HEMORHEOLOGICAL ASPECTS OF REDUCED OSMOLARITY

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
WILLIAM M. PORTEOUS II
1971

ABSTRACT

HEMORHEOLOGICAL ASPECTS OF REDUCED OSMOLARITY

By

William M. Porteous II

In this laboratory, vascular flow resistance is being studied *in vivo* as a function of blood osmolarity. It is therefore desirable to investigate the effect of reduced osmolarity on viscosity. In this work, a small hemodialyzer was used to obtain canine blood samples with osmolarities between 227 and 320 mOsm/l. Flow measurements on the samples were made with a capillary viscometer using a 400 micron diameter tube.

This study indicates that, at least in vessels of 400 micron diameter or larger, blood osmolarity has no significant effect on high shear viscosity, while the yield stress increases with decreased osmolarity. This change in yield stress appeared to be related to the hematocrit. However, since shear stresses *in vivo* are normally quite high, both viscosity and yield stress changes would appear to be unimportant in flow resistance measurements involving small osmolarity changes. Still open to question is the

effect of osmolarity on flow through capillaries of the size found in the vascular bed.

HEMORHEOLOGICAL ASPECTS OF REDUCED OSMOLARITY

By

William M. Porteous II

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Chemical Engineering

1971

G 84703

to

Mary Beth

ACKNOWLEDGEMENTS

I would like to express my appreciation to Dr. D. K. Anderson for his guidance and patience and to thank Dr. F. J. Haddy for his suggestions.

I also extend my gratitude to the Michigan State University Division of Engineering Research, to Mom and Dad, and to Grandpa for financial support.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Though the word hemorheology is less than two decades old, the study which it entails, that of blood deformation and flow, is in its second century. Continual progress is being made in describing the flow of blood through both living bodies and inanimate vessels. Work in this area aids in determining pressure requirements for flow. It helps man to interpret and thus better understand the reasons for many cardiovascular disorders. In addition rheological techniques are used to study blood clotting. Hemorheology, by nature, is interdisciplinary and therefore both engineers and physiologists become involved.

Current research efforts at Michigan State University include studying the effect of blood osmolarity on vascular flow resistance. These studies are being conducted *in vivo* by altering the osmolarity of the blood entering the gracilis muscle of the dog and observing the resulting change in the perfusion pressure at constant flow. Normally the osmolarity is assumed to affect the resistance by constricting or dilating the vessels. However some of the change may be due to changes in viscosity. Reducing the osmolarity of blood might increase the viscosity by causing water to move from the plasma into the erythrocytes. This in turn results in an increased hematocrit and it has been shown that viscosity is an exponential function of hematocrit.

It should be pointed out that most studies on viscosity with respect to hematocrit have been done by varying the number of erythrocytes. The effect is not necessarily the same when hematocrit is altered by changing the size of the erythrocyte.

Thus in order to aid in the interpretation of *in vivo* data on osmolarity effects, this study was undertaken to measure the effects of osmolarity on the rheological properties of blood. To duplicate experimental conditions of the *in vivo* studies, blood samples were taken immediately after the *in vivo* experiments had been performed.

Though the effects on viscosity are probably dependent on tube diameter, this study was limited to the use of a single tube. Data are presented on the apparent viscosity of blood samples with reduced osmolarity. The corresponding data on the apparent viscosity of control samples are also presented for comparison.

BACKGROUND

Rheological Models

Newtonian. For laminar flow of a Newtonian fluid one can utilize a constant viscosity in describing the flow resistance. The flow rate through a circular tube is then given by the Hagen-Poiseuille equation:

$$Q = \frac{\pi R^4 \Delta P}{8\ L\ \mu_p} \tag{1}$$

where Q is the volumetric flow rate, R is the radius, ΔP is the pressure drop, L is the length, and μ_p is the viscosity.

Because blood is a non-Newtonian fluid, one cannot utilize a constant viscosity. Therefore the apparent viscosity is sometimes used to describe the flow resistance. The apparent viscosity for flow through a circular tube is given by:

$$\mu_a = \frac{\pi R^4 \Delta P}{8\ L\ Q} \tag{2}$$

Casson. Though the viscosity of blood is not constant, the use of a yield stress along with a limiting high sheer viscosity, often will describe flow. Reiner and Scott Blair [10] found that for blood the Casson equation:

$$\tau^{1/2} = \tau_y^{1/2} + \mu_b^{1/2} \left(\frac{-dv_z}{dr}\right)^{1/2} \tag{3}$$

gave a satisfactory relation between shear stress and shear rate. Here, τ is the shear stress, τ_y is the yield stress, and μ_b is the limiting viscosity at high shear rates. When Equation 3 is integrated for flow through circular tubes, one obtains the volumetric flow rate:

$$Q = \frac{\pi R^4 \Delta P}{8\ L\ \mu_b} \left[1 - \frac{16}{7}\left(\frac{\tau_y}{\tau_w}\right)^{1/2} + \frac{4}{3}\left(\frac{\tau_y}{\tau_w}\right) - \frac{1}{21}\left(\frac{\tau_y}{\tau_w}\right)^4\right] \tag{4}$$

Here τ_w is the shear stress at the wall and is given by:

$$\tau_w = \frac{R \Delta P}{2\ L} \tag{5}$$

Substitution of Equation 4 into Equation 2 gives the apparent viscosity of a Casson fluid flowing in a circular tube:

$$\mu_a = \mu_b \Big/ \left[1 - \frac{16}{7}\left(\frac{\tau_y}{\tau_w}\right)^{1/2} + \frac{4}{3}\left(\frac{\tau_y}{\tau_w}\right) - \frac{1}{21}\left(\frac{\tau_y}{\tau_w}\right)^4\right] \tag{6}$$

The Casson equation, which was originally used to describe the flow of India ink [1], is frequently used to describe blood flow. Though the Casson equation predicts no flow when the shear stress at the wall is less than the yield stress, what actually happens at these low stresses is in question.

Effect of Hematocrit on Apparent Viscosity

Einstein's Equation. Einstein [3] has shown theoretically that for rigid spheres in a suspending liquid the viscosity of the bulk fluid, μ_b, is related to the viscosity of the suspending fluid, μ_p, by:

$$\mu_b = \mu_p\ (1 + 250H) \tag{7}$$

where H is the percent by volume of spheres. Note that neither the size nor the number of spheres are explicitly important. Because erythrocytes are neither rigid nor spheres, better results are obtained for blood when the constant in Equation 7 is other than 250.

Exponential Equation. An exponential equation which has also been used to correlate viscosity with hematocrit [5] is:

$$\mu_b = \mu_p \exp\ (\lambda H) \tag{8}$$

where λ is an adjustable parameter. This is an empirical equation that was developed by varying the number of erythrocytes and hence the hematocrit.

Cell Size versus Number. With either Equation 7 or 8 viscosity has a large dependence on hematocrit. Unfortunately these equations and their respective parameters have only been used to correlate viscosity with hematocrits altered by changing the number of cells. Therefore the correlations are not necessarily valid when hematocrit is altered by changing cell size. However because Equation 7 has both erythrocyte size and number as implicit variables, it should describe viscosity when hematocrit is changed by either procedure. Even though Equation 8 is empirical and based on changing the number of erythrocytes, the reasoning involved in the derivation of Equation 7, lends support to the use of Equation 8 when cell size is altered.

In small capillaries (8 micron diameter) the question of cell size versus number has different implications. Because the erythrocytes are larger than the small capillaries, they must deform to flow through them. Increasing the hematocrit through cell size influences the apparent viscosity by altering the ability of the erythrocytes to deform and flow through the capillaries. Thus the change in apparent viscosity is probably not the same as when hematocrit is changed by increasing the number of cells.

Effects of Osmolarity on Viscosity

Erythrocyte Size and Deformability. One of the most important ways in which osmolarity affects blood is by changing the erythrocyte size and shape. The normal shape, that of a biconcave disc with rounded edges, may be altered for various reasons. Lowering the osmolarity tends to make the erythrocytes more spherical. This in turn increases the hematocrit which may increase the apparent viscosity as mentioned in the preceding discussion.

In addition Murphy [9] has found that the spherically shaped erythrocytes of hereditary spherocytosis are less deformable and thus increase blood viscosity. A similar effect has been found by Schmid-Schönbein *et al.* [13] for erythrocytes with reduced size. Using Millipore filters they found that osmotic crenation increased viscosity. With packed erythrocytes Wells and Schmid-Schönbein [16] also found an increase in viscosity with extreme increases or decreases in osmolarity. Thus large alterations in erythrocyte size and/or shape seem to reduce deformability and hence increase viscosity.

Electrokinetic Charge and pH. Blood pH may also be altered when the osmolarity is reduced. According to Rand *et al.* [11], pH changes may alter plasma water content enough to affect plasma viscosity, and to a minor but measurable degree, blood viscosity. However no such relation was found by Masin [6].

Both osmolarity and pH affect the electrokinetic charge of cells and decreasing the charge of the erythrocytes was found by Seman and Swank [15] to increase the viscosity. Another factor affecting the electrokinetic charge is that of relative ion concentration. Masin [6] has found that certain ions, in particular potassium, may have an effect on the apparent viscosity.

Reduced osmolarity may also affect the charge of proteins. It is not known whether this will in turn affect viscosity. However, Mayer *et al.* [7] have studied various proteins, including fibrinogen, and found that protein concentration correlated with viscosity.

A possible reason for the electokinetic charge affecting the apparent viscosity is its ability to affect the deformability of and aggregations of erythrocytes.

Previous Results. Using the Casson relationship, Meiselman *et al.* [8] studied the effect of increased osmolarity on blood viscosity. They found an increase in limiting viscosity and a decrease in yield stress. A similar study was later performed by Schmid-Schönbein *et al.* [13] and they confirmed these results. Schmid-Schönbein *et al.* [14] also used ultrafiltration to increase osmolarity and reported an increase in apparent viscosity.

The effect of reduced osmolarity on blood viscosity was also studied by Meiselman *et al.* They found an increase in yield stress that was related to the increased hematocrit. The limiting viscosity showed a small decrease. However using a viscometer with shear rates greater than 400 sec^{-1} Cox and Su. [2] found no change in limiting viscosity with reduced osmolarity. Because Meiselman *et al.* made no measurements with shear rates greater than 25 sec^{-1} these results are not necessarily in conflict.

EXPERIMENTAL METHODS AND APPARATUS

A hemodialyzer of the type described by Grimsrud and Babb [4] and by Roth [12] was used to decrease the osmolarity of blood samples. The dialyzer was fed blood from the femoral artery of a dog using a constant displacement pump. Normal Ringer's solution was used as a dialysate for control samples. Ringer's solution with a reduced NaCl concentration was used to produce low osmolarity blood samples. After the blood had passed through the dialyzer, 50 cc samples were collected in polyethylene bottles which were capped after collection.

Within two hours of collection samples were treated with 0.0025 g of heparin to prevent coagulation. One sample was then refrigerated while pH and flow measurements were made on the other at room temperature. The second sample was allowed to come to room temperature and measured immediately after the first. Each run took about 3 hours.

The procedure and apparatus (see Figure 1) for determining the viscosity were those used by Masin [6] and in short are as follows. A capillary viscometer with a tube diameter of 397.9 microns and a length of 15.796 cm

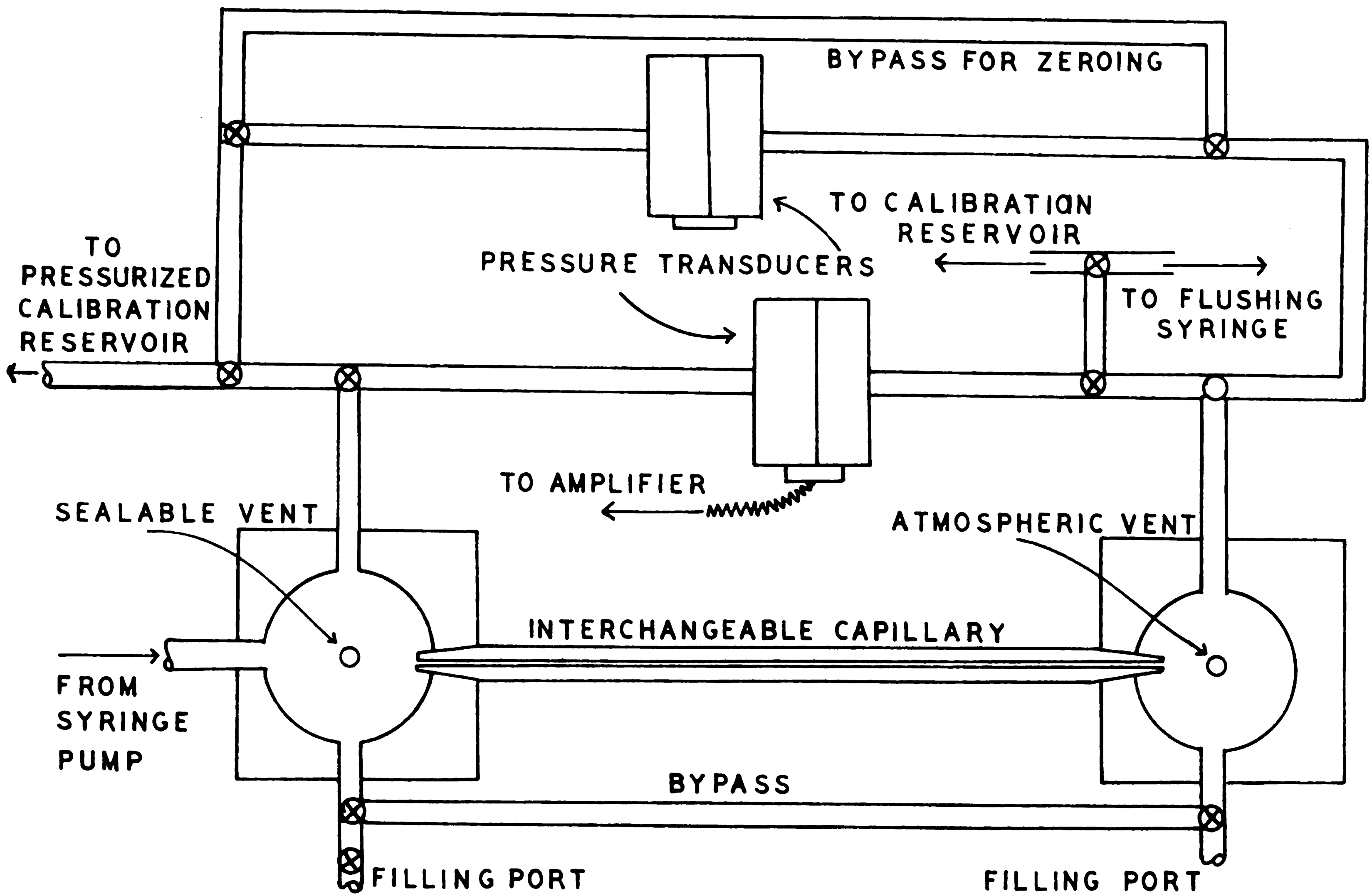

Figure 1. Flow-Pressure Apparatus Diagram.

was used. Blood was placed in both upstream and downstream reservoirs. The upstream reservoir was fed blood using a 1.0 cc syringe and a syringe drive. The pressure drop was measured with a pressure transducer, and voltage output was recorded continuously. Thus for a series of flow rates the pressure drop was measured.

The results were then fit by a least squares technique to the Casson equation in integrated form for flow through circular tubes. Thus a limiting viscosity and a yield stress were obtained for eight low osmolarity blood samples and the corresponding controls.

RESULTS

Osmolarity Changes

The average osmolarities for the control and low osmolarity samples were 306 mOsm/l and 265mOsm/l respectively (see Table 1). The mean percent change in osmolarity was -13.4 and the standard deviation was 5.5.

Hematocrit

The percent change in the hematocrit was always positive (see Table 1) and had a mean of 6.2 and a standard deviation of 3.8. The correlation coefficient of percent change in hematocrit with percent change in osmolarity was -0.76. Thus a significant change in hematocrit and correlation with osmolarity was observed.

Limiting Viscosity

The limiting viscosity decreased in five of the eight low osmolarity samples (see Table 2). The mean of the percent change in limiting viscosity was -0.60 and the standard deviation was 15.5. The percent change in limiting viscosity and the percent change in osmolarity had a correlation coefficient of -0.029. Thus the limiting

TABLE 1.--Experimental Osmolarities and Hematocrits.

Data Set	Osmolarities (mOsm)			Hematocrits		
	Control	Low Osmolarity	Percent Change	Control	Low Osmolarity	Percent Change
1	309	255	-17.5	47	52	10.6
2	300	227	-24.3	32	36	12.5
3	302	273	- 9.6	55	57	3.64
4	319	269	-15.7	58	60	3.45
5	297	265	-10.8	56	60	7.14
6	289	257	-11.1	36	38	5.56
7	314	283	- 9.87	49	49.5	1.02
8	316	291	- 7.19	53	56	5.66

TABLE 2.--Experimental Viscosities and Yield Stresses.

Data Set	Limiting Viscosities (cp)			Yield Stresses (dyne/cm^2)		
	Control	Low Osmolarity	Percent Change	Control	Low Osmolarity	Percent Change
1	4.76	5.70	19.8	0.028	0.094	236
2	4.45	3.80	-14.6	0.035	0.040	14.3
3	7.56	7.40	- 2.1	0.235	0.296	26.0
4*	5.83	6.12	5.0	0.258	0.240	- 7.5
5*	7.24	8.81	21.7	0.115	0.170	47.7
6*	4.93	4.73	- 4.0	0.079	0.099	25.3
7	6.46	5.02	-22.3	0.105	0.105	0.0
8	8.32	7.64	- 8.2	0.152	0.208	36.9

*Indicates low osmolarity sample measured first.

viscosity showed no significant change or correlation with osmolarity.

<u>Yield Stress</u>

The yield stress increased with reduced osmolarity in all but one case (see Table 2). The mean of the percent change was 47.4 while the standard deviation was 78.3. The correlation coefficient with percent change in osmolarity was only -0.214. Thus a significant change in yield stress does not seem to correlate well with osmolarity. However the change in hematocrit and the change in logarithm of the yield stress had a correlation coefficient of 0.74. Thus though the change in yield stress does not seem to correlate directly with the change in osmolarity, there is probably an indirect correlation based on hematocrit.

When logarithm of the yield stress versus hematocrit was fit by a least squares straight line to all data points (both control and low osmolarity) a slope of 0.062 was obtained. The correlation coefficient was 0.65 with a p of 0.04 (see Figure 2). Thus yield stress also had an overall dependence on hematocrit. The average slope of the individual changes in logarithm of the yield stress versus hematocrit was 0.083. A t-distribution was applied to this average and the least squares slope fell within the 75 percent confidence interval. Thus the individual yield stress changes agree with the expected change due to hematocrit.

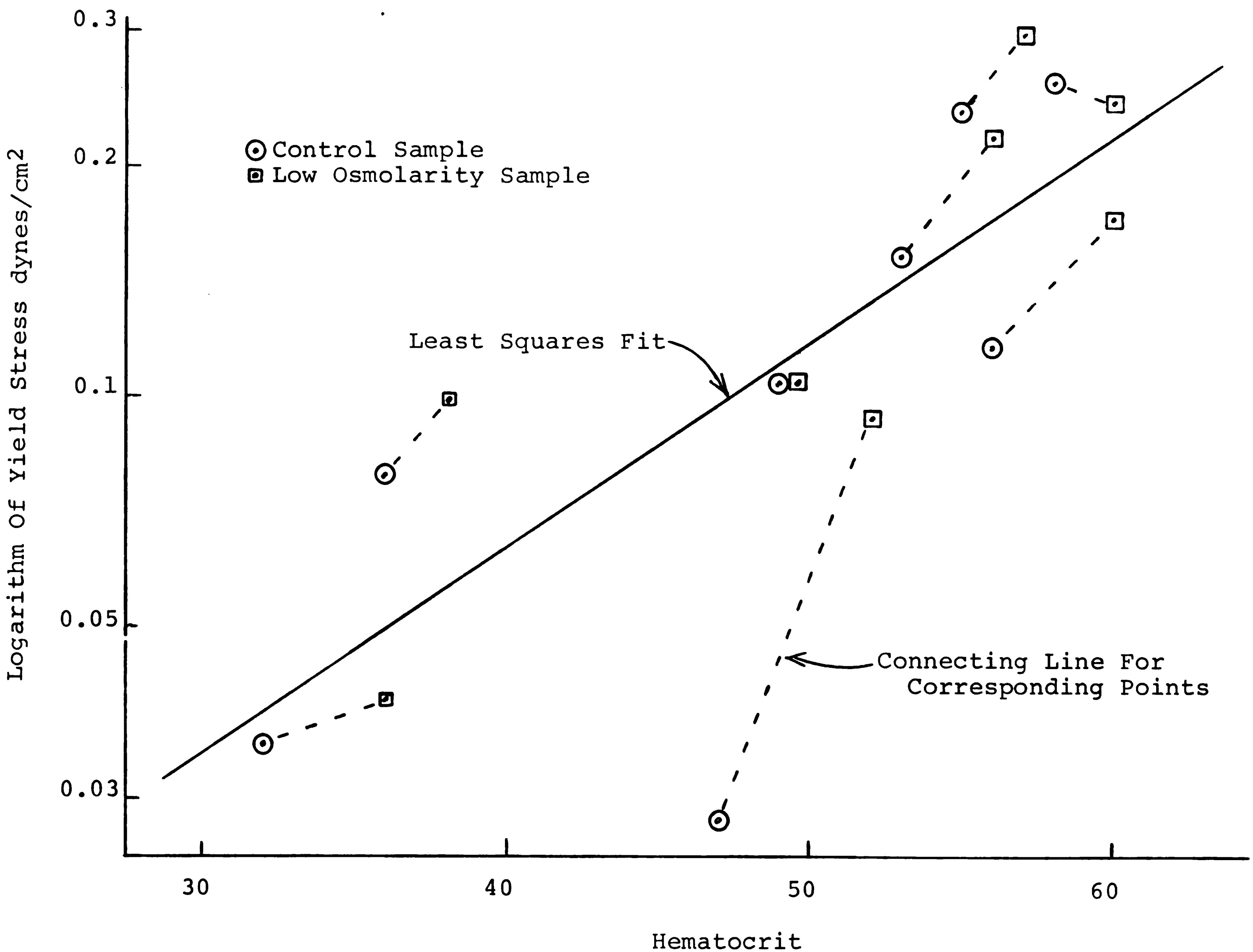

Figure 2. Yield Stress Versus Hematocrit.

pH

With the exception of one case, the pH increased with reduced osmolarity (see Table 3). The increase was significant since the average was 0.134 and the standard deviation was 0.084. However the correlation coefficient with percent change in osmolarity was only -0.186. Thus though the increase in pH appears to be significant, the correlation with osmolarity change is poor.

TABLE 3.--Experimental pH's and Temperatures.

Data Set	pH's			Temperatures (C)		
	Control	Low Osmolarity	Change	Initial	Final	Change
1	7.16	7.38	0.22	20.95	22.85	1.90
2	7.38	7.45	0.07	21.94	23.15	1.21
3	7.38	7.49	0.11	21.72	22.79	1.07
4	7.27	7.48	0.21	20.1	21.08	0.98
5	7.37	7.51	0.14	23.24	24.86	1.62
6	7.10	7.30	0.20	20.74	22.2	1.46
7	7.19	7.34	0.15	23.12	23.76	0.64
8	7.33	7.30	-0.03	22.95	24.15	1.20

TABLE 4.--Means and Sample Standard Deviations for Percentage Changes.

Percentage Change	Mean	Sample Standard Deviation	p*
Osmolarity	-13.4	5.48	.0005
Hematocrit	6.2	3.81	.005
Limiting Viscosity	- 0.6	15.5	---
Yield Stress	47.4	78.3	.10
pH**	0.134	0.084	.005

*Probability of having a mean with the same sign and a larger absolute value for a series of random numbers with the given sample standard deviation.

**Not percentage change, but actual change.

TABLE 5.--Correlation Coefficients for Control to Low Osmolarity Changes.

Change			
Independent	Dependent	Correlation Coefficient	p**
Osmolarity*	Hematocrit*	-0.76	0.015
Osmolarity*	Limiting Viscosity*	-0.029	0.48
Osmolarity*	Yield Stress*	-0.214	0.31
Osmolarity*	pH	-0.186	0.34
Hematocrit	Log Yield Stress	0.74	0.017

*Percentage change.

**Probability of having a correlation coefficient with the same sign and a larger absolute value for two uncorrelated series.

DISCUSSION

Limiting Viscosity

The hematocrit is normally an important factor affecting the viscosity. Though the low osmolarity samples had increased hematocrits and were less deformable [9] (i.e. had more spherical erythrocytes), they showed no significant increase in limiting viscosity. This result corresponds with that obtained by Cox and Su. [2]. Any hematocrit effect may have been in part counteracted by the increase in pH which, according to Rand *et al.* [11], reduces viscosity. However Masin [6] found little effect of pH on viscosity.

In addition changing the concentrations of various ions in the blood could have had an effect on the erythrocytes and proteins, and in turn on the limiting viscosity. Unfortunately except for the study of Masin [6] this area is relatively unexplored.

Apparent Viscosity

The decrease in limiting viscosity, though insignificant, and the increase in yield stress obtained in this study correspond with the results obtained by

Meiselman *et al.* [8] for reduced osmolarity. Because of the effect on yield stress one would expect to see an increase in apparent viscosity with reduced osmolarity at low shear rates (see Equation 6). However the yield stress does not usually manifest itself *in vivo* due to the high shear rates encountered. Therefore in vessels of 400 microns or larger, reducing the osmolarity appears to have no significant effect on *in vivo* apparent viscosity.

It should be pointed out however that *in vivo* the erythrocytes must deform to flow through the small capillaries (8 micron diameter). Since osmolarity effects the deformability of erythrocytes, the effect of osmolarity on apparent viscosity in the small capillaries may be different. However if the experiments of Wells and Schmid-Schönbein on packed erythrocytes hold for whole blood, the effect of small reductions in osmolarity on apparent viscosity will be insignificant in the small capillaries also.

Thus, with the possible exception just mentioned, when measuring changes in vascular flow resistance due to blood osmolarity, changes in apparent viscosity do not have to be taken into account.

SUMMARY AND CONCLUSIONS

Reductions of 10 to 20 percent in the osmolarity of blood did not appear to have any significant effect on the limiting high shear viscosity obtained using a glass capillary with a diameter of 400 microns. However the yield stress showed a significant increase that appeared to be related to the change in hematocrit. Thus one would expect an increase in apparent viscosity with reduced osmolarity at low shear rates. Because of the high shear rates normally encountered with *in vivo* blood flow, changes in apparent viscosity due to reduced osmolarity do not seem to be significant.

RECOMMENDATIONS

To better understand the effects of reduced osmolarity on hemorheology, viscosity measurements should be made over a larger range of hematocrits and some experimental work should be done with a larger change in osmolarity. In addition to using a regular viscometer, flow measurements should be made with a Millipore filter to help answer the question of what happens in small capillaries.

Greater accuracy would be obtained by adding temperature control. The most desirable temperature would be one close to body temperature, about 37 C. To obtain low osmolarity and control samples alike in all respects other than osmolarity, the samples should be taken as soon as possible after cannulation.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Casson, N., in Rheology of Disperse Systems, Chap. 5, C. C. Mill (Editor), Pergamon Press, Oxford (1959).

2. Cox, H. A. and G. J. Su., in Proc. Intern. Congr. Rheology-Symp. Biorheology, 1965, part 4, 337.

3. Einstein, A., Ann. Physik. 19, 289 (1906).

4. Grimsrud, L. and A. L. Babb, Trans. Am. Soc. Artificial Int. Organs 10, 101 (1964).

5. Haynes, R. H., Am. J. Physiol. 198(6), 1193 (1960).

6. Masin, J. G., Ph.D. Thesis, Michigan State University (1971).

7. Mayer, G. A., *et al.*, Biorheology 3, 177 (1966).

8. Meiselman, H. J., *et al.*, J. Appl. Physiol. 22. 772 (1967).

9. Murphy, J. R., in Hemorheology, 469, A. L. Copley (Editor), Pergamon Press, London (1968).

10. Reiner, M. and G. W. Scott Blair, Nature, Lond. 184, 354 (1959).

11. Rand, P. W., *et al.*, J. Appl. Physiol. 25(5), 550, (1968).

12. Roth, S. A., Ph.D. Thesis, Michigan State University (1970).

13. Schmid-Schönbein, *et al.*, Circ. Res. 25, 131 (1969).

14. Schmid-Schönbein, *et al.*, Pflueger Arch., 312, R76 (1969).

15. Seman, G. V. F. and Swank, Biorheology 4, 47 (1967).

16. Wells, R. and Schmid-Schönbein, J. Appl. Physiol. 27, 213 (1969).

APPENDIX

APPENDIX A

NOMENCLATURE

Symbol	Meaning	Units
H	Hematocrit	--
L	Length	cm
p	Probability	--
ΔP	Pressure drop	$dyne/cm^2$
Q	Volumetric Flow Rate	cc/sec
r	Radius	cm
R	Radius of Vessel	cm
v_z	Velocity in z Direction	cm/sec
π	3.14159. . . .	--
μ_a	Apparent Viscosity	cp
μ_b	Limiting Viscosity of Bulk Fluid	cp
μ_p	Viscosity of Suspending Fluid	cp
τ	Shear Stress	$dyne/cm^2$
τ_w	Shear Stress at the Wall	$dyne/cm^2$
τ_y	Yield Stress	$dyne/cm^2$