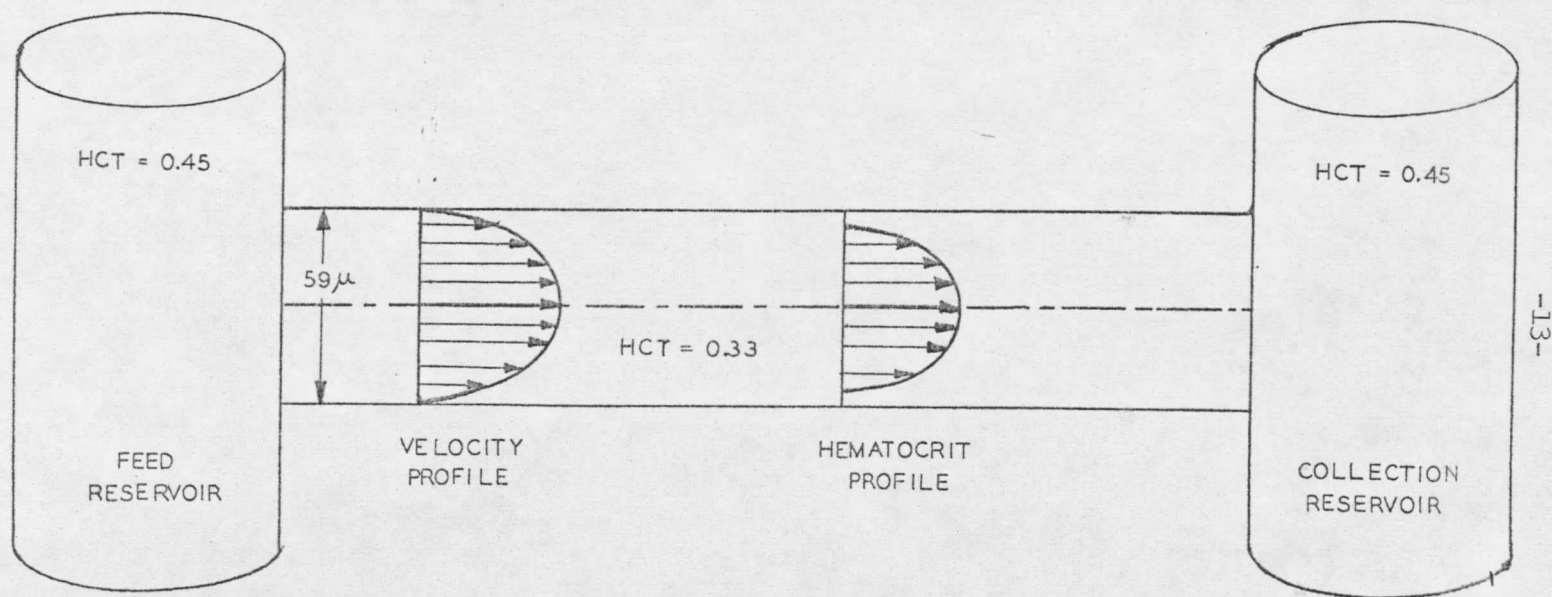


Fig. 6 Comparison of Hematocrit Profile and Velocity Profile in a Capillary Tube

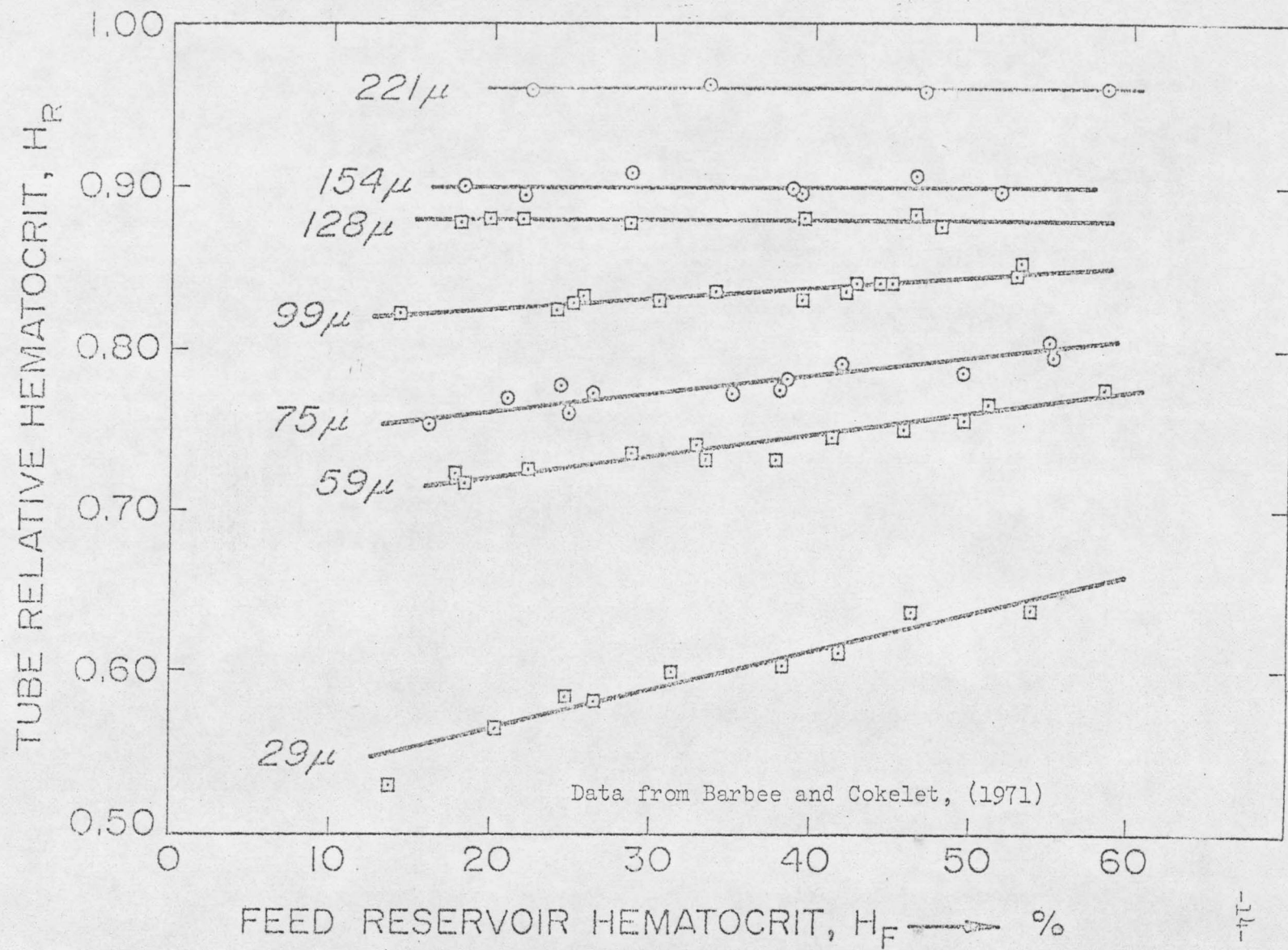


Fig. 7 Decrease in Tube Hematocrit with Decreasing Tube Diameter

of a 23 micron diameter were investigated. For smaller tubes it was found that some effect was causing screening of the cells at the tube entrance, because the exit hematocrits were less than the feed hematocrits.

The present investigation was undertaken to further study this decrease in exit hematocrit. Data from Barbee (2) are available for tubes with diameters of 8.1, 12.5, and 23 microns, as shown in Fig. 8. This graph assumes that no flow occurs for normal cells through diameters less than 2.8 microns, as reported by Gregersen, et. al. (9). The strange behavior between 8.1 microns and 12.5 microns was assumed to occur because of "crowding" at the entrance of the 12.5 micron tube--two cells struggling to enter the tube, and thus blocking it. This shouldn't occur at the 8.1 micron tube, as the cells can enter only one at a time, and must proceed in single file down the tube.

This study was done to obtain further data using five tube sizes: 15.1, 16.1, 19.4, 28.5, and 36.5 microns. The data obtained fills in some of the gaps on the graph in Fig. 8, and hopefully will show exactly where the drop-off in exit hematocrit occurs.

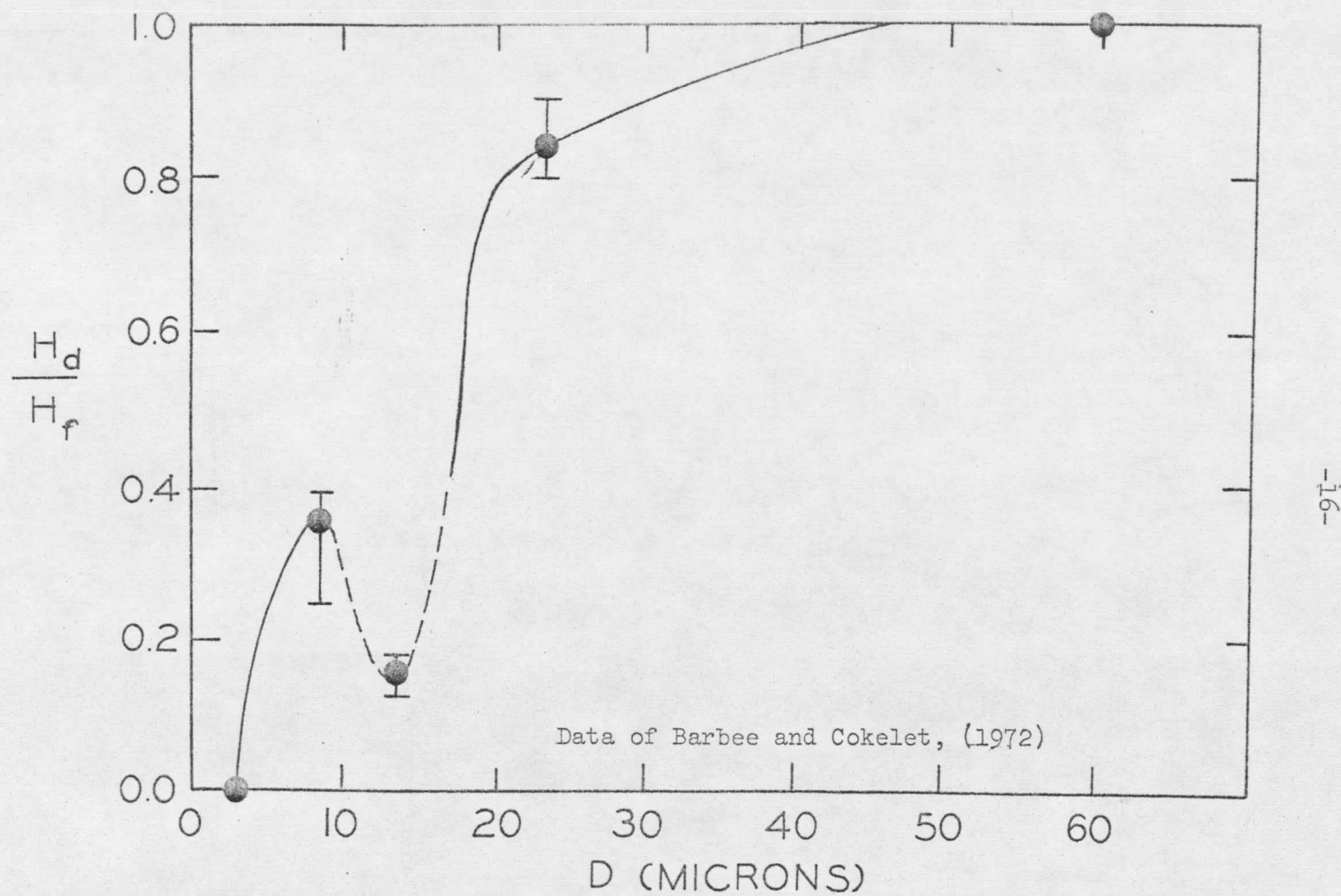


Fig. 8 Ratio of Discharge Hematocrit to Feed Hematocrit vs. Tube Diameter

RESEARCH OBJECTIVES

This research was undertaken to investigate the discrepancies found in the relationship between entrance and exit hematocrits. Five tube sizes were studied: 15.1, 16.1, 19.4, 28.5, and 36.5 microns. In previous studies, the drop-off effect was found to occur somewhere in the range between 23 and 59 microns. By using these tube sizes, it was hoped to pinpoint this range, and further study the region of tubes smaller than this range.

By adding high molecular weight Dextran to the blood plasma, a relationship of the blood cells to various suspending media could be found. If the effect occurred at a given tube diameter, a change in the blood viscosity might either increase or decrease the effect.

Different flow rates and feed hematocrits were also investigated to see if this could have any influence on the effect.

APPARATUS

Blood Preparation

Blood was drawn from healthy donors by standard blood banking methods. Acidified-citrate-dextrose (ACD) solution was used as an anti-coagulant and the blood was stored at 4°C. until used.

Plugging of the capillary tubes was one of the major problems, and a number of steps were taken to avoid this effect. The samples of blood were filtered through Swank Blood Microemboli filters to remove any microemboli aggregates which may have formed during storage. These filters remove any debris down to 10 microns and allow the red cells to pass through with no hemolysis (bursting of cells) or damage to the cells.

Coagulation, presumably initiated by the platelets, was causing plugging, especially with units of blood one to two days old. So, the samples were centrifuged and the buffy coat removed. This contains the white cells, which may range up to 22 microns in diameter, and the platelets, which play an important role in coagulation. Plugging was most serious with the smallest tube sizes. It was found that storing the blood 4-5 days allowed most of the platelets to disintegrate, thereby reducing the plugging problems in these tubes.

For the whole blood runs, the cells were resuspended in the plasma after removing the buffy coat. When the two Dextran solutions were used, the viscosity of the plasma was measured in a Wells-Brookfield

viscometer at 37°C. The plasma was weighed, Dextran was added, and the viscosity of the plasma-Dextran solution was measured in the viscometer. Concentrations of the Dextran were 2.0% plasma weight Dextran-40, and 0.5% plasma weight Dextran-250. The plasma was then remixed with cells to give a hematocrit range between 35 and 45%.

Tube Preparation

Five sizes of capillary tubes were used. Measurement of the exact tube diameters was done in two ways. Initially, distilled water was run through the 29 and 37 micron tubes at steady state, and the pressure drop was measured. This was used with Poiseuille's equation to determine the tube diameter. A diagram of the apparatus used appears in Fig. 9. These tubes were measured to be 29.2 and 38.2 microns by this method.

Photographs of the ends of the tubes were also used to calibrate the tube diameters. Tube lengths approximately 1.0 cm. long were balanced on a microscope slide in clear nail polish. When the polish dried, the tubes were snapped off close to the surface of the hardened enamel. These were then photographed with a microscope and an oil immersion lens at 1000X magnification, along with a micrometer, which was used as a ruler to measure the inside diameter of the tubes. The measured tube sizes were 15.1, 16.1, 19.4, 28.5 and 36.9 microns.

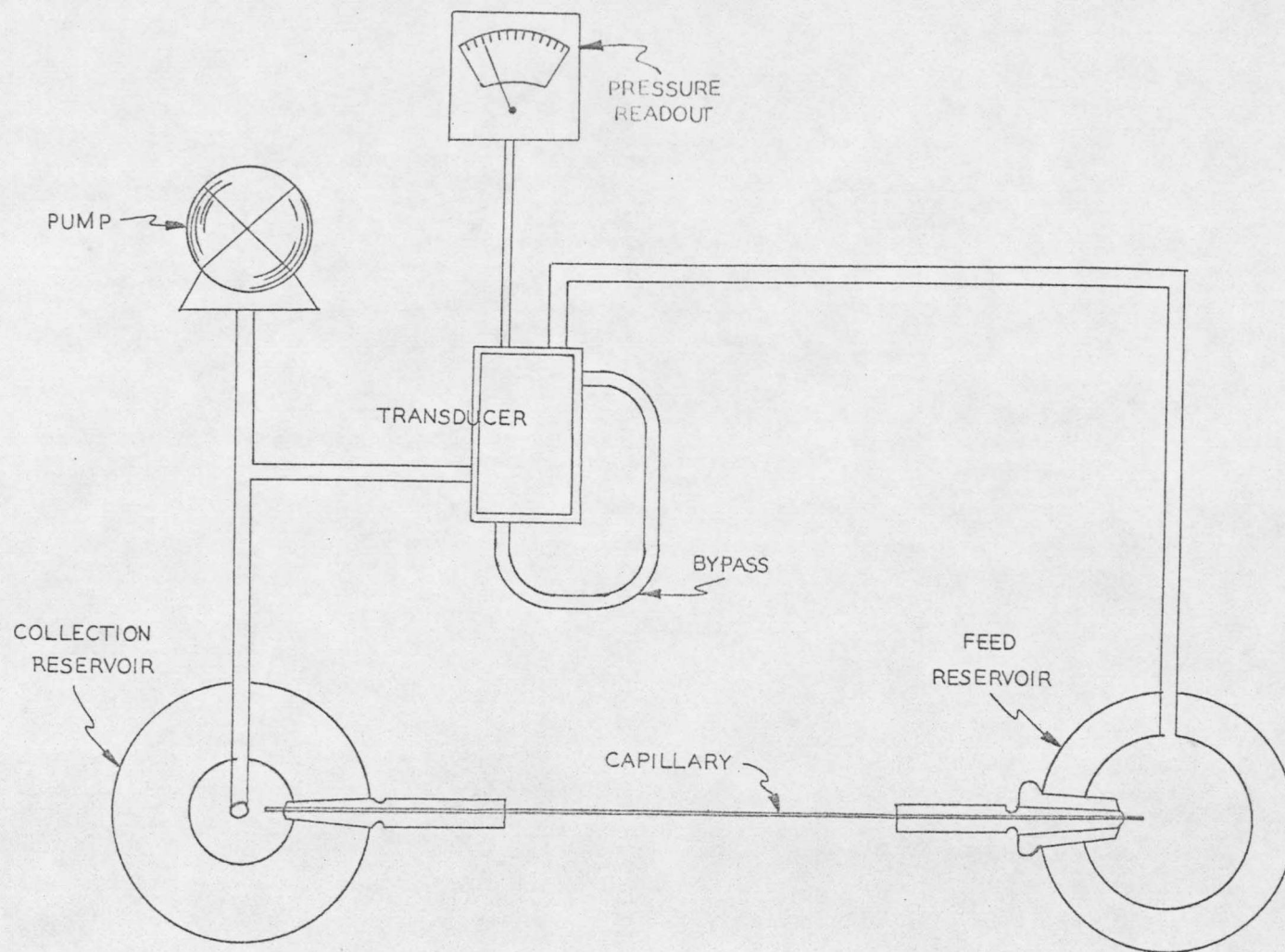


Fig. 9 Apparatus for Pressure Drop - Flow Rate Measurements

There was a difference of 2.40% for the 29 micron tube and 3.31% for the 37 micron tube by these two methods.

Photographs were also taken of the tubes at 100X magnification. These show that the tubes snap off perpendicular to the axial line of flow in most cases. Even a slight angle does not cause much change in the tube opening, as this is such a small area of the entire end of the tube. This precludes any possible variation in entrance effects due to cells entering the tube at an angle.

The tubes were cut into approximately 10.0 cm. lengths and glued into standard taper joints with epoxy glue. One tube was used for the 29 and 37 micron tube sizes. By gluing two tubes in parallel in a standard taper joint, two microhematocrit samples were obtained in the same time. This was done for the 15, 16, and 19 micron tube sizes. The fittings were re-enforced with brass wire, glued to the standard taper joint with epoxy, parallel to the capillary tubes.

Frequently the tube plugging seemed to occur at the entrance or exit of the tube. A possible reason for this is that the sharp edges of the capillary aggravated the platelets and induced the clotting action. In any case, snapping off a millimeter or two of the ends and forcing isotonic saline through the tube could sometimes restore it to utility.

Flow Apparatus

The flow equipment consists of a pump, a gas-tight syringe, a feed reservoir, the glued tubes, and a microhematocrit tube used as a collection reservoir. The pump forces liquid into or out of the syringe at various flow rates. Various syringe sizes may be used; this allows greater variation in flow rate.

The liquid from the syringe is forced into the feed reservoir, which in turn forces the blood out through the capillary into the microhematocrit tube. Three-way valves are located along the tubing between the syringe and the feed reservoir to allow refilling and cleaning the syringe or the reservoir without disassembling the entire apparatus.

The reservoir is a plexiglass cylinder positioned over a magnetic stirrer to insure uniform distribution of the red cells in the feed. It is fitted with a 5/20 standard taper joint and metal screws for holding the capillary tube in place.

The entire apparatus is surrounded by a plexiglass box, which allows for accurate temperature and humidity control. Water can be evaporated into the atmosphere of the box to keep it humid.

A diagram of the entire apparatus appears in Fig. 10.

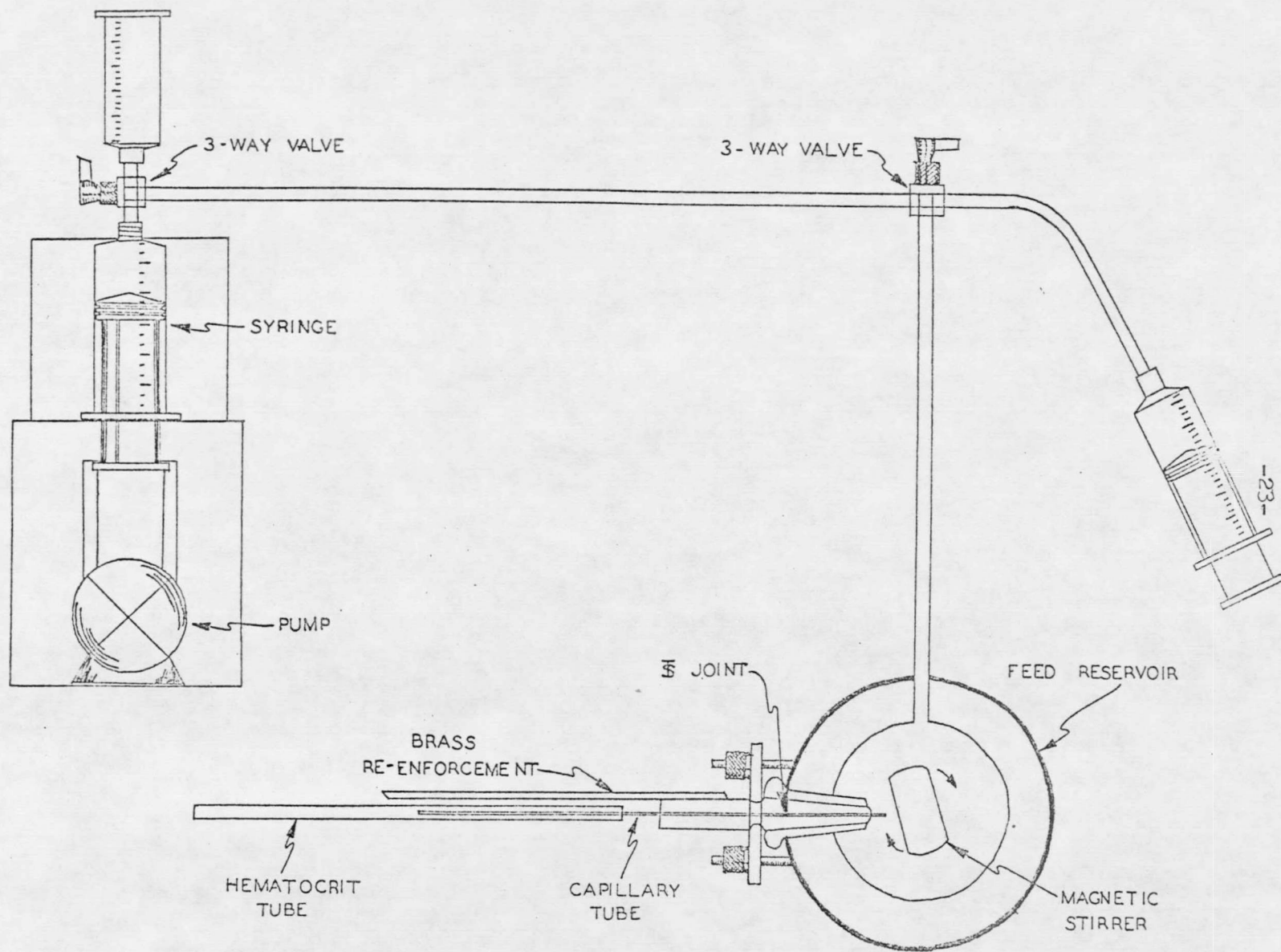


Fig. 10 Diagram of Blood Flow Apparatus

PROCEDURE

The entire system is filled with liquid except for the feed reservoir. The blood sample is poured into the reservoir and some is drawn up into the system towards the syringe. Originally, isotonic saline was used in the system; however, it was found that this was changing the feed hematocrit. A silicone fluid of a viscosity of about 5 cp. was used instead, as this is immiscible with the blood.

The hematocrit is taken of the sample in the feed reservoir, the system is sealed, and the pump is turned on. Hematocrit tubes are placed on the ends of the capillary tubes and sealed, either at the reservoir end with Vaseline, or at the other end with Critoseal, to prevent evaporation of plasma from the sample. When a large enough sample is obtained, the tubes are removed and centrifuged to obtain the discharge hematocrit.

Microhematocrit Method

Well-mixed anti-coagulated blood is drawn up into a microhematocrit tube (75 x 1.5 mm) to approximately three-fourths of its length. One end of the tube is sealed with Critoseal molding clay, and the tube is centrifuged in a microhematocrit centrifuge at 5500g for ten minutes. The hematocrit is calculated by measuring the length of the red cell volume (excluding the buffy coat, if any) and dividing this by the length of the entire sample. This fraction is reported as a percent. The normal range in healthy humans is 37 to 54%.

Either just before, or just after, a run is made, two microhematocrit tubes are filled to obtain the H_f value. These are averaged. Except where noted in the results, two tubes were filled from the capillaries to obtain the H_d values. If the sample obtained for the H_d was too small to be accurately measured with a machinist's rule, the distance was measured with a vernier scale on a microscope.

RESULTS AND DISCUSSION

Whole Blood Runs

The results of the whole blood runs are given in Table I. This table contains all the runs performed, including the runs at various flow rates and feed hematocrits. The values reported are the tube diameter in microns, the approximate flow rate in tube diameters per second calculated from the syringe pumping rate, the feed hematocrit H_f , the discharge hematocrit H_d , and the ratio of these last two, H_f/H_d . The values of this ratio were all very close to 1.0 for the range of tubes studied, indicating that little or no entrance effect occurs to cause a decrease in the discharge hematocrit.

Table II gives the averages of the hematocrit ratios for the various tube diameters. When a Student t test was done on this data, the differences between the averages were not significant. These data are plotted in Fig. 11. This graph shows the tube diameter plotted versus the ratio of the exit and entrance hematocrits, H_d/H_f . The curve does not drop at all before about 15 microns. The present data indicate that the curve may be beginning to drop off at this point. The results are entirely different from the results found earlier, as can be seen by comparing Fig. 11 with Fig. 8. It is thought, however, that the data for 8 microns on the earlier graph is correct, and therefore Fig. 11 is drawn using these data.

The differences in the two graphs can be explained by comparing the two methods used in obtaining the data. The earlier study (Fig. 8)

TABLE I
WHOLE BLOOD RUNS

TUBE SIZE (microns)	FLOW RATE (TD/sec.)*	H _f	H _d	$\frac{H_d}{H_f}$	COMMENTS
36.9	3644	41.25	41.50	1.006	
		27.95	27.65	0.989	blood dil. w/ saline
		36.65	36.10	0.985	
		29.80	29.10	0.976	hemolysis in bagged
		29.25	28.65	0.979	blood
28.5	7911	42.30	42.10	0.995	
		43.90	43.07	0.981	
		46.20	46.00	0.996	only one H _d tube
		29.37	28.45	0.968	
		41.30	41.20	0.997	
19.4	28,124	33.50	33.00	0.985	
		38.00	36.40	0.958	
		35.90	34.85	0.971	
	25,074	42.40	42.10	0.993	only one H _d tube
		37.50	36.50	0.973	very slight hemolysis
	14,062	44.67	44.50	0.996	
		38.00	37.75	0.993	

*calculated using pumping rate, see text for discussion

TABLE I (CONT)
WHOLE BLOOD RUNS

TUBE SIZE (microns)	FLOW RATE (TD/sec.)*	H _f	H _d	$\frac{H_d}{H_f}$	COMMENTS
19.4	14,062	35.90	35.60	0.992	
		38.75	39.23	1.012	humidified
	12,537	40.25	40.80	1.014	only one H _d tube
	7031	35.90	35.75	0.996	
16.1	49,213	27.70	27.30	0.986	only one H _d , hum.
	24,607	27.75	27.05	0.975	hum.
	12,303	38.75	37.00	0.955	only one H _d , hum.
		27.60	26.85	0.973	hum.
15.1	59,653	37.80	36.50	0.966	hum.
		38.10	36.85	0.967	hum.
		36.20	35.35	0.976	hum.
		42.65	41.25	0.967	hum., very slt. hemolysis
	28,828	38.75	38.25	0.987	hum.
		25.10	25.30	1.007	hum.
	14,414	25.05	24.25	0.968	hum.

*calculated using pumping rate, see text for discussion

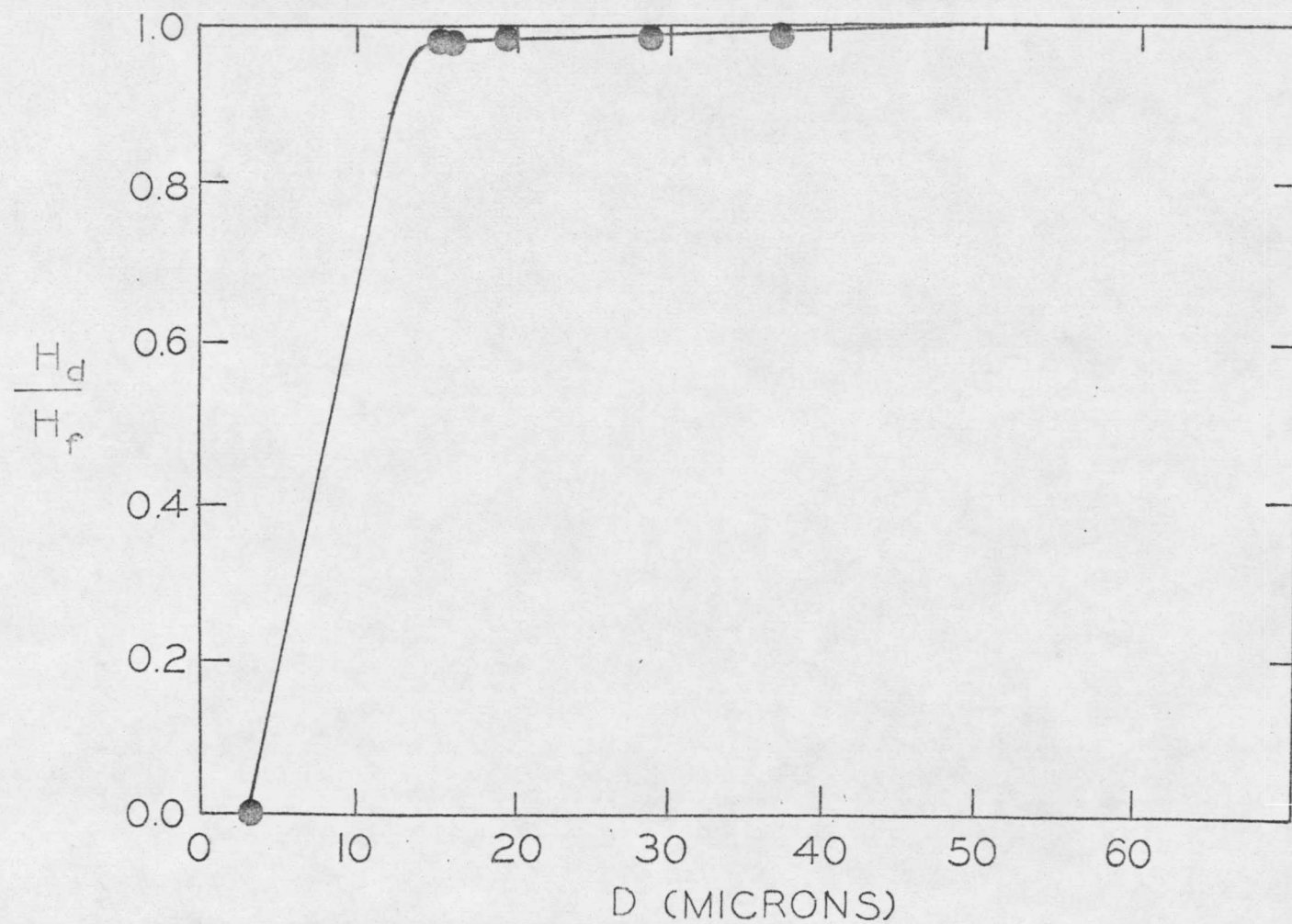


Fig. 11. Results of Whole Blood Runs

TABLE II
AVERAGES FOR WHOLE BLOOD RUNS

TUBE SIZE	NO. RUNS	AVERAGE $\frac{H_d}{H_f}$
36.9	5	0.987
28.5	5	0.987
19.4	11	0.989
16.1	4	0.972
15.1	7	0.976

used a cinemaphotography technique to count the number of cells flowing in the capillary tube. It can be shown that the ratio of the average velocity of the red cells to the average velocity of the blood is equal to the ratio of the discharge hematocrit to the hematocrit in the capillary tube, or

$$\frac{\bar{U}_c}{\bar{U}_B} = \frac{H_d}{H_t}$$

This is done by relating the particle velocity in the tube to the volume of liquid in the tube to get a measure of tube hematocrit, H_t .

The discharge hematocrit is calculated by relating the blood velocity to the fluid flux across a given section of the tube. These two are compared and the relation between bulk velocity and particle velocity is found. This calculation is shown in the Appendix. Using this information, it should be possible to calculate the discharge hematocrit by

knowing both the cell velocity and the blood velocity.

However, in the calculations of the data shown in Fig. 8, the numbers used were average cell velocities rather than blood velocity. This gives discharge hematocrit values which in actuality are the same as the tube hematocrits. This does not completely explain the discrepancies between the two graphs, but it does explain the large difference, especially in the 15 micron range.

The rapid drop-off in Fig. 11 at the smaller tube diameter is more easily explained than the previous graph. Earlier, it was thought that at approximately two red cell diameters, or 14-16 microns, the red cells were crowding each other and allowing only plasma to get into the tube. But since cells are flexible, they can easily slip over one another to enter the various sized tubes. A crowding effect would merely deform them more and force them into the tube, with little or no change in the relation between the plasma and the red cells. As the tube sizes approach the cell diameter, that is, in the range of 8 microns, the actual size of the cell may impede entrance to the tube. At the range of tubes studied, however, there would not seem to be any actual blockage effect of the cells. Hardened cells may show this effect, and thus a reduction in the hematocrit ratio, at the entrance of a tube which is two particle diameters in diameter.

The range of H_d/H_f values obtained is very narrow; the variation could be due only to the error involved in the microhematocrit method.

When the tubes plugged up, it seemed to be an all or nothing situation, with neither plasma nor red cell flow. Apparently, cells aggregate into a clot that fills the whole tube diameter, not just one region which the cells may slip around. This is of physiological interest, as the body has to have a mechanism for removing any such emboli which might form in the circulatory system, or the flow of blood would be restricted. This is indeed the case; a system of enzymes breaks down any such clots that form in the system.

TABLE III
COMPARISON OF HEMATOCRIT RATIOS AT DIFFERENT FLOW RATES

TUBE SIZE (microns)	FLOW RATE* (TD/sec.)	NO. RUNS	AVERAGE $\frac{H_d}{H_f}$
19.4	28,124	3	0.971
	14,062	4	0.998
15.1	59,653	4	0.976
	28,828	2	0.997

*calculated using pumping rate, see text for discussion

Various different flow rates were studied for the smaller tube sizes. These are compared in Table III. The ratio seems to increase slightly as the flow rate is decreased, although this may be due to

the error involved in the microhematocrit method. A Student t test done on these data shows no significance in the differences between these averages.

One of the drawbacks of the apparatus used was that such a large pressure drop had to be maintained across the capillary to get any data within a reasonable length of time. This caused much leaking to occur-- at the standard taper joint, at the various connections within the system, and around the gas-tight syringe. Thus the flows measured using the pumping rate were off by a large factor. The flows for the 16.1 micron tube were measured by calculating the rate which a volume is obtained in the hematocrit tube, and converting it to capillary tube diameters per second. The pumping rate, calculated flow rate and actual flow rate are compared in Table IV. This shows the

TABLE IV
COMPARISON OF CALCULATED AND MEASURED FLOW RATES
FOR 16.1 MICRON TUBES

GEAR RATIO	CALCULATED FLOW RATE (TD/sec.)	MEASURED FLOW RATE (TD/sec.)
128/1	49,213	1990
256/1	24,607	991
512/1	12,203	528

TABLE V
COMPARISON OF HUMIDIFIED AND NON-HUMIDIFIED DATA

TUBE SIZE (microns)	NO. RUNS	AVERAGE $\frac{H_d}{H_f}$	COMMENT
19.4	2	0.986	non-humidified
	2	0.996	humidified

error involved in using the flow rate calculated from the pumping rate.

It should also be noted that the actual flows were proportional to the pumping rate, that is, for twice the pumping rate, the flow will be twice as large.

These actual flows were closer to physiological flows, which range from 2500-5500 tube diameters per second in tubes from 20 to 30 microns in diameter (8). Thus the experiments were closer to an in vivo system than the pumping rate calculations would indicate.

Since the exit reservoir (the hematocrit tube) took so long to fill up, it was thought that there could possibly be some evaporation of plasma taking place, thus increasing the measured exit red cell concentrations and the ratio of the two hematocrits. A calculation of the maximum possible evaporation shows that only 0.2% of the plasma could evaporate from the hematocrit tube in the amount of time available. This means that 99.7% of the blood volume would remain, and the change in the H_d/H_f ratio would be within the range of values obtained for the

various runs. However, the apparatus was surrounded with a plexi-glass box which allowed for constant temperature and humidity control. By evaporating water into the box, it was possible to keep the relative humidity up to about 85-90%, thus minimizing the possibility of evaporation from the hematocrit tube. A comparison of the data for the 19.4 micron tube, both with and without the humidifier going, is shown in Table V. Since the results show the opposite effect of what would be expected if evaporation were occurring, it can be assumed that this is due only to errors with the microhematocrit method, and not due to evaporation. However, the humidifier proved to be an additional bonus. The small amount of evaporation that was occurring was causing the exit end of the tube to plug more easily, and less plugging occurred with the humidifier going.

To determine whether there was an effect due to variations in the feed hematocrit, the concentration of red cells in the feed reservoir was varied by changing the relative amounts of plasma and red cells. The values are compared in Table VI. Again, a Student t test shows the differences in the averages are not significant. It can be seen from these data that a very slight decrease in the hematocrit ratio occurs with an increase in feed hematocrit. This could be due to a crowding effect at the entrance to the tube, causing a relatively larger amount of plasma to enter the tube. It also may be an artifact due to inaccur-

TABLE VI
COMPARISON OF HEMATOCRIT RATIOS
WITH VARIOUS FEED HEMATOCRITS

TUBE SIZE (microns)	H_f	$\frac{H_d}{H_f}$	AVERAGE $\frac{H_d}{H_f}$
15.1	25.10	1.007	0.982
	25.05	0.968	
16.1	27.60	0.973	
	27.70	0.986	
	27.75	0.975	
15.1	37.80	0.966	0.968
	38.10	0.967	
	38.75	0.987	
	42.65	0.967	
16.1	38.75	0.955	

acies in the microhematocrit method.

Addition of Dextran-40

The plasma viscosity was increased by adding Dextran-40 to the plasma. With a concentration of 2.0% by weight of the plasma, the viscosity was found to be about 1.4 times that of the original plasma. This was used to increase the deformation of the red cells, and possibly allow them to enter the tubes more easily. This would give a higher

TABLE VII
DEXTRAM - 40 RUNS

TUBE SIZE (microns)	FLOW RATE (TD/sec.)*	H _f	H _d	$\frac{H_d}{H_f}$	COMMENTS
36.9	3644	36.95	36.45	0.986	slight hemolysis in H _d
		49.85	49.30	0.989	only one H _d tube
		36.65	36.05	0.984	some hemolysis
28.5	7911	36.20	35.80	0.989	small H _d sample
		50.45	49.70	0.985	
		41.20	41.65	1.010	
		37.95	37.05	0.976	some hemolysis
19.4	14,062	44.80	45.80	1.020	
		38.50	38.00	0.987	only one H _d tube
		38.90	37.50	0.964	only one H _d tube
15.1	59,653	42.13	36.90	0.876	some hemolysis, hum.
	28,828	41.50	40.60	0.978	only one H _d , hum.
		39.50	36.90	0.935	only one H _d , hum.

*calculated using pumping rate, see text for discussion

TABLE VIII
AVERAGES FOR DEXTRAN - 40 RUNS

TUBE SIZE (microns)	NO. RUNS	AVERAGE $\frac{H_d}{H_f}$
36.9	3	0.986
28.5	4	0.990
19.4	3	0.990
15.1	3	0.930

hematocrit ratio than would be expected for whole blood. The results of these runs are shown in Table VII. These data show that the effect of the plasma was not great enough to cause any large effect in the ratio. In fact it actually decreased for the 15.1 micron tube, although a Student t test reveals that this decrease is not significant. Thus any variations were assumed to be because of the microhematocrit method. These data are averaged in Table VIII and plotted in Fig. 12. This figure can be superimposed almost exactly on Fig. 11.

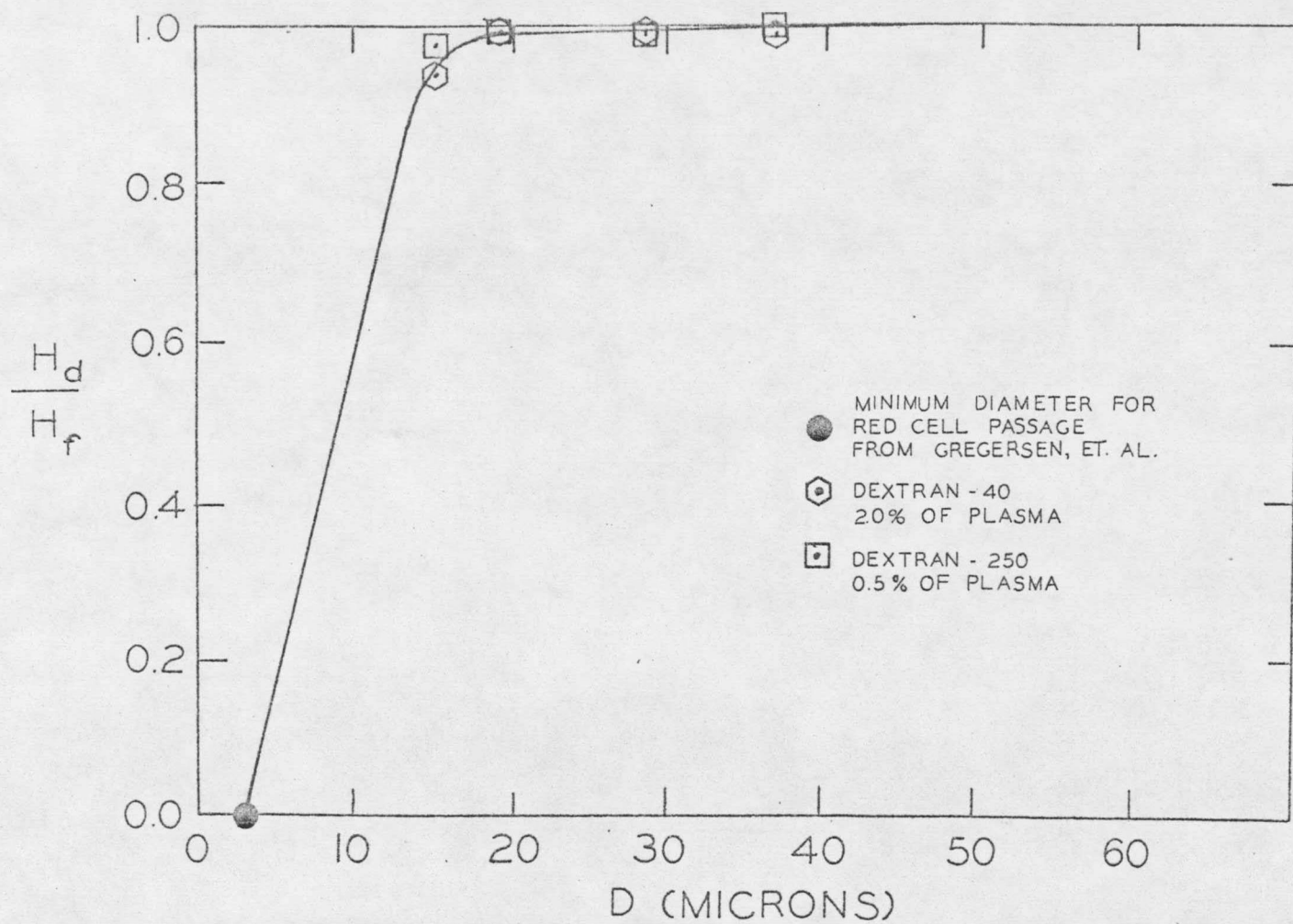


Fig. 12 Results of Dextran-40 and Dextran-250 Runs

TABLE IX
DEXTRAN - 250 RUNS

TUBE SIZE (microns)	FLOW RATE (TD/sec.)*	H _f	H _d	$\frac{H_d}{H_f}$	COMMENTS
36.9	3644	39.95	39.75	0.995	
		40.20	40.40	1.005	
		44.50	44.50	1.000	only one H _d tube
		41.65	41.95	1.007	
28.5	7911	38.70	38.00	0.982	only one H _d tube
		38.00	38.00	1.000	
		38.35	37.50	0.978	only one H _d tube
19.4	28,124	42.20	41.70	0.988	
	14,062	42.50	41.80	0.984	
		40.65	40.70	1.001	hum.
		40.65	40.30	0.991	hum.
15.1	59,653	40.15	39.50	0.984	hum.
		36.23	34.50	0.952	hum.

*calculated using pumping rate, see text for discussion

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TABLE X
AVERAGES FOR DEXTRAN - 250 RUNS

TUBE SIZE (microns)	NO. RUNS	AVERAGE $\frac{H_d}{H_f}$
36.9	4	1.002
28.5	3	0.987*
19.4	4	0.991
15.1	2	0.968

Addition of Dextran-250

Table IX shows the data found using Dextran-250, 0.5% by weight of plasma. It was thought that aggregation of the red cells was occurring due to the high molecular weight Dextran, and the entrance of cells into the capillary tube might be inhibited. It was not known, however, exactly how much aggregation was occurring, as the cells had to be stirred to maintain an accurate feed hematocrit. The aggregates cause the cells to settle out and change the concentration at the entrance of the tube, but stirring causes the aggregates to break up and act as individual cells. At any rate, a decrease in the hematocrit did not occur, and the range in values obtained is broad enough to make it impossible to detect any difference between the Dextran-250 runs and the whole blood runs.

The average values of the hematocrits are shown in Table X, and

these are plotted in Fig. 12. Again, note the similarity to Fig. 11. There is little difference between the data for the Dextran-250 runs and the whole blood runs.

Note on Data Collection

The apparatus used for this study was fraught with inadequacies. The pressure drop across the smallest tube sizes is extremely high. This caused a number of difficulties, including leaking of both blood and silicone out of the various valves and connections in the equipment. The time involved in filling one exit reservoir tube made the data collection long and tedious. A better investigation of this phenomenon at even smaller capillary diameters would require a redesign of the equipment to allow for the high pressure flows involved. It would be beneficial to investigate other means of measuring the exit hematocrits, possibly by a spectrophotometric technique comparing the hemoglobin concentrations in feed and exit reservoirs. This would require a much smaller sample of blood to be forced through the tubes, and take much less time. Also, a better method to prevent tube plugging would save much time and effort and would be less costly.

CONCLUSIONS

Based on the results given in the previous pages, a number of conclusions may be drawn about the effect of small diameters on the exit hematocrit in relation to the entrance hematocrit.

1. The drop-off effect of the hematocrit ratio at 15 microns previously reported does not occur; in fact, no measurable change occurs in this range.

2. The flow rate of the blood does not cause significant difference in the hematocrit ratio at any of the diameters studied.

3. The flow rates calculated by using the pumping rate were much larger than those measured by calibrating the rate which the hematocrit tube filled. However, the two methods gave results that were proportional. The flows measured by the hematocrit tube method were closer to physiological flow rates.

4. Variations in the feed hematocrit did not give a significant difference in the results obtained.

5. Increasing the deformation of the red cells by adding Dextran-40 to the plasma did not alter the hematocrit ratio.

6. Adding Dextran-250 to cause red cell aggregation did not change the hematocrit ratio. This aggregation may not have been great, as the rouleaux may have been broken up by stirring the feed reservoir to keep the red cell concentration constant.

7. Evaporation of plasma was not great enough to cause any error

in the results obtained. Humidifying the apparatus minimized evaporation and helped cut down some of the tube plugging.

Thus it can be concluded that no entrance effect occurs to facilitate the entrance of plasma rather than red cells into capillary tubes, at least down to tube diameters of 15 microns. It may occur at tubes smaller than 15 microns, but this cannot be concluded from this study.

RECOMMENDATIONS

It is obvious that a further study of this phenomenon in the 2.8 to 15 micron range would clarify the lower regions of the graph in Fig. 11. However, the equipment would have to be redesigned to a degree to withstand the pressures involved. If stronger valves were added, much of the leaking could be avoided.

The spectrophotometric technique mentioned earlier would make the data collection easier, as a smaller discharge sample would be needed. It could then be determined whether a drop-off would occur at the limiting diameter for red cell flow--2.8 microns--or more in the range of the red cell diameter--8 microns.

Addition of Dextran-40 and Dextran-250 may change the results in this range, and investigations involving these should be continued.

If an effect is found to occur, it would be interesting to see if cells hardened with acetaldehyde or glutaraldehyde would change the effect. Also, other forms of red cells abnormalities would be interesting to study, for example, sickle cells may react differently than than normal cells.

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APPENDIX

CALCULATION OF DISCHARGE HEMATOCRIT FROM CELL VELOCITY MEASUREMENTS

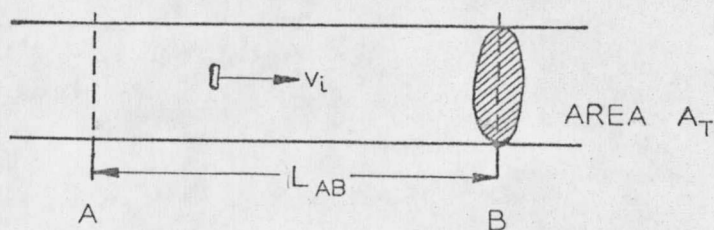


Fig. 13 Diagram of Tube for Material Balance

A material balance is done on a section of a capillary tube shown in Fig. 13. The tube has an area A_T and length L_{AB} between the points A and B, as shown. In time interval θ , the number of particles crossing the plane B with a velocity v_i is

$$\left(\frac{v_i \theta}{L_{AB}} \right) n_i$$

where n_i is the number of cells between A and B with a velocity v_i .

This assumes that the cells are uniformly distributed.

The total number of particles crossing B is

$$\begin{aligned} n_t &= \sum v_i n_i \left(\frac{\theta}{L_{AB}} \right) \\ &= \left(\frac{\theta}{L_{AB}} \right) \sum n_i v_i \end{aligned}$$

This is not the same as the average particle velocity, i. e.

$$\bar{v} \neq \frac{\sum n_i v_i}{n_t} = \frac{L_{AB}}{\theta}$$

Q is the total flux across section B. This can be expressed in terms of the blood velocity, $\bar{U}_B$

$$Q_B \theta = \bar{U}_B A_T \theta$$

The discharge hematocrit hematocrit, or the particle concentration in the fluid flowing out of B is

$$H_d = \frac{n_t V_c}{Q_B \theta}$$

where V_c is the volume of a particle. Substituting gives

$$H_d = \frac{\left(\frac{\theta V_c}{L_{AB}} \right) \sum n_i v_i}{\bar{U}_B A_T \theta} = \frac{V_c}{\bar{U}_B (L_{AB} A_T)} \sum n_i v_i$$

But $(L_{AB} A_T)$ is the capillary volume between A and B, or V_T . So

$$H_d = \frac{V_c}{\bar{U}_B} \sum \frac{n_i}{V_T} v_i$$

or

$$H_d \bar{U}_B = \frac{V_c}{V_T} \sum n_i v_i \quad (1)$$

This relates the discharge hematocrit and blood velocity to the cell volume, tube volume, and particle number and velocity.

We now define a tube hematocrit for the concentration of particles with a velocity v_i .

$$H_{Ti} = \frac{V_c n_i}{V_T}$$

This, substituted into Eq. 1, gives

$$\begin{aligned} H_d \bar{U}_B &= \frac{V_c}{V_T} \sum n_i v_i \\ &= \sum H_{Ti} v_i \end{aligned}$$

This will equal $H_t \bar{U}_c$, where H_T is the tube hematocrit, and $\bar{U}_c$ is the average red cell velocity, only if all the particles have the same velocity v_i , in which case

$$\frac{\bar{U}_c}{\bar{U}_B} = \frac{H_d}{H_T}$$

But the average tube hematocrit, H_T , is defined as

$$H_T \equiv \frac{\sum n_i V_c}{V_T} = \frac{V_c}{V_T} \sum \bar{n}_i \quad (2)$$

Dividing Eq. 1 by Eq. 2 gives

$$\frac{H_d \bar{U}_B}{H_T} = \frac{\frac{V_c}{V_T} \sum n_i v_i}{\frac{V_c}{V_T} \sum n_i} = \bar{U}_c$$

So, in general, it is true that

$$\frac{\bar{U}_c}{\bar{U}_B} = \frac{H_d}{H_T} \quad (3)$$

This could be used to determine the discharge hematocrit, as is used in Fig. 8. However, if the red cell velocity is used instead of the blood velocity, a tube hematocrit will be calculated instead.

The supposed discharge hematocrit is given by

$$H_d' = \frac{V_{RBC}}{V_{blood}} = \frac{V_{RBC}}{(\Delta\theta) \frac{\pi D^3 \bar{U}}{4}}$$

where

V_{RBC} = total red cell volume

V_{blood} = total blood volume

$\Delta\theta$ = time lapse over which cells were counted

D = tube diameter

$\bar{U}$ = average red cell velocity in tube diameter per second

This really should be

$$H_d'' = \frac{V_{\text{RBC}}}{(\Delta\theta) \left(\frac{\pi D^3}{4} \right) \kappa \bar{U}_B}$$

where $\kappa = \bar{U}_B / \bar{U}$, and $\bar{U}_B$ = average blood velocity in tube diameters per second. Taking the ratio of the last two equations gives

$$H_d'' = H_d' (1/\kappa)$$

Based on Eq. 3, $\kappa = H_T / H_d''$. So

$$H_d'' = H_d' \frac{H_d''}{H_T}$$

or

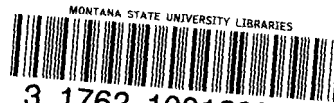
$$\frac{H_d''}{H_T} = 1$$

This shows that the values given on Fig. 8 are no values at all,

rather they are H_T values. The data for the 15 micron point on Fig. 8 could be plotted on Fig. 7, the graph relating tube diameter to tube hematocrit and feed hematocrit. It would show up as a line underneath the line for the 29 micron tube data.

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Flow of human blood
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