The Effect of Blood Chemistry on the Rheological Properties of the Fluid

by

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(ABSTRACT)

A four variable constitutive equation was developed utilizing the method first presented by Schneck and Walburn. Spearman rank correlation coefficients were calculated on whole blood samples within a narrow range of hematocrit to investigate further the effect of the various plasma constituents on whole blood viscosity.

Viscosity measurements were made on one hundred anticoagulated blood samples of known hematocrit and chemical composition. The constitutive equation was developed using a power law functional form similar to that employed by Schneck and Walburn. This equation contains two parameters, the consistency index and the non-Newtonian index. A computerized multiple regression technique with apparent viscosity as the dependent variable was used to determine the particular form of these parameters.

The one, two and three variable models developed confirmed the results of the previous work of Schneck and Walburn. The four variable model included the total lipids in combination with the concentration of total protein minus albumin and hematocrit. Spearman rank correlation coefficients showed the highest correlations between whole blood viscosity and the plasma constituents to be those of the globulins, total protein and fibrinogen.

The constitutive equation developed did not show as high a correlation between experimental data and theory as did the Schneck-Walburn three variable model. The addition of a fourth variable did produce a statistically significant increase over the best three variable model of the present study.

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CHAPTER 1

INTRODUCTION

The modern clinical pathology laboratory has become an indispensable, efficient and accurate source of information to aid the physician in diagnosing many disease states. The chemical composition of blood plasma, the morphology and concentration of the various blood cells, and the rate of coagulation of blood can all be relied upon to provide evidence of the state of health of the body. Despite the increasing number of chemical blood tests now available, measurements of the physical properties of blood (in particular, blood viscosity) have only recently gained some acceptance as an indicator of cardiovascular disorders, malignancies and other pathologic conditions. It has been suggested (1) that detection of impending cardiovascular disease or impending cancer metastasis can often be made by estimation of the blood viscosity factors, even at a time when the usual clinical methods present a normal picture. In addition to the prediction of disease, viscosity measurements could also be of prognostic value (2,3).

The concept of measuring plasma viscosity as a clinical pathology procedure results from the research of Whittington and Miller as reported in the article by Harkness (4). Whittington and Miller showed in 1942 a correlation between increased plasma viscosity and the severity of pulmonary tuberculosis. Since then numerous reports have linked abnormally high viscosity values to such diverse conditions as

multiple sclerosis (5), sickle cell anemia (6), diabetic microangiopathy (7) and Wilm's Tumor (8). Therapeutic manipulation of blood viscosity has also been considered (9,10). Most investigations on whole blood viscosity, however, are still aimed at providing information on its involvement in the etiology of occlusive vascular disease, which remains one of the leading health hazards in Western society.

Study of the pathogenesis of cardiovascular disease now concentrates on the flow properties of blood rather than driving pressures, histology of the vessels or biochemistry of the fluid (1). A useful approach to the investigation of blood flow is through mathematical modeling of the in vivo situation, which necessitates the development of a constitutive function. This equation describes the deformation behavior of a material in terms of its physical properties. In the case of whole blood, it relates the shear stress to the shear rate and, in some cases, to the hematocrit and other variables.

The function first employed by Schneck and Walburn (11) has been reported (12) to be the most successful in describing the flow properties of whole blood. It is the intent of this thesis to improve still further on their model by providing a more in-depth investigation into the effects on blood of the lipid fractions and fibrinogen concentration, which were not significantly included in the development of the Schneck-Walburn function. The possible contributions of these variables to the existing model will be considered and any significant correlation among these variables and whole blood viscosity will be determined.

CHAPTER 2

REVIEW OF LITERATURE

A. Early Work

The discovery in 1915 by Hess (13) that blood exhibits non-Newtonian behavior meant that viscosity measurements on whole blood can only be meaningful if carried out at a range of constant shear rates. This led to a lack of interest in the clinical hemorheologic aspects of the viscosity of whole blood until the 1960's when variable shear rotational viscometers were introduced (4). Research since then has focused primarily on identifying the changes in viscosity associated with various disease states which affect the cellular constituents of blood, and also the biochemistry of the plasma (see Appendix A, reproduced from Schneck, Daniel J., "Blood", Virginia Tech College of Engineering, Department of Engineering Science and Mechanics, Biomedical Engineering Program, Technical Report Number VPI-E-84-33, Blacksburg, Virginia, October 15, 1984). Interest in providing a more rigorous constitutive equation to describe blood flow properties adequately has only occurred in recent years. For a more comprehensive review of the history of blood viscometry and constitutive functions related to blood flow prior to 1976, the reader is referred to reference (14). A few important points will be repeated here for the sake of clarity.

B. Modern Developments

Blood has been found to behave as a combination of a pseudoplastic and Bingham plastic fluid. Its pseudoplastic behavior is characterized by the fact that as the shear rate increases the resisting shear stress increases in disproportionately smaller amounts (see Figure 1). A Bingham plastic fluid is one which exhibits a yield stress (15). This characteristic has been reported in blood (16,17). Such a combination in blood of pseudoplastic and Bingham plastic behavior shaped the development of the earliest constitutive functions (14). Constitutive equations proposed more recently include those of Quemada (18), Phillips and Deutsch (19) and Huang and Fabisiak (20). Phillips and Deutsch proposed a non-linear viscoelastic equation based on the work of Thurston (21) whose study of pulsatile flow through tubes convinced him of blood's viscoelastic nature. Two simplified forms of the equation with a hematocrit dependence introduced appear below (12).

$$\tau = X_1 \exp(X_4 H) \frac{1 + X_2 \dot{\gamma}^2}{1 + X_3 \dot{\gamma}^2} \dot{\gamma} \qquad (2.1)$$

$$\tau = X_1 \exp(X_4 H) \frac{1 + X_2 H \dot{\gamma}^2}{1 + X_3 H \dot{\gamma}^2} \dot{\gamma} \qquad (2.2)$$

where τ = shear stress

$\dot{\gamma}$ = shear rate

H = hematocrit (%)

X_i, i = 1,2,3,4 = set of parameters which determine the specific form and allow adjustment of the function in question.

The equation proposed by Huang and Fabisiak, which developed from the earlier work of Huang (22), introduced a molecular arrangement parameter related to the entropy. The rheological equation for a pseudoplastic fluid was subsequently developed from the model for thixotropic (time dependent) fluids. The equation is presented in the following form (12).

$$\tau = \mu_p \dot{\gamma} \pm cA\dot{\gamma}^n \exp(-c\dot{\gamma}^n t_o) \tag{2.3}$$

where μ_p = plasma viscosity

c,A,n,t_o = specified numerical constants

The (+) sign is taken for a dilatant fluid and the - sign for a pseudoplastic or shear-thinning fluid. Quemada derived the following function (12):

$$\tau = X_4\dot{\gamma} \quad \frac{1 + X_1^{-1}\dot{\gamma}^{-\frac{1}{2}}}{1 - X_2H + (1 - X_2X_3H)\, X_1^{-1}\dot{\gamma}^{-\frac{1}{2}}} \tag{2.4}$$

The aforementioned equations and eight others based on these were compared to that of Schneck and Walburn (11) in a study conducted by Easthope and Brooks (12) in 1980. The Schneck-Walburn function (see

below) was found to provide the most accurate fit to the clinical data against which it was tested.

Schneck and Walburn developed their empirical constitutive function from a power law equation of the form

$$\tau = k\dot{\gamma}^n \text{ dynes/cm}^2 \tag{2.5}$$

where

k = consistency index, and

n = non-Newtonian index.

The parameters k and n were examined as a function of hematocrit, plasma proteins and total lipids using a computerized multiple regression technique. This technique provided the function of best fit in the linearized (logarithmic) form of the equation. The resultant three variable model showed a shear-stress dependence on shear rate, hematocrit and total protein minus albumin (TPMA), with the parameters k and n taking the form:

$$k = C_1 e^{C_2(\text{Hematocrit}) + C_4 \text{TPMA}/(\text{Hematocrit})^2} \tag{2.6}$$

$$\text{and } n = 1.0 - C_3(\text{Hematocrit})$$

where $C_1 = 0.00797$

$C_2 = 0.0608$

$C_3 = 0.00499$

$C_4 = 145.85$ for <u>human</u> blood. The C's are species - specific constants.

This model has an R^2 value of .9049 (where a value of 1.0000 would represent a perfect fit between experimental data and regression analysis) and a T value equal to 0.0001 (the probability that the R^2 value is wrong).

In order to derive an empirical equation similar to the one above, it is necessary to identify those chemical constituents most likely to affect the viscosity of blood. Most investigators agree that the constituent which affects plasma viscosity the most is total protein (4,23,24,25). The non-protein constituents in plasma contribute very little to the viscosity. Even the extreme changes associated with uremic and diabetic comas have little significant effect on plasma viscosity (4).

When the three protein fractions are present in equal concentrations in saline solution and are tested separately, it is the protein fibrinogen which exerts the most influence on plasma viscosity. This is reasonable considering its high molecular weight (Table 1), asymmetry, and the ability to cause aggregation of erythrocytes. Increased concentrations of fibrinogen, and consequent increases in plasma and whole blood viscosity have been identified in many pathologic conditions (6,26-29) and in the normal state (29-33). Both Mayer (32) and Dintenfass (29) found correlations between plasma or whole blood viscosity and the fibrinogen concentration. Mayer's correlation coefficients range in value from 0.314 to 0.464 with $p \leq .001$ and Dintenfass reports values in the range of 0.250 to 0.486 with $p \leq .001$.

Both investigators used Spearman correlation coefficients (see Appendix B).

When the protein fractions are present in their normal concentration ratios (Table 1), the globulin fraction has been found to have the most significant effect on plasma viscosity (32, 29). An increase in the globulin fraction of plasma is associated with severe chronic infections, malignancies or any disease state which excessively stimulates the body's immune system. The relationship of these conditions to increased blood viscosity has been well documented (23,29,33,34).

Albumin has the least effect on viscosity and it is proposed that it may actually decrease the viscosity of plasma (23). Mayer (32) also reports a negative correlation between albumin and plasma viscosity. Despite being recognized as a major risk factor in atherosclerosis (35), the rheologic effects of the plasma lipids have not been investigated as fully as the other constituents of plasma. Leonhardt (36) reports positive correlations (Spearman correlation coefficients) between plasma viscosity and the individual lipid fractions (triglycerides, cholesterol, phospholipids) and also a significant correlation r=0.719, between total lipids and plasma viscosity. This study, however, failed to provide information on the fibrinogen concentrations of the samples tested. This is important in the light of a subsequent investigation (27) involving patients with type II hyperlipoproteinemia. In this study Lowe, et al., show an association between increased whole blood and plasma viscosity and

increased fibrinogen concentration. They also mention the lack of a relationship between increased viscosity and lipid or lipoprotein concentrations. Seplowitz, et al. (37) report correlations between plasma viscosity and plasma triglyceride concentration ($r=0.56$, $p \leq 0.01$), cholesterol concentration ($r=0.29$, $p \leq 0.05$) and chylomicron concentration ($r=0.92$, $p \leq .001$). This investigation involved hyperlipidemic patients with subsequent chylomicronemia. It is significant to note, however, that after removal of the chylomicrons, viscosity values returned to baseline levels. These reports conflict somewhat with the work of others. Dintenfass (24) studied lipid poor and lipid rich plasma at low temperatures and at 37°C. He found very little difference in viscosity at 37°C. In a later study Dintenfass also reports no significant correlation between cholesterol concentration and plasma viscosity (38). Schneck and Walburn (14) also report a limited involvement of plasma lipids in the development of their constitutive equation. Mayer (32) showed no significant correlation between plasma viscosity and the beta lipoproteins (LDL). A review of the literature, however, reveals the lack of a comprehensive study relating all of the measurable plasma constituents to whole blood viscosity.

Most other investigations have focused on the rheological properties or concentration of the erythrocytes. Ramcharan, et al. (38), related whole blood viscosity to red cell charge or zeta potential. Thomas, et al. (39), and Friedland, et al. (40) have studied the effect of hematocrit on cerebral blood flow. Nicolaides, et al.

(41) identified increased red cell flexibility as a factor in counteracting the effects of increased plasma fibrinogen in patients with angina. Studies have also been made to determine the causes and effects of exercise on plasma viscosity (42).

C. Summary

The flow behavior of whole blood is dependent upon the rate at which it is sheared, its hematocrit, the viscosity of the plasma, its temperature, the vascular geometry and circulatory driving pressures, and erythrocyte aggregation and flexibility. The last two factors are prime determinants of the non-Newtonian behavior of the fluid (43). Quantification of these complex phenomena presents many difficulties and challenges. In an attempt to model flow behavior, recent constitutive equations concentrated mainly on nonlinear viscoelastic functions with thermodynamic considerations. The function developed by Walburn and Schneck incorporates the hematocrit, shear rate, and globulin concentration of plasma into a practical and realistic equation which has been found to be reasonably successful in describing the clinical data. Other research has been aimed at relating the plasma proteins and lipid fractions to the viscosity. Investigators have also studied the properties of the erythrocytes, i.e. erythrocyte charge, flexibility, aggregation and their internal viscosity. The work which follows represents an attempt to improve the Schneck-Walburn model still further, as stated in Chapter 1.

CHAPTER 3

METHOD OF ANALYSIS

A. Materials

One hundred whole blood and serum samples from non-fasting surgical admissions were obtained from the hematology laboratory of Montgomery County Hospital, Blacksburg, Virginia. For the sake of confidentiality, no information was provided as to the age, sex or state of health of the donors. Each whole blood sample, anticoagulated with EDTA (see Appendix A) was carefully mixed and two aliquots of 1.2 mls each extracted. When compared with natural plasma, all anticoagulants have a measurable effect upon the plasma viscosity. EDTA has been found to have the least effect (4). Viscosity measurements were made as described below at strain rates of 46.56, 116.40 and 232.80 reciprocal seconds, and the results at each shear rate were averaged for the two aliquots tested to minimize errors due to incomplete mixing. If large discrepancies occurred due to possible clot formation, or other factors, another aliquot was withdrawn and tested.

Viscosity measurements were performed at 37°C using a Wells-Brookfield Cone and Plate Viscometer with a Brookfield Model N recirculating constant temperature water bath. The instrument was calibrated daily using the Brookfield calibration oil standard (10 Cp) provided with the machine. The cone on plate configuration of this instrument ensures that the whole sample is exposed to the same shear rate (14).

The velocity difference between the cone and plate surfaces will increase toward the periphery but so will the distance between them, thereby ensuring a constant velocity gradient or shear rate. The coefficient of variation of individual readings varies from 2% at the highest shear rate (230 sec^{-1}) to 8% at 23 sec^{-1} (4).

Total cholesterol, triglycerides, total protein and albumin determinations were performed on a Dupont ACA autoanalyzer and generously supplied by the hospital. The remaining chemical variables were determined in the Biomechanics Laboratory at VPI&SU by the methods summarized below.

Total lipid determinations were performed on the serum sample within 24 hours after collection utilizing Dade total lipid reagent and total lipid standard (American Scientific Products, 8855 McGaw Road, Columbus, Maryland 21045). The determination depends on the method first described by Zollner and Kirsch (44).

Cholesterol esters were determined by the ferric chloride-sulfuric acid reaction (Leffler modified) outlined in Tietz (45).

HDL cholesterol was determined with the use of a Stanbio "SR-Plus" Total and HDL Cholesterol Test Set (Stanbio Laboratory, Inc., 2930 E. Houston St., San Antonio, Texas 78202). In this procedure low density and very low density lipoprotein cholesterol is removed by precipitation with magnesium chloride-dextran sulfate and the supernatant is analyzed for total cholesterol by the Liebermann-Burchard reaction (46).

VLDL and LDL cholesterol concentrations were determined using the equation of Friedewald, Levy and Fredrickson (47).

Phospholipid determinations were made using a Boehringer-Mannheim Phospholipid Test Set (available from Boehringer-Mannheim Diagnostics, 9115 Hague Road, Indianapolis, Indiana 46250). In this procedure the phospholipids are precipitated from serum by trichloroacetic acid. The precipitate is digested at 235°C with perchloric acid and a catalyst. Addition of ammonium molybdate results in formation of a yellow phosphovanadomolybdate complex (48).

Fibrinogen values were determined by the heat precipitation method (49) carried out at the Virginia-Maryland College of Veterinary Medicine clinical pathology laboratory. Results were supplied to the author.

In order to monitor accuracy and precision, all the above determinations were checked against Fischer-Scientific's "Sera Chem" Clinical Chemistry Control Serum (Fischer Scientific, Raleigh, North Carolina 27604).

Hematocrit determinations were made using the microhematocrit method. In this procedure a capillary tube is filled by capillary attraction from a well mixed anticoagulated blood sample. The tube should be at least half full. One end of the tube is sealed and it is then centrifuged for 5 minutes at 10,000 to 12,000 g in order to pack the red blood cells and consequently separate them from the plasma. The tube is then placed in the radial groove of an adjustable measuring device which facilitates the accurate reading of the percentage of red blood cells present in the sample, i.e., the hematocrit.

B. Variables

This investigation examined all those variables considered in the development of the Walburn-Schneck model; that is, shear rate, hematocrit, total protein, albumin, total lipids and total protein minus albumin. Going one step further, however, the study now included, as well, fibrinogen, total cholesterol, cholesterol esters, HDL, VLDL and LDL cholesterol, triglycerides and phospholipids. The whole blood "apparent" viscosity (see below) was considered to be the dependent variable, and the shear rate, hematocrit, and chemical parameters were considered to be the independent variables. A multiple regression computer technique identical to that used by Walburn and Schneck (14) was utilized to determine the variables having the greatest influence on whole blood viscosity. Spearman correlation coefficients (Appendix B) were also calculated to elucidate further the contribution of each variable to the viscosity.

C. Statistical Analysis

The development of the constitutive function followed identically the procedure described by Walburn and Schneck, beginning with the basic functional form of equation 2.5. This equation is modified by introducing the concept of an "apparent viscosity", (μ_a) which is defined in a Newtonian sense to be the viscosity of the fluid at a given shear rate:

$$\mu_a = \frac{\tau}{\dot{\gamma}} \text{ poise} \qquad (3.1)$$

Substituting equation 3.1 into equation 2.5, we obtain:

$$\mu_a = k\dot{\gamma}^{n-1} \text{ poise} \tag{3.2}$$

Equation 3.2 is now linearized to facilitate a least squares regression analysis:

$$\log_e \mu_a = \log_e k + (n-1) \log_e \dot{\gamma} \tag{3.3}$$

This is of the form $y = mx + b$

where y = dependent variable = $\log_e \mu_a$

x = independent variable = $\log_e \dot{\gamma}$

m = slope = $n-1$

b = y intercept = $\log_e k$

The parameter $\log_e \mu_a$ (the dependent variable) was examined in the regression analysis as a function of all of the independent variables mentioned in section B above, where all but $\dot{\gamma}$ enter implicitly through their effect on k and n. The analysis examined as well the squares of all independent variables, their inverses, the squares of their inverses and all nonlinear interaction terms. Some interaction terms were not considered for inclusion in the model. For example, terms such as (1/Hematocrit) x $\log_e \mu_a$, when included in the basic regression equation, imply that the viscosity at a constant shear rate decreases with increasing hematocrit. This is known to be physically unrealistic and so variables such as these were rejected on such grounds.

A stepwise regression procedure contained in the Statistical Analysis System S.A.S. (50) of the VPI 370 IBM digital computer was used to analyze the data. The linear regression analysis initially

determines the one independent variable which, when included in equation (3.3) provides the highest R^2 statistic (see Appendix D, reference 14) for the assumed form of $\log_e \mu_a$. The program then proceeds to choose a combination of two variables which will increase this R^2 statistic towards unity (representing a perfect fit between theory and experiment). This procedure is repeated until all the desired variables are included in the model, and the maximum R^2 statistic is reported. The above analysis determines an equation of the form:

$$\log_e \mu_a = C_1 + C_2 Y_1 + C_3 Y_2 \ldots \tag{3.4}$$

where

C_1 is the intercept and the values of the coefficients C_2, C_3, ... are reported along with the variable they precede.

The Spearman rank correlation coefficient will also be calculated using the S.A.S. system. The following formula is used to compute the coefficient of rank correlation (51).

$$r_s = \frac{1 - 6 \sum d_i^2}{N(N^2-1)} \tag{3.5}$$

where

d_i = difference between the rankings of the i-th observations

N = number of observations

For a more detailed explanation of the calculation of this coefficient and the probable error associated with it, see Appendix B.

CHAPTER 4

RESULTS AND DISCUSSION

A. Preliminary Remarks

As mentioned previously, it is the intent of this investigation to improve on the Schneck-Walburn constitutive function by examining those variables not significantly included in their model. This goal will be achieved by extending their constitutive equation to include a fourth variable, with the intent of affecting a significant increase in the R-square statistic. We will also examine the contributions of the plasma constituents to whole blood viscosity through the calculation of their respective correlation coefficients. Considering the similarity of the method of investigation, it was expected that the initial results of this investigation should coincide with those of the previous authors. With few exceptions this was found to be the case.

B. One Variable Model

The best one variable model shows the shear rate to be the single most significant independent variable. The function is of the same form as the Schneck-Walburn best one variable model which is:

$$\mu_a = k\dot{\gamma}^{n-1} \text{ poise} \qquad (4.1)$$

where

$k = .156$ = constant, and

$n = .763$ = constant.

Equation 4.1 has an R-square value of .5278 and a mean square error of 0.0219. As was pointed out by the previous authors, it is significant to note that both n (the non-Newtonian index) and k (the consistency index) are constants in this model for all shear rates and hematocrits. This is physically unrealistic as the non-Newtonian behavior of blood varies with both parameters. Obviously, then an equation involving only shear rate is insufficient to describe adequately the flow behavior of blood. This is reflected in the low value of R^2.

C. Two Variable Model

The best two variable model generated by the multiple regression technique involves both the shear rate and hematocrit. It is also of the same form as the Schneck-Walburn B2VM. The parameters k and n now show a hematocrit dependence of the form:

$$k = C_1 e^{C_2 \text{Hematocrit}} \tag{4.2}$$

$$n = 1.0 - C_3 \text{Hematocrit} \tag{4.3}$$

where

$C_1 = .01533$

$C_2 = 0.05457$

$C_3 = 0.00552$

The R-square value for this model is .8252 and the mean square error is 0.0081. The T value of the coefficients is 0.0001. The T value is a measure of the probability that a variable is not statistically significant, i.e., a T value of 0.0001 means that the probability of a variable being statistically significant is 99.99%. The above equations clearly show the significant effect of the hematocrit on the values of k and n and consequently on the value of the apparent viscosity. This is not surprising considering that the hematocrit dependence of whole blood viscosity has been observed experimentally by a number of authors (12,30,52).

D. Three Variable Model

The best three variable model was also of the same form as that reported by Schneck and Walburn and includes the variable TPMA or total protein minus albumin. The concentration of TPMA can be considered to be a good approximation of the concentration of the globulin fraction of the total proteins. This statement is reasonable in consideration of the fact that the total protein determinations were made on serum and as such do not include the fibrinogen concentration. All that remains after subtraction of albumin, therefore, are the globulins. The equation has the same basic form as (4.1) but now k and n are of the form:

$$k = C_1 e^{C_2 \text{Hematocrit} + C_4 \frac{\text{TPMA}}{\text{Hematocrit}^2}} \tag{4.4}$$

$$n = 1.0 - C_3 \text{Hematocrit} \tag{4.5}$$

where

$C_1 = .01212$

$C_2 = .05889$

$C_3 = .00559$

$C_4 = 44.637$

The R-square for this model is .8434 and the mean square error is 0.00732. The T values for the coefficients are 0.0001 each.

A comparison of the results reported by Schneck and Walburn and those presented here is provided in Tables 2, 3 and 4. It's obvious that the most significant difference is that of the value of C_4 in the three variable model. It should be pointed out that this coefficient precedes the term involving TPMA and may reflect a difference in the range of normal values used by the previous authors and those used in the current investigation. The values of total protein and albumin used in the development of the Schneck-Walburn function were determined by a SMA 10 autoanalyzer. This machine reports values within a different normal range and standard deviation from those values determined by the Dupont ACA autoanalyzer which is currently in use (53). Appendix C addresses this issue of quality control and the determination of normal values for the clinical laboratory. The coefficients present in the B1VM and the B2VM do not show a significant variation. Discrepancies in the values of the R-square statistic are reasonable, considering the difference in sample size.

The results of the present study thus far, then, at least confirm the basic form of the Schneck-Walburn constitutive function with the three most significant variables being shear rate, hematocrit and TPMA.

E. Four Variable Model

The best four variable model (B4VM) was developed by adding to those in the B3VM (known to have the most significant effect on whole blood viscosity), a fourth variable which increased the R-square statistic. The variables examined for inclusion consisted of all those mentioned previously, and also combinations of TPMA, hematocrit, and the individual variables i.e. triglycerides, total lipids, and so on. The addition of an individual variable by itself failed to yield a significant increase in the R-square statistic over the B3VM. However, when considered in combination with each other, some variables did provide a significant increase in the R-square statistic. For example, the combination Phospholipid/Total lipid^2 produced an R-square value of 0.8602, which is a significant increase of 0.0268 over the B3VM.

Further analysis of the constitutive equation, however, suggests that this particular combination of variables is not relevant because it leads to a lack of consistency. To explain further, consider equation (4.4). As hemotocrit increases, the first term dominates and the value of TPMA becomes less significant. This is reasonable since, when hemotocrit is high, one would expect the chemistry of the fluid to have correspondingly less effect on viscosity. When hematocrit is low, the second term dominates, i.e. TPMA becomes the prime factor in affecting

viscosity. This is also a physically reasonable finding. In a four variable model, the equation for k now contains an extra variable, which for purposes of explanation we will now assume to be phospholipid/Total Lipid^2, making the equation of the form:

$$k = C_1 \, e^{C_2 \text{Hematocrit} + C_4 \frac{\text{TPMA}}{\text{Hematocrit}^2} + C_5 \frac{\text{Phospholipid}}{\text{Total Lipid}^2}} \qquad (4.6)$$

The new term affects the value of k at all values of hematocrit which is not consistent with our earlier finding (and those of others) that the chemistry of the fluid has little affect on viscosity at high values of hematocrit. Also, the term never becomes insignificant as the phospholipids are a fraction of the total lipids. As total lipids increase, so do phospholipids. Therefore, in order to obtain a reasonably self-consistent constitutive function, it was decided to constrain the variables examined to include combinations with TPMA and hematocrit. These combinations incidently, also yielded the highest values of the R-square statistic, suggesting still further that the constraining precedures was justified.

Using the above criteria, the B4VM yielded equations for k and n of the forms:

$$k = C_1 \, e^{C_2 \text{Hematocrit} + C_4 \frac{\text{TPMA}}{\text{Hematocrit}^2} + C_5 \frac{\text{TPMA}^2 \cdot \text{Total Lipid}}{\text{Hematocrit}^2}} \qquad (4.7)$$

and

$$n = 1.0 - C_3(\text{Hematocrit}) \tag{4.8}$$

where

$C_1 = .01713$

$C_2 = .05227$

$C_3 = .00559$

$C_4 = -71.97268$

$C_5 = .04466$

and R-square = 0.8693

mean square error = .0061

T value for C_1, C_2, C_3, C_5 = 0.0001

T value for C_4 = 0.0003

It can be seen in equation (4.7) that as hematocrit increases, both TPMA and total lipids exert little effect on the value of k. It can be inferred that total lipids only have a fourth order effect on viscosity, after shear rate, hematocrit and globulins (in the form of TPMA). they also exert this effect in combination with the other prime determinants of viscosity, hematocrit and TPMA. We can perhaps conclude that the individual effect of total lipid on the consistency of whole blood is minimal, as are the lipid fractions.

Note further that the coefficient preceding the variable TPMA/Hematocrit^2 may now be put in the form (TPMA x Total Lipids x C^5 - C_4) where, the quantity in parentheses is positive for most of the data

used in this study (i.e., for the normal physiologic range of hematocrit, TPMA and total lipids). In effect, the B4VM has added the term in parentheses to "modify" the value of the variable TPMA/Hematocrit^2 which appears as the third most significant variable in the B3VM. It is conceivable that under certain conditions, such as low hematocrit plus abnormally low values of TPMA or total lipids or both, a negative value for the exponent may occur. This phenomenon could be attributed to a mathematical "artifact" (produced by fitting all the available data to the regression line), rather than any physiologically significant reason.

Observe also that in each of the models considered thus far, to the order of this analysis, the non-Newtonian index, n, shows a dependency on the hematocrit, H, alone, whereas the value of the consistency index, k, is dependent upon not only the hematocrit, but also on the chemistry of the fluid. This is reasonable to the extent that the non-Newtonian index measures the suspension characteristics of the fluid--i.e., the deviation of the fluid from having the characteristic behavior of a homogeneous solution--rather than the specific properties of the solution, itself. This is as opposed to the consistency index, which, as the name implies, is more of a measure of the "viscous" behavior of the fluid, and, therefore, more sensitive to chemical composition, per se. The non-Newtonian behavior (n) is determined for the most part by the presence of large numbers of geometrically complex erythrocytes, by their aggregation and flexibility, and by the hematocrit, which is a measure of the percentage of erythrocytes. We should therefore expect

the most significant variable affecting the value of n to be the hematocrit, H. The consistency index, however, is dependent upon the actual nature of the solution, and therefore shows more of a dependency upon not only hematocrit but also the chemistry of the fluid as reflected by TPMA, the total lipids, and so on.

F. Spearman Correlation Coefficients

In order to examine further the effects of the individual plasma constituents on the viscosity of whole blood, Spearman correlation coefficients for whole blood apparent viscosity versus concentration of the various chemical variables were calculated. To obtain meaningful results only those blood samples in the narrow hematocrit range of 39% to 41%, examined at the common shear rate of 232.8 sec^{-1}, were selected for comparison. This particular shear rate provides the most accurate viscosity readings when using the Wells' Brookfield Viscometer (4). Selection of those samples in the desired narrow hematocrit range reduced the sample size to 25, which is still statistically meaningful. The results are presented in Table 5.

Observe that the most significant correlation is between whole blood viscosity and the concentration of total protein minus albumin, which is essentially equivalent to the golbulin fraction of plasma (r_s = 0.66087, $p \leq 0.0003$). This result is not surprising considering that the third most significant variable which appears in the Schneck-Walburn constitutive function is total protein minus albumin, the first two variables being shear rate and hematocrit. This result also confirms

the observations of numerous other investigators (28-34). Dintenfass (29) reports correlation coefficients for plasma viscosity versus globulin in the range of 0.497 to 0.695 ($p \leq 0.0001$) for 615 subjects. Mayer (32) reported significant correlations between whole blood viscosity and the individual globulin fractions in the same range as those reported by Dintenfass ($r = 0.377$ to $r = 0.436$, $p \leq 0.001$).

The second highest correlation coefficient is that between whole blood viscosity and total protein ($r = 0.489$, $p \leq 0.13$). The effect of the total protein on plasma and whole blood viscosity has been well documented (4,23,24,25). Our results show a higher level of correlation than those reported by Mayer ($r = 0.311$, $p \leq 0.001$) but at a much lower level of significance.

The effect of fibrinogen on whole blood and plasma viscosity has been frequently reported (6,26-33). Mayer (32) and Dintenfass (29) also report correlation coefficients between plasma viscosity and fibrinogen concentration of ($r = 0.488$ $p \leq 0.001$) and ($r = 0.374$ $p \leq 0.001$) respectively. These results agree with those of the present study ($r = 0.428$, $p \leq 0.033$).

None of the lipid fractions or total lipids show correlations of any significance when compared to blood viscosity. These results conflict with those of Leonhardt (36) and Seplowitz, et al. (37), who report significant positive correlations between both total lipids and the individual lipid fractions and plasma viscosity. The results, however, do agree with those of Dintenfass (24,29) and Mayer (32).

The conflicting studies were discussed fully in Chapter 2. In addition, it should be pointed out that the present study used whole blood at a (relatively) constant hematocrit to obtain the values for the coefficients, whereas the previously mentioned works utilized plasma. Although the presence of red blood cells does present some difficulty, using whole blood samples at a constant hematocrit clearly models the in vivo situation more accurately than does the investigation of plasma alone.

Despite the fact that the correlation between HDL cholesterol and whole blood viscosity is not significant, it is interesting to note that higher than normal levels of this plasma component have often been associated with a lower risk of heart disease (35). However, this is probably not due to their effects on plasma velocity, but to the fact that HDL are less likely to attach to the arterial wall than are LDL, which are more asymmetric molecules.

The only other negative correlation, albeit not significant, is that between albumin and whole blood viscosity. Mayer (32) reports a negative correlation between albumin and plasma viscosity and it has been suggested by others that albumin may actually decrease the viscosity of plasma (23).

To summarize, then, the results of the study on ranked correlation coefficients utilizing samples within a very narrow hematocrit range confirm that the plasma components which affect viscosity to the highest degree are, in this order: the globulins, total protein and fibrinogen. Thus, these results reaffirm the complicated nature of the blood flow

problem by pointing out the fact that some individual plasma components may not affect blood viscosity by themselves, but may have a significant effect when considered in combination with another variable, as is evident in the four variable model developed in the present study.

It should be pointed out that the computerized regression analysis used in this study assumes the independence of all the chemical variables. A recent report by Schneck & Flatten (54) demonstrates that this may not be an entirely accurate assumption. Their study involved the calculation of Pearson rank correlation coefficients between total protein and the individual protein fractions (see table 6). It is significant to note that the correlation between TP (total protein) and globulin is higher than that between TP and TPMAL (globulin + fibrinogen), and, since the correlation between TP and fibrinogen is negative, the implication is that fibrinogen may "temper" the effect of the globulins on total protein. Note further that the albumin + globulin fraction (i.e. TPMFB) correlates better with changes in the globulins (r_p = 0.6777) than with changes in albumin (r_p = 0.5492). This may be due to the fact that when one considers albumin and globulin together, there may be an inverse relationship between the two (r_p for albumin vs. globulin = -0.1554), that causes one to go up as the other goes down, thereby having a somewhat cancelling effect on the sum of the two. Thus, albumin + globulin could hypothetically remain unchanged for slight changes in either albumin or globulin, thereby giving low correlation values. These points lead us to postulate whether increasing or decreasing concentration of globulins (TPMA) are the

direct caues of changes in the plasma viscosity or are merely a secondary effect of the change in the concentration of some other protein fraction. For a first order approximation of the effects of the plasma constituents on whole blood viscosity, however, our method of analysis offers a reasonable solution.

CHAPTER V

SUMMARY AND CONCLUSIONS

A. Constitutive Equations Development

The present study confirmed the results of Schneck and Walburn (11) through the development of a one variable, two variable, and three variable model similar to those proposed by the previous authors. A four variable model was also developed in order to investigate the effect of those plasma constituents not included in the development of the Schneck-Walburn constitutive function. Spearman rank correlation coefficients were calculated using samples of relatively constant hematocrit subjected to a strain rate of 232.80 reciprocal seconds. These results were used to elucidate further on the effect of the various individual plasma components on whole blood viscosity. The equations for k and n were developed from a relation of the form:

$$\mu_a = k\dot{\gamma}^{n-1} \text{ poise} \tag{5.1}$$

where

μ_a = apparent viscosity

k = consistency index

n = non-Newtonian index, and

$\dot{\gamma}$ = shear rate

The constitutive function was developed from a power law functional form:

$$\tau = k\dot{\gamma}^{n} \text{ dynes/cm} \tag{5.2}$$

where

τ = shear stress

The best one variable model gave the following results

$$k = 0.156 = \text{constant} \tag{5.3}$$

$$n = 0.763 = \text{constant} \tag{5.4}$$

R-square = 0.5278

Mean square error = 0.0219

T = 0.0001

The best two variables model yielded:

$$k = C_1 e^{C_2 \text{Hematocrit}} \tag{5.5}$$

$$n = 1.0 - C_3(\text{Hematocrit}) \tag{5.6}$$

where

C_1 = 0.01533

C_2 = 0.05457

C_3 = 0.00552

and,

R-square = 0.8252

Mean square error = 0.0076

T values = 0.0001

The best three variable model has the form

$$k = C_1 \, e^{C_2 \text{Hematocrit} + C_4 \frac{\text{TPMA}}{\text{Hematocrit}^2}} \tag{5.7}$$

$$n = 1.0 - C_3(\text{Hematocrit}) \tag{5.8}$$

where

C_1 = .01212

C_2 = .05889

C_3 = .00559

C_4 = 44.637

and

R-square = .8434

Mean square error = .0073

T value for C_1, C_2, C_3 = .0001

T value for C_4 = .0003

The best four variable model yielded:

$$K = C_1 \, e^{C_2 \text{Hematocrit} + C_4 \frac{\text{TPMA}}{\text{Hematocrit}^2} + C_5 \frac{\text{TPMA}^2 \cdot \text{Total Lipid}}{\text{Hematocrit}^2}} \qquad (5.9)$$

$$n = 1.0 - C_3(\text{Hematocrit}) \qquad (5.10)$$

where

C_1 = .01713

C_2 = .05227

C_3 = .00559

C_4 = -71.97268

C_5 = .04466

and

R-square = 0.8693

Mean Square error = 0.00611

T value for C_1, C_2, C_3, C_5 = .0001

T value for C_4 = .0003

B. Spearman Rank Correlation Coefficients.

Further analysis through the calculation of Spearman rank correlation coefficients showed the most significant variables to be:

TPMA (globulins)	$r_s = 0.66087$, $p \leq 0.0003$
Total protein	$r_s = 0.42843$, $p \leq 0.0326$
Fibrinogen	$r_s = 0.48851$, $p \leq 0.0132$

The results of this study indicate that the total lipids and lipid fractions have no significant effect on the consistency of whole blood for samples of given hematocrit. The total lipids do appear in the constitutive function, however, as part of the fourth variable which includes TPMA and hematocrit.

C. Results Summarized and Conclusions

The results of the present study are similar to those of Schneck and Walburn in the development of a one, two and three variable model. A four variable model was developed to include the value of total lipids in combination with TPMA and hematocrit. There was an accompanying significant increase in the R-square statistic of 0.0259. Although this new variable included total lipids, no significant correlation coefficient was calculated for total lipids with whole blood viscosity. This was reasonable in consideration of the fact that the total lipids are only significant in the constitutive function when they are in combination with TPMA and hematocrit. Other significant correlation coefficients were calculated for TPMA, fibrinogen and total protein. No other plasma constituents showed a significant correlation coefficient or appeared in the development of the constitutive equation. This

result leads us to conclude that the prime determinants of whole blood viscosity are shear rate, hematocrit and the globulins (in the form of TPMA).

D. Direction of Future Studies

Although the four variable model developed showed a significant increase in the R-square statistic in comparison to the three variable model, it failed to provide an almost perfect fit of the experimental data to the equation (i.e. an R-square statistic of 1). This result suggests that rather than focusing on the plasma constituents, further studies should be aimed at analyzing the contributions of the leukocytes and platelets. Moreover, other factors such as red blood cell flexibility and aggregation could also be included to develop a constitutive function which best describes the rheological behavior of blood. Furthermore, the development of a constitutive function utilizing the concepts of continuum mechanics may not provide the most realistic description of blood flow in vivo, the reason being that blood is a complex, inhomogeneous fluid, perfusing a complex network of vessels of grossly different diameters under extremely variable driving pressures. Quantification of all these factors will be challenging.

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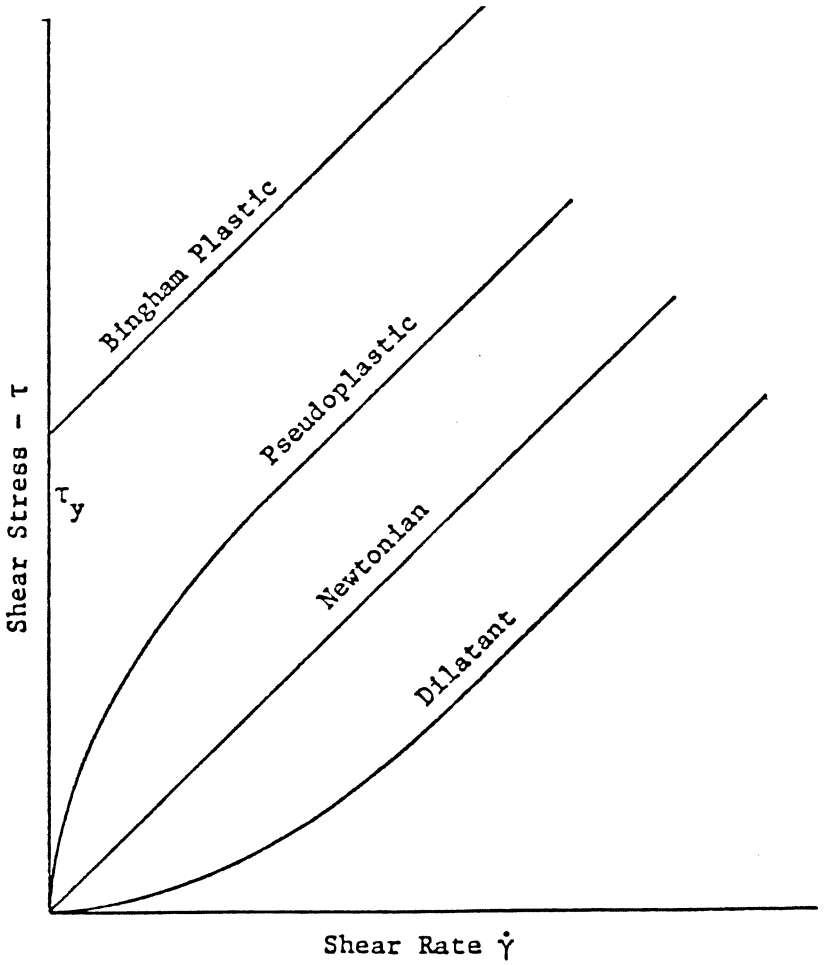


Figure 1. Types of non-Newtonian Fluids. (Reproduced from reference 24)

Table 1. Contribution of plasma constituents to the increase of the mean normal plasma viscosity (1.64 m Pa. s @ 25°C) over the viscosity of water (0.8937 m Pa. s @ 25°C)). Reproduced from reference 4.

	Mean Concentration g/liter	% of Total	% Effect on plasma Viscosity	Characteristics of protein molecules			
				Molecular Weight	Molecular Dimensions Length	Diameter	L/D Ratio
Protein	78		99				
Albumin	45	58	36	69,000	150	38	3.95
Globulin	30	38	41	160,000	235	44	5.34
Fibrinogen	3	3.8	22	341,000	700	38	18.4

Table 2. Values of k, n, R-square statistic and mean square error for Best One Variable Model

	Schneck-Walburn	Best One Variable Model
k	0.134	0.156
n	0.785	0.763
R-square	0.6187	0.5278
Mean-Square Error	0.0218	0.0219

k = constant

n = constant

Table 3. Values of C_1, C_2, C_3, R-square statistic and mean square error for Best Two Variable Model

	Schneck-Walburn	Best Two Variable Model
C_1	0.0148	0.01533
C_2	0.0512	0.05457
C_3	0.00499	0.00552
R-square	0.8789	0.8252
Mean-Square Error	0.0069	0.0081

$$k = C_1 e^{C_2(\text{Hematocrit})}$$

$$n = 1.0 - C_3(\text{Hematocrit})$$

Table 4. Values of C_1, C_2, C_3, C_4, R-square statistic and mean square error for Best Three Variable Model

	Schneck-Walburn	Best Three Variable Model
C_1	0.00797	0.01212
C_2	0.0608	0.05889
C_3	0.00499	0.00559
C_4	145.85	44.637
R-square	0.9049	0.8434
Mean-Square Error	0.00546	0.00732

$$k = C_1 e^{C_2(\text{Hematocrit})} + C_4 \quad \frac{\text{TPMA}}{\text{Hematocrit}^2}$$

$$n = 1.0 - C_3(\text{Hematocrit})$$

Table 5. Correlation coefficients of chemical constituents with whole blood viscosity at a shear rate of 232.8 sec^{-1} and hematocrit range of 39% to 41%.

Chemical Constituent	Spearman Corr. Coeff.	p	
TPMA	0.66087	0.0003	
Total Protein	0.48851	0.0132	
Fibrinogen	0.42843	0.0326	
Cholesterol	0.32436	0.1137	n.s.
Phospholipids	0.30583	0.1371	n.s.
Low Density Lipoproteins	0.26785	0.1955	n.s.
Lipids	0.26120	0.2072	n.s.
Very Low Density Lipoprotein	0.24827	0.2315	n.s.
High Density Lipoprotein	-0.24542	0.2370	n.s.
Triglycerides	0.24456	0.2387	n.s.
Cholesterol esters	0.20392	0.3282	n.s.
Albumin	-0.08857	0.6738	n.s.

n.s. no significant correlation

Table 6. Pearson Correlation Coefficients of Total Protein and Protein Fractions

	TPROT	ALBUM	GLOBU	FIBRIN	HEMAT	TPMAL	TPMFB	TPMGL
TPROT	1	.5002	.7038	-.1053	.2355	.6814	.9765	.5101
ALBUM	.5002	1	-.1554	-.2492	.1748	-.2161	.5492	.9654
GLOBU	.7038	-.1554	1	-.0499	.1578	.9741	.6777	-.1730
FIBRIN	-.1053	-.2492	-.0499	1	.0226	.1703	-.2947	-.0164
HEMAT	.2355	.1748	.1578	.0226	1	.1475	.2290	.1934
TPMAL	.6814	-.2161	.9741	.1703	.1475	1	.6121	-.1781
TPMFB	.9765	.5492	.6777	-.2947	.2290	.6121	1	.5089
TPMGL	.5101	.9654	-.1730	-.0164	.1934	-.1781	.5089	1

TPMAL = GLOBULIN + FIBRINOGEN
TPMFB = ALBUMIN + GLOBULIN
TPMGL = ALBUMIN + FIBRINOGEN

TABLE 7. Pearson Spearman Kendall Correlation

OBS	HCT	K	N	TP	ALB	TA	TGL	CHOL	CHOLE	HDL	LDL	VLDL	LIP	PLIP	FIB
1	38	10.971	0.8037	6.2	3.8	2.4	46	184	68	86	89	9	522	263	400
2	40	10.113	0.8389	5.9	3.5	2.4	264	270	181	49	168	53	750	275	400
3	36	10.649	0.8118	6.7	2.7	4.0	62	130	95	20	98	12	401	159	300
4	40	11.325	0.8359	6.6	4.1	2.5	312	226	146	57	108	62	845	319	100
5	38	7.323	0.8800	5.8	3.7	2.1	160	220	80	62	126	32	828	315	100
6	40	8.521	0.8728	6.8	4.3	2.5	304	268	114	46	161	61	836	367	200
7	44	16.137	0.7769	6.9	3.9	3.0	192	233	58	24	171	38	632	311	300
8	27	7.677	0.8073	6.6	3.9	2.7	76	168	99	35	118	15	442	295	100
9	41	16.286	0.7724	7.3	3.4	3.9	122	175	96	34	117	24	513	287	600
10	40	9.267	0.8278	6.7	3.9	2.8	184	209	73	58	114	37	647	264	200
11	47	15.971	0.7768	7.2	3.8	3.4	288	203	113	46	99	58	713	297	300
12	38	11.972	0.7711	6.9	3.9	3.0	110	107	74	32	53	22	341	153	100
13	44	13.569	0.7770	6.6	2.6	4.0	64	125	99	26	86	13	303	157	300
14	39	12.199	0.7869	7.2	4.0	3.2	136	247	198	53	167	27	568	228	300
15	39	20.206	0.7025	6.9	4.6	2.3	356	231	124	43	117	71	803	335	200
16	44	14.944	0.7903	6.7	3.8	2.9	58	240	149	57	171	12	607	278	300
17	47	17.380	0.7613	6.9	3.7	3.2	240	138	42	38	52	48	630	297	400
18	48	10.839	0.7491	7.8	4.3	3.5	195	432	357	71	322	39	815	314	200
19	43	18.305	0.7496	6.4	4.0	2.4	308	213	115	36	115	62	692	311	200
20	44	12.908	0.7989	6.5	3.8	2.7	190	173	88	43	92	38	622	240	300
21	48	14.477	0.8275	6.3	5.0	1.3	493	393	230	71	233	99	1191	460	400
22	45	12.792	0.7949	7.0	4.3	2.7	76	152	92	38	99	15	427	250	300
23	52	18.770	0.7847	6.5	4.1	2.4	408	244	128	33	129	82	897	364	100
24	44	14.778	0.7783	7.0	4.6	2.4	140	337	215	62	247	28	913	457	200
25	37	19.768	0.6872	6.8	3.4	3.4	140	207	131	61	118	28	611	299	500
26	42	13.275	0.8040	7.0	6.2	0.8	214	283	160	42	198	43	897	308	200
27	47	17.927	0.7657	7.1	3.5	3.6	54	136	56	47	78	11	414	222	500
28	44	25.504	0.6811	6.9	3.9	3.0	182	221	140	56	129	36	681	289	300
29	46	23.920	0.9661	7.0	4.0	3.0	116	193	115	28	142	23	647	237	200
30	42	13.353	0.7641	6.8	4.3	2.5	40	182	100	100	74	8	564	264	300
31	46	21.452	0.7077	6.0	2.9	3.1	114	147	13	145	21	23	724	393	500

OBS = Observation Number
HCT = Hematocrit (%)
K = Consitency index
N = Non-Newtonian index
TP = Total Protein (g/dl)
ALB = Albumin (g/dl)
TA = Total Protein minus Albumin (g/dl)
TGL = Triglycerides (mg/dl)
CHOL = Cholesterol (mg/dl)
CHOLE = Cholesterol Esters (mg/dl)
HDL = High density lipoprotein cholesterol (mg/dl)
LDL = Low density lipoprotein cholesterol (mg/dl)
LIP = Total lipids (mg/dl)
PLIP = Phospholipids (mg/dl)
FIB = Fibrinogen (mg/dl)

Table 7. (Continued)

OBS	HCT	K	N	TP	ALB	TA	TGL	CHOL	CHOLE	HDL	LDL	VLDL	LIP	PLIP	FIB
32	33	11.433	0.7885	6.5	2.0	4.5	118	94	29	8	62	24	379	193	400
33	36	11.738	0.7775	7.3	4.3	3.0	34	161	92	50	104	7	468	303	100
34	41	18.979	0.7222	7.1	4.2	2.9	28	126	70	61	59	6	339	166	100
35	46	15.707	0.7973	7.5	4.2	2.3	398	150	78	50	20	80	718	262	400
36	33	10.818	0.7893	8.2	4.3	3.9	82	204	127	64	124	16	525	324	300
37	47	16.824	0.7602	8.1	4.6	3.2	84	168	101	51	100	17	450	276	100
38	41	16.357	0.7672	7.1	4.1	2.0	156	294	184	41	222	31	840	345	300
39	44	14.376	0.7923	7.8	4.6	3.2	184	292	212	64	191	37	720	317	100
40	43	16.560	0.7485	6.4	3.7	2.7	206	203	119	44	118	41	641	258	200
41	46	21.405	0.7106	7.1	4.0	3.1	174	185	98	34	116	35	620	241	100
42	41	13.806	0.7867	6.5	4.1	2.4	110	211	92	34	155	22	603	241	300
43	43	21.979	0.7122	6.6	4.3	2.3	242	318	168	78	192	48	813	372	100
44	46	22.003	0.6934	6.6	3.9	2.7	140	297	145	74	195	28	732	331	100
45	43	18.638	0.7174	7.0	4.1	2.9	96	223	94	64	140	19	580	241	200
46	52	29.345	0.6790	7.6	4.4	3.2	74	249	140	90	144	15	580	350	100
47	38	14.383	0.7409	6.8	4.0	2.8	50	158	89	103	45	10	455	214	200
48	40	22.707	0.6461	6.3	4.1	2.2	66	158	93	69	76	13	403	271	100
49	48	31.290	0.6670	6.5	4.0	2.5	248	171	99	58	63	50	592	271	300
50	45	32.206	0.6528	7.2	3.6	3.6	186	170	98	49	84	37	550	271	300
51	46	32.763	0.6364	7.0	4.3	2.7	268	328	219	25	250	54	883	400	200
52	39	17.535	0.7063	6.0	3.8	2.2	174	185	106	71	79	35	650	336	100
53	43	15.250	0.7818	6.3	3.8	2.5	124	221	128	74	122	25	692	303	200
54	45	18.956	0.7561	5.4	2.2	3.2	176	280	164	54	191	35	883	293	400
55	34	10.129	0.8120	4.4	2.2	2.2	187	294	207	29	228	37	653	307	400
56	40	15.667	0.7670	6.1	3.3	2.8	480	271	188	93	82	96	1000	414	300
57	37	14.107	0.7996	7.2	3.9	3.3	80	202	138	59	127	16	533	251	100
58	48	15.780	0.7682	4.2	2.2	2.0	84	171	106	53	101	17	500	245	200
59	39	11.110	0.8111	6.6	3.9	2.7	106	207	129	49	137	21	583	270	100
60	40	18.296	0.7311	6.7	3.9	2.8	84	232	138	61	154	17	548	232	400
61	47	21.439	0.7231	7.2	4.1	3.1	70	247	173	93	140	14	647	330	100
62	42	18.049	0.7567	6.5	4.0	2.5	292	282	208	29	185	58	797	223	100

OBS = Observation Number
HCT = Hematocrit (%)
K = Consitency index
N = Non-Newtonian index
TP = Total Protein (g/dl)
ALB = Albumin (g/dl)
TA = Total Protein minus Albumin (g/dl)
TGL = Triglycerides (mg/dl)
CHOL = Cholesterol (mg/dl)
CHOLE = Cholesterol Esters (mg/dl)
HDL = High density lipoprotein cholesterol (mg/dl)
LDL = Low density lipoprotein cholesterol (mg/dl)
LIP = Total lipids (mg/dl)
PLIP = Phospholipids (mg/dl)
FIB = Fibrinogen (mg/dl)

Table 7. (Continued)

OBS	HCT	K	N	TP	ALB	TA	TGL	CHOL	CHOLE	HDL	LDL	VLDL	LIP	PLIP	FIB
63	48	20.838	0.7561	7.5	3.6	3.9	180	2.3	124	28	149	36	881	488	200
64	42	22.543	0.7425	8.4	3.0	5.4	130	143	71	37	80	26	563	290	100
65	39	12.973	0.7914	6.2	2.8	3.4	78	208	121	54	138	16	581	295	400
66	44	24.938	0.6934	6.6	4.1	2.5	82	173	93	57	100	16	484	229	100
67	40	21.422	0.7100	6.7	3.9	2.8	173	203	131	35	133	35	604	228	500
68	51	20.651	0.7545	7.2	4.3	2.9	247	132	23	39	44	49	630	251	200
69	42	12.262	0.8084	6.9	4.6	2.3	92	216	122	51	137	18	602	294	100
70	43	15.823	0.7685	6.4	4.2	2.2	94	184	119	66	99	19	426	148	300
71	41	11.121	0.8193	6.2	4.1	2.1	136	253	148	52	174	27	648	259	200
72	41	13.130	0.7945	6.8	4.4	2.4	112	200	117	52	126	22	519	207	100
73	43	12.903	0.7949	6.9	4.5	2.4	102	181	130	66	94	20	481	198	300
74	40	10.785	0.8101	5.7	4.0	1.7	214	203	114	39	121	43	639	222	200
75	45	21.331	0.7319	6.2	3.9	2.3	118	185	104	35	126	24	519	216	200
76	43	13.728	0.7811	7.5	4.2	3.3	26	138	89	70	63	5	355	191	300
77	43	13.060	0.7855	7.6	4.2	3.4	68	173	83	62	97	14	372	131	200
78	38	12.443	0.7977	7.3	4.8	2.5	52	128	16	33	85	10	375	195	500
79	43	15.377	0.7658	7.1	3.8	3.3	94	185	75	33	133	19	442	163	200
80	39	12.161	0.8120	7.6	4.2	3.4	100	154	53	61	73	20	408	154	100
81	37	11.841	0.7952	7.5	4.3	3.2	68	236	95	71	151	14	550	246	300
82	44	19.255	0.7393	8.0	4.4	3.6	82	225	80	40	169	16	517	210	300
83	43	15.919	0.7805	8.0	4.7	3.3	104	190	89	71	98	21	475	181	200
84	40	11.638	0.8071	7.5	4.4	3.1	206	155	83	43	71	41	450	89	100
85	41	14.805	0.7815	6.6	4.4	2.2	326	274	147	38	171	65	700	100	400
86	40	12.746	0.7667	6.6	4.3	2.3	46	171	100	52	110	9	462	245	200
87	39	10.844	0.8059	6.5	4.0	2.5	104	170	80	67	82	21	529	255	100
88	44	23.123	0.7161	6.9	4.3	2.6	134	188	47	57	104	27	550	228	200
89	39	15.903	0.7508	6.8	4.2	2.6	46	152	58	57	86	9	375	177	100
90	43	14.984	0.7887	6.9	4.2	2.7	74	160	70	48	97	15	500	414	300
91	40	18.743	0.7122	6.5	4.2	2.3	104	133	64	40	92	21	437	200	200

OBS = Observation Number
HCT = Hematocrit (%)
K = Consitency index
N = Non-Newtonian index
TP = Total Protein (g/dl)
ALB = Albumin (g/dl)
TA = Total Protein minus Albumin (g/dl)
TGL = Triglycerides (mg/dl)
CHOL = Cholesterol (mg/dl)
CHOLE = Cholesterol Esters (mg/dl)
HDL = High density lipoprotein cholesterol (mg/dl)
LDL = Low density lipoprotein cholesterol (mg/dl)
LIP = Total lipids (mg/dl)
PLIP = Phospholipids (mg/dl)
FIB = Fibrinogen (mg/dl)

TABLE 8. Data Table for Spearrman Rank Correlation

OBS	HCT	K	N	TP	ALB	TA	TGL	CHOL	CHOLE	HDL	LDL	VLDL	LIP	PLIP	FIB
1	40	10.113	0.8389	5.9	3.5	2.4	264	270	181	49	168	53	750	275	400
2	41	11.534	0.8102	6.5	4.0	2.5	80	164	109	46	102	16	474	239	200
3	40	11.325	0.8359	6.6	4.1	2.5	312	226	146	57	102	62	845	319	100
4	40	8.521	0.8728	6.8	4.3	2.5	304	268	114	46	161	61	836	367	200
5	41	16.286	0.7724	7.3	3.4	3.9	122	175	96	34	117	24	513	287	600
6	40	10.557	0.8256	5.9	3.6	2.3	272	234	189	37	143	54	735	306	300
7	39	20.206	0.7025	6.9	4.6	2.3	356	231	124	43	117	71	803	335	200
8	41	18.979	0.7222	7.1	4.2	2.9	28	126	70	61	59	6	339	166	100
9	41	16.357	0.7672	7.1	4.1	3.0	156	294	184	41	222	31	840	345	300
10	41	13.806	0.7867	6.5	4.1	2.4	110	211	92	34	155	22	603	241	300
11	40	22.707	0.6461	6.3	4.1	2.2	66	158	93	69	76	13	403	271	100
12	39	17.535	0.7063	6.0	3.8	2.2	174	185	106	71	79	35	650	336	100
13	40	15.667	0.7670	6.1	3.3	2.8	480	271	188	93	82	96	1000	414	300
14	39	11.110	0.8111	6.6	3.9	2.7	106	207	129	49	137	21	583	0	100
15	40	18.296	0.7311	6.7	3.9	2.8	84	232	158	61	154	17	548	0	400
16	39	12.973	0.7914	6.2	2.8	3.4	78	208	121	54	138	16	581	0	14
17	40	21.422	0.7100	6.7	3.9	2.8	173	203	131	35	133	35	604	0	500
18	41	11.121	0.8193	6.2	4.1	2.1	136	253	148	52	174	27	648	0	200
19	41	13.130	0.7945	6.8	4.4	2.4	112	200	117	52	126	22	519	0	100
20	40	10.785	0.8101	5.7	4.0	1.7	214	203	114	39	121	43	639	0	200
21	39	12.161	0.8120	7.6	4.2	3.4	100	154	53	61	73	20	408	0	100
22	40	12.746	0.7667	6.6	4.3	2.3	46	171	100	52	110	9	462	0	200
23	39	10.844	0.8059	6.5	4.0	2.5	104	170	80	67	82	21	529	0	100
24	39	15.903	0.7508	6.8	4.2	2.6	46	152	58	57	86	9	375	0	100
25	40	18.743	0.7122	6.5	4.2	2.3	104	133	64	40	92	21	437	0	200

Symbols and units are identical to Table 7.

APPENDIX A

BLOOD

INTRODUCTION

Blood consists of a complex, heterogeneous suspension of formed elements in a continuous, straw-colored suspending medium. The formed elements are comprised of blood cells, collectively termed hematocytes, from the Greek "haima", meaning "blood", and "kytos", meaning "cell". Hematocytes carry out many complex functions in the circulatory system -- one of the most important being the delivery of oxygen (which is not very soluble in water) to the tissues. They include three basic types of cells: Red Blood Cells (Erythrocytes, from the Greek "erythros", meaning "red"), White Blood Cells (Leukocytes, from the Greek "leukos", meaning "white"), and Platelets (Thrombocytes, from the Greek "thrombos", meaning "lump").

The suspending medium (plasma) is composed of a saline (0.15 Normal Salt) solution of four major types of protein -- Fibrinogen, Globulin, Albumin, and Prothrombin -- and contains many other chemical constituents (e.g., nutrients, dissolved gases, electrolytes other than sodium, and other

substances) which will be mentioned briefly below. The study of blood, both in states of normal health and in diseased states (particularly those states where the blood is the origin of the disease) is called Hematology.

The average adult male possesses between five and six liters of whole blood, while the average adult female generally has about a liter less, and the total volume of blood in any given individual is kept conspicuously constant at about 69 milliliters (cc's) per kilogram (2.205 pounds) of body weight for males and 64 milliliters per kilogram of body weight for females. At a mass density of 1.057 grams per cubic centimeter (Specific Gravity = 1.052 to 1.062), this represents about 7.0% (range, 7.00 to 8.00 percent) of the total human body weight. The plasma portion of the fluid accounts for nearly 18% of the body's total Extracellular Fluid Space (i.e., that fluid space located outside of cells), while the fluid contained within hematocytes accounts for some 10% of the body's total Intracellular Fluid Space (i.e., that quantity of fluid contained within cells).

Students of physiology are generally well-versed and trained to understand the <u>metabolic</u> (mass transport) function of the cardiovascular system. Blood is, indeed, the river of life -- bringing nutrients absorbed from the gastrointestinal tract, oxygen absorbed from the lungs, and other biochemical supplies to all the cells of the body,

while continuously collecting carbon dioxide and other toxic metabolic waste products from all the tissues of the body and transporting them to the lungs, kidneys and gastrointestinal tract for disposal and removal from the body.

By comparison, the thermoregulatory function of the cardiovascular system receives much less emphasis, and the student comes away from a physiology course with little appreciation for how important this role really is in terms of the sustenance of life. Yet, when one envisions the anatomical configuration of the vascular network of pipes and channels through which the blood flows as it courses through the body, and compares this anatomy with, for example, the radiator of an automobile, the potential for thermoregulation afforded by the cardiovascular system becomes immediately obvious. Like water in a radiator, blood in the physiologic system can absorb the heat generated by the complex chemical reactions of life; and, like water in a radiator, blood can carry (convect) this heat to the surface of the organism to be expelled to the environment. Indeed, various estimates suggest that as much as 95% of the heat generated by the body at any given time flows to the surface (skin) through vascular convection, and, conversely, this thermoregulatory role can be reversed when heat is to be conserved by the body. Again, acting like the thermostat in the radiator of an automobile (which keeps the engine coolant away from the heat-dissipating

surface area when the engine is cold), vascular smooth muscle tissue can completely shut off (or significantly reduce) flow to the periphery when this becomes necessary to maintain core temperature and release more heat to the internal organs of the body.

Even its metabolic function is not totally independent of the role of blood in thermoregulation. Many of the processes which are called upon to generate additional heat at a time of need are controlled by biochemical constituents (hormones, enzymes, substrates, "activating" factors, and so on) which are brought to their site of utilization by the mass transport capabilities of this "river of life". One must therefore be constantly aware of the fact that blood has both mass transport and thermoregulatory functions in physiologic systems, and it therefore has properties that are consistent with these two functions.

CELLULAR ELEMENTS OF BLOOD

All Hematocytes originate in the bone marrow from undifferentiated stem cells called hemocytoblasts (G. "blastos" = "germ"). In the adult, only the red bone marrow located principally in the skull, vertebrae, ribs, sternum, and pelvis, retains the ability to make blood cells. Hemocytoblasts undergo alterations in nuclear and cytoplasmic characteristics as they divide and become

differentiated into one of the three major types of blood cells, before entering the circulation. The total productive bone marrow in the adult is about 1400 grams, and the process of making blood cells is called hematopoiesis (G. "poiesis" = "production"), or sometimes, hematocytopoiesis.

Erythrocytes

Erythrocytes account for over 99 percent of the total number of formed elements in blood. They mature from Hemocytoblasts through a sequence of morphologic stages that include: (i) Rubriblasts, (ii) Prorubricytes, (iii) Rubricytes, (iv) Metarubricytes, and (v) Reticulocytes. Of these, only the Reticulocytes (which are the immediate forerunners of Erythrocytes) are found in relatively small numbers (about 1.5% of the total red blood cell count, with a range of 0.1 - 2.1%) in the blood. The others are generally confined to the bone marrow. There are about 5 million total red blood cells (range: 4,200,000 to 5,400,000 for females and 4,600,000 to 6,200,000 for males) per cubic millimeter of blood, or, for an average five liters of blood, there are 25 trillion red cells circulating throughout the body. This represents about 25% of the total number of cells that comprise the entire human organism (some 10 to the 14th power), so the immense importance of

these tiny masses of protoplasm to the maintenance of life is put into immediate perspective. The red blood cells are shaped like biconcave discs ("life saver candies" with a filled-in hole in the middle). They have a diameter of 6-9 microns, a maximum width of 1.84-2.53 microns (which tapers to 0.92 - 1.08 microns at their indented "neck"), and a cell membrane thickness of 75 to 80 Angstroms. Their peculiar shape is attributed to the collapse of the central portion of the red cell membrane as the nucleus is extruded just before the cell reaches maturity, and to the role of the erythrocytes as oxygen carriers. That is to say, their shape is intended to provide a large absorption surface, the total surface area of these cells representing approximately 3000 to 4000 square meters (roughly, 120 to 160 micrometers, squared, per cell) or, on the order of 1500 times the total surface area of the human body.

The average density of erythrocytes is 1.098 grams per cubic centimeter and their cell volume ranges from 80 to 100 cubic microns. Thus, we can compute the volume of whole blood which is occupied by red blood cells. If each cell has a mean volume of, say 90 cubic microns, and if there are 5 million cells per cubic millimeter, then the volume of cells per cubic millimeter of whole blood turns out to be 0.45 cubic millimeters, or, on the average, the erythrocytes occupy 45 percent by volume of whole blood. This figure is called the "hematocrit" (G. "krinein" = "to separate") of the fluid. It normally ranges from 37 to 47 percent for

females and 40 to 54 percent for males, with 45% representing a fairly good overall average. In Polycythemia, where the number of red cells is excessive, hematocrits of 60 to 70 percent are found, and there is then considerable interference with the free movement of blood in the circulation. At the other extreme, in conditions such as Hypoplastic or Aplastic Anemia (reduced red blood cell production in the bone marrow) or Pernicious Anemia (reduced red blood cell production due to a deficiency of Vitamin B-12), hematocrits can get as low as 30% or less, and the cells produced are large (macrocytic), oddly shaped, and very fragile (i.e., easily subject to rupture, or "Hemolysis" from the Greek "lysis", meaning "dissolution").

Oxygen Transport

The single primary function of red blood cells is to transport oxygen to the living tissues, via the hemoglobin content of the cell. As mentioned earlier, Oxygen does not readily dissolve in water. Its solubility in aqueous fluid, as measured by its "Henry's Law Coefficient", k, (Henry's Law states that C = kP, where C is the number of milliliters of gas dissolved in one milliliter of fluid at a partial pressure of P atmospheres) is only 0.024 at 37 degrees Centigrade. Now, moist Alveolar air contains 13.6% Oxygen, 5.3% Carbon Dioxide, 74.9% Nitrogen, and 6.2% Water Vapor,

and is at a pressure of 760 mm Hg (one atmosphere) at sea level. Blood travelling through the lungs thus tends to equilibrate its gaseous partial pressures at: p(oxygen) = 0.136 x 760 = 103 mm Hg, and p(carbon dioxide) = 0.053 x 760 = 40 mm Hg. Thus, for P = (103/760) = 0.136 atmospheres, and k = 0.024, only about 0.003 milliliters of dissolved oxygen can be carried per milliliter of whole blood. This is usually expressed as 0.30 ml per 100 ml of arterial blood (i.e., the unit "volume percent"), and is hardly enough to meet the metabolic needs of the organism. Therefore, oxygen must be actively carried by binding it to a non-protein, iron-containing group called "heme", which is folded into and carried by a larger protein called globin, to form the hemoglobin complex. Hemoglobin has a molecular weight of 68,000, a viscosity of 6.00 centipoise (about twice that of whole blood), and occupies about one third of the red blood cell. The remaining two-thirds consists mainly of water (65%), with some proteins, lipids, gluthathione, Adenosine Diphosphate (ADP), Adenosinetriphosphate (ATP) and ions making up the rest.

One molecule of hemoglobin can combine with four molecules of oxygen, and each red blood cell contains some 280 million hemoglobin molecules, so that every red blood cell can carry about 1.12 billion (ten to the ninth power) oxygen molecules. Thus, five million red blood cells (i.e., one cubic millimeter of whole blood) can carry 5.6 E15 oxygen molecules, one milliliter (cc) of whole blood can

carry 5.6 E18 oxygen molecules, and 100 milliliters of whole blood can carry 5.6 E20, or, .0056 E23 molecules of oxygen. Since the Avogadro Number of molecules, 6.0225 E23, represents one mole of a substance (in the case of Oxygen, 32 grams), 0.0056 E23 molecules represents 0.00093 moles, or, about 0.03 grams. In bound form, at body temperature (310 degrees Kelvin), a partial pressure of 100 mm Hg (133,316 dynes per square centimeter), and a pH of 7.396 (normal for arterial blood), this converts to approximately 20.1 ml of oxygen per 100 ml of blood (commonly expressed as 20.1 volumes percent), assuming that the hemoglobin in the blood stream is completely saturated with oxygen. Compared with 0.3 ml/100 ml for dissolved oxygen, we see, then, that hemoglobin increases the oxygen-carrying capacity of blood by a factor of 67! Moreover, since one gram of hemoglobin can combine with 1.34 cubic centimeters (ml) of oxygen (the resulting combination being called "oxyhemoglobin"), the usual concentration of Hemoglobin is approximately (20.1/1.34) = 15 grams per 100 milliliters of whole blood, which compares favorably with measured values (14-18 grams/100 ml for Men; 12-16 grams/100 ml for Women).

The extent to which hemoglobin molecules are carrying oxygen at any given time is called the "oxygen saturation" of the complex and it is very sensitive to a variety of factors. For example, if the oxygen content of hemoglobin solution is measured after equilibration at different partial pressures of the gas, and the results are plotted as

percent saturation (oxygen content divided by oxygen capacity, multiplied by 100, ordinate) versus oxygen partial pressure (mm Hg, abscissa), an S-shaped sigmoid curve, called the oxyhemoglobin dissociation curve, is obtained. The curve rises relatively slowly at first (up to about 8-10 mm Hg of oxygen partial pressure), then steepens (up to about 50-60 mm Hg), and finally flattens out as it approaches complete saturation.

At a partial pressure of 103 mm Hg, the partial pressure of oxygen in blood leaving the lungs is sufficient to almost saturate hemoglobin, i.e., hemoglobin leaving the lungs is about 97.5 percent saturated. Hence, arterial blood leaving the lungs contains approximately 0.975 x 20.1 = 19.6 ml of oxygen bound to 100 ml of blood (19.6 volumes percent), at normal body temperature and pH. The dissociation curve for hemoglobin is very steep below 60 mm Hg, which illustrates the ability of this oxygen-carrier to release large amounts of its cargo for small decreases in partial pressure of the gas, as happens in the tissues. At rest, for an average tissue partial pressure of 40 mm Hg, Hemoglobin releases to the tissues about 5 ml of oxygen per 100 ml of blood. During exercise, two to three times this amount can be released in the capillaries of active muscle as the partial pressure of oxygen falls to as low as 20 mm Hg. On the other hand, the flatness of the dissociation curve for hemoglobin above 60 mm Hg. allows this substance to still remain pretty well saturated even at high altitudes

(up to 10,000 feet), where the partial pressure of oxygen in blood leaving the lungs is significantly reduced below the value of 103 mm Hg that it has at sea level.

The dissociation of the oxyhemoglobin complex is further facilitated by the simultaneous increases in the partial pressure of carbon dioxide, Hydrogen ion concentration (decreased pH) and temperature which occur in active tissues. A decrease in pH shifts the oxygen dissociation curve to the right (and vice versa); an increase in carbon dioxide tension from normal values does likewise (and vice versa, the so-called "Bohr Effect", which facilitates the uptake of oxygen in the lungs as carbon dioxide is released, and the release of oxygen to the tissues as carbon dioxide enters the blood); and an increase in temperature also shifts the oxygen dissociation curve to the right (and vice versa), facilitating the transfer of oxygen to active cells around which the temperature is highest. Hemoglobin carries over 98 percent of the oxygen transported by blood; less than 2 percent is carried in simple solution in the plasma.

As it is for oxygen, the carbon-dioxide-carrying capacity of blood is also enhanced by red blood cells, but not entirely because of their hemoglobin concentration. Indeed, only about 20% of the carbon dioxide diffusing out of tissues is bound to hemoglobin together with amine groups, and carried in the form of Carbaminohemoglobin.

Some 70% of the expelled gas finds its way into the red blood cell to be converted into carbonic acid in a reaction catalyzed by the enzyme Carbonic Anhydrase contained within Erythrocytes. The carbonic acid dissociates into bicarbonate and hydrogen ions. The bicarbonate ions (negatively charged) diffuse back out into the plasma, in exchange for an influx of chloride ions (the so-called "chloride shift" from plasma into red blood cells), while the hydrogen ions remain behind to drop the pH level of the cell and thereby encourage more dissociation of the oxyhemoglobin complex (and, concurrently, encourage more hemoglobin to combine with carbon dioxide). The remaining 10% of expelled carbon dioxide is in solution.

When blood reaches the lungs, all of these processes are reversed (oxygen enters, bicarbonate ions are reabsorbed in the red blood cell, carbonic acid is reconstituted and broken down into carbon dioxide and water) and about 4 ml of carbon dioxide per 100 ml of blood are released to the alveoli for expulsion from the organism.

Erythropoiesis

In man, red blood cells survive in the circulation for an average of 120 days (about four months), after which they are destroyed (mainly by the macrophages of the reticuloendothelial system of the spleen). Parts that can

be reused to make new red blood cells are delivered back to the bone marrow. The remainder is excreted through the liver (mainly bilirubin in bile) and large intestines (feces). Remarkably, some two to ten million red cells are being destroyed each second (a daily turnover rate of about 1 percent), but they are being replaced just as fast (42,500 per cubic millimeter of blood per day, or, 3,065,000,000 per kilogram of body weight per day) by the process of erythropoiesis. The control of erythropoieses is by a feed-back control system which is inhibited by an increase in the circulating concentration of red cells (hematocrit) and stimulated by anemia (below normal hematocrit).

The set point for control of the specific red cell concentration or hematocrit in the blood is adjusted by the level of hypoxia (lack of oxygen) at the tissue site. Erythropoiesis is controlled by a hormone called Erythropoietin. In response to an imbalance between available oxygen and tissue oxygen requirements, Erythropoietin (or, Erythropoietic Stimulating Factor, ESF) is formed in the blood by the action of an enzyme, Renal Erythropoietic Factor (REF), which is released principally from the kidney. This hormone acts on the bone marrow to promote the differentiation of certain stem cells into red cells, thus increasing the production of erythrocytes. The increased number of red blood cells thus produced augments the oxygen carrying capacity of the blood and tends to restore adequate oxygenation of renal tissue. The loss of

kidney function will result in lowered hematocrit in the human. At some specific lower level, approximately 20% hematocrit, the control of red cell production is taken over by some other organ of the body.

Blood Typing

In 1901, Landsteiner showed that the membranes of human red blood cells contain specific antigens called agglutinogens, and the plasma contains corresponding antibodies called agglutinins. Erythrocytes are classified according to whether they possess type A agglutinogens, or type B, or both (type AB), or neither (type O), and as to whether they do (Rh-Positive) or do not (Rh-Negative) contain an additional antigen called antigen-D -- named the "Rh-Factor" because it was originally discovered by Wiener and Peters in 1940 in the blood of Rhesus monkeys. Plasma corresponding to type A cells contains antibodies for type B cells (so-called Anti-B or Beta-Agglutinins) while plasma corresponding to type B cells contains antibodies for type A cells (so-called Anti-A or Alpha-Agglutinins). The blood of a type AB person contains no plasma agglutinins, while the blood of a type O person contains both anti-A and anti-B agglutinins. Anti-D agglutinin does not occur naturally in the blood. Blood types are genetically inherited traits and must not be mixed.

Nearly half (46%) of the white population of this world contains type O blood, and some 85% is Rh-D Positive. These individuals are called universal donors, since their red cells contain no antigens and thus cannot react with the antibodies contained in the plasma of the recipient. Another 42% of the population is type A, with the rarest blood types being type B (9%), type AB (3%), and Rh-Negative (15%). Type AB individuals are sometimes called universal recipients, because their plasma contains no antibodies, they possess significant amounts of both type A and type B red blood cells, and the relatively little amount of plasma that gets infused into their system through a transfusion is diluted to the extent that there are only a negligible amount of agglutinins present to cause significant agglutination. These, however, are the only individuals who can arbitrarily receive blood from anyone. In all other cases, when blood is transfused into an individual with an incompatible blood type, transfusion reactions occur. Usually, these reactions form agglutinations (aggregates) of red cells which are irreversibly chemically bonded to one another. The transfusion recipient develops an elevated temperature and, in severe cases, can actually die!

Blood is typed and stored in the hospital by ABO-Rh groups. When a recipient needs blood, a cross-match is done in the hospital laboratory to check the compatibility of the transfusion unit with the recipient's blood. A small amount

of the patient's blood is mixed with a small amount of the donor's blood and the technician looks for signs of red cell aggregation. Today, there are some 370 blood types beyond the ABO-Rh groups. Most of these 370 types do not cause problems when transfused. The cross-match is designed to check for any incompatibility in this group.

Rouleaux Formation

Agglutination is an irreversible chemical reaction that causes red blood cells to form large masses or aggregates. There is a less serious form of red cell interaction which is reversible and due not to chemical reactions but to electrostatic attraction resulting from the charge distribution on the cell surface, and from the geometry of the cells, themselves. This latter form of interaction causes groups of red blood corpuscles to arrange themselves in stacks, like a roll of coins, and hence goes by the name of Rouleaux Formation (from the French word for "Roll").

The tendency for Rouleaux Formation is dependent on the concentration of large proteins in the plasma, particularly fibrinogen and alpha-2-macroglobulin. However, this process is time-dependent (blood has to sit around long enough for these aggregates to form), and, the rolls of cells break up very easily once the fluid is sheared. In fact, for shear rates above 50 reciprocal seconds, no aggregates remain,

and, for shear rates well below this, red cell aggregation is still quite negligible. Thus, Rouleaux Formation is not considered to be of great consequence *in vivo*, but may have some clinical significance in terms of diagnosing red blood cell disorders that may show up as changes in Rouleaux patterns.

Leukocytes

Leukocytes constitute less than one-half of one percent of the total number of hematocytes, and account for a volume fraction of 0.003 (or 0.3%) of the total blood volume. There are some 4,000 to 11,000 of these cells per cubic millimeter of blood (this is referred to as the total white blood cell count), so that their concentration is of order one one-thousandth that of the erythrocytes (making their presence of lesser rheological importance). Again, considering an average five liters of blood, we find that there are about 37.5 billion white cells normally circulating throughout the body. This number, however, can increase by orders of magnitude in cases of disease or infection, such as Leukemia, where white blood cell counts as high as 500,000 per cubic millimeter of whole blood are routinely reported. The leukocytes come in a variety of shapes and sizes, but are classified basically in terms of whether or not numerous granules can be identified in the

cell cytoplasm. Thus, we have Granulocytes (polymorphonuclear phagocytes, or PMN's), and, Agranulocytes, which consist of two types of leukocytes: Lymphocytes, and, Monocytes.

It was established in the 1960's that the granules seen in granulocytes are actually lysosomes, i.e., cell organelles containing digestive enzymes (see, Schneck, Daniel J., "Principles of Bioenergetics and Metabolism in Physiologic Systems," Virginia Polytechnic Institute and State University, College of Engineering, Department of Engineering Science and Mechanics, Biomedical Engineering Program, Technical Report Number VPI-E-83-42, Blacksburg, Virginia, November 1, 1983). The Granulocytes are further classified on the basis of the staining properties of their granules. Thus, Basophils, are readily stained with the basic dye methylene blue, Acidophils, or, Eosinophils, are readily stained with the red acidic dye eosin, and Neutrophils, stain only very weakly with both acid and basic types of dyes.

Lymphocytes and The Defense Mechanisms of the Human Machine

Lymphocytes normally account for 20 to 50% of the total number of white blood cells, but may increase to as much as 90% in pathologic cases of lymphatic leukemia or lymphocythemia. These 1,000 to 4,800 cells per cubic

millimeter of whole blood have an average diameter ranging from 7 microns up to 10 or 12 microns, but may be as large as 20 microns. They are characterized by a deeply staining, compact, dark blue nucleus which occupies almost all of the cell. Derived from stem cells in bone marrow that proceed through stages including lymphoblasts and prolymphocytes, the lymphocytes, themselves, reach maturity in the bone marrow (so-called, B-Lymphocytes), or in the Thymus Gland (so-called, T-Lymphocytes), or in the lymph nodes of the lymphatic system (which is why these cells were called lymphocytes to begin with). They are a somewhat heterogeneous group of corpuscles that have a circulatory life span of some 100 to 300 days (to as much as a year; some can even remain for the entire life span of an individual) and they play an important role in the process of immunity, producing antibodies and other agents involved in the immune process. A brief description of this process follows.

In a simple sense, the human machine is an aggregation of organic compounds bathed in electrolytes. However, the geometric configuration and structural characteristics of these compounds are so complicated, due to the astronomical variability of genetic possibilities, that the tissues of any given individual are endowed with a unique chemical identity. The body has thus developed sophisticated mechanisms by which it can easily distinguish its own constituents from foreign substances, and herein lies the

essence of its immune responses to foreign invasion. Each cell contains long, polypeptide chains, called human leucocyte antigens (HLA) embedded in its outer membrane. This membrane consists of a bilayer of lipid (fat) molecules (see Schneck, Daniel J., "Principles of Mass Transport Across Biological Membranes," Virginia Polytechnic Institute and State University, College of Engineering, Department of Engineering Science and Mechanics, Biomedical Engineering Program, Technical Report Number VPI-E-83-07, Blacksburg, Virginia 24061, March 15, 1983). One part of the antigen molecule extends into the interior of the cell, where it may interact with cytoplasmic proteins, and another part extends out from the cell surface, where it is available for interactions with other cells. Human leucocyte antigens (also called histocompatibility or transplantation antigens) have a high degree of genetic diversity, such that exact matches in individuals other than identical twins are believed to be extremely rare. Identical twins contain the same genetic information and possess identical histocompatibility antigens. The basis of the immune responses of the human body lies in the ability of the HLA of the host to recognize foreign antigens, to attack, and to destroy the latter. The recognition scheme, in turn, is mostly geometric -- that is, the foreign antigens do not "fit" into the geometric pattern that is characteristic of the host -- like an odd piece attempting to become part of a complicated jig-saw puzzle.

The control system for the immune response is composed of white blood cells (leukocytes). Specialized white blood cells, called Lymphocytes, recognize and respond to the presence of a foreign substance. Lymphocytes circulate in the body, moving freely between the blood and the lymphatic tissues by squeezing in between the endothelial cells of the walls of blood vessels. As they travel through the body, lymphocytes encounter other cells in a random fashion. If a lymphocyte contacts a cell containing the correct histocompatibility antigens unique to the individual, the lymphocyte will remain in an inactive, metabolically quiescent state. If, however, a lymphocyte contacts a foreign antigen, it may become activated and trigger an immune response leading to the removal of the foreign substance.

Two types of lymphocytes, B-Lymphocytes and T-Lymphocytes, can trigger an immune response in the human body. Both B-and-T-Lymphocytes contain on their surface histocompatibility antigens and receptors for foreign antigens. Neither Lymphocyte is a Phagocytic cell, but both originate in the bone marrow as mentioned earlier. Maturation of B-Lymphocytes also takes place in the bone marrow, but maturation of T-Lymphocytes occurs in the thymus gland. Thus, the names of the respective Lymphocytes are derived from their sites of maturation.

The B-Lymphocytes protect the body by producing

antibodies, providing what is known as humoral immunity. When antigen molecules bind to the surface receptors (thought to be bound antibodies) of a small B-Lymphocyte, the lymphocyte becomes "activated". The exact mechanism by which this occurs is not clear, but when they are activated by a foreign substance these B-cells first grow larger and then begin a series of cell divisions. Over a period of several days, this cell proliferation gives rise to numerous memory cells that resemble the original lymphocyte, and specialized plasma cells that secrete antibodies. It is the antibody molecules that attack the foreign antigen. Each antibody molecule secreted can form bonds with two antigen molecules, thereby holding the antigens together in large clumps through a process called agglutination. The agglutinated antigens are subsequently engulfed by large phagocytic macrophages, which are the Polymorphonuclear Granulocytes (see below). Memory cells, genetically identical to the lymphocyte originally stimulated, are responsible for the more rapid production of antibody upon re-exposure to the same antigen.

Antibodies are globulin proteins consisting of four polypeptide chains: two identical "heavy" chains and two identical shorter "light" chains. The chains are linked by disulfide bonds. About three fourths of each heavy chain and half of each light chain show great constancy of amino acid sequence; the variability lies mostly in the remaining portions of each chain, at the free amino ends. The binding

sites for antigens (two on each antibody molecule) are at the ends of the variable portions. Each binding side is a pocket or cleft bounded partly by the heavy chain and partly by the light chain.

In many respects, the action of T-lymphocytes parallels the action of B-lymphocytes. T-Lymphocytes are believed to have surface receptors for antigen whereby antigen recognition takes place and activation of T-cells is triggered. The composition of these receptors is not clear, but is thought to differ somewhat from that of B-Lymphocytes. Activated T-cells become enlarged and divide, producing effector cells and memory cells. Effector cells, also called "killer" T-Cells, may combine directly with target cells to bring about their destruction. Instead of directly destroying the antigen, the effector cells can, upon re-encounter with the antigen, release soluble mediators called lymphokines. Although the biological activity of lymphokines is varied, one of its major functions is the activation of macrophages. Activated macrophages are larger and more phagocytic than nonactivated macrophages. Activated macrophages are sometimes called "angry" macrophages because antigens that cannot be phagocytized by nonactivated macrophages can be destroyed by angry macrophages. Memory cells formed by activated T-Lymphocytes act in a similar manner to those derived from activated B-Lymphocytes. Immunological responses due to T-Lymphocytes are caused by antibodies that remain bound to

the surface of the T-cells that produce them. The entire antibody-bearing cell attacks the antigen. For this reason, the T-Lymphocytes are said to provide the body with cell-mediated immunity.

Each kind of antigen stimulates synthesis of antibodies specific for it. Estimates of how many different kinds of lymphocytes would be required to endow the body with the capability of synthesizing antibodies against virtually any antigen run as high as 10 billion or more. At first, this may sound preposterous, but since each antibody is composed of four polypeptide chains, only 100,000 genes for light chains and 100,000 genes for heavy chains are necessary to produce 10 billion different antibodies. Thus, 200,000 genes can code for 10 billion antibodies. It has been calculated that 200,000 genes occupy less than 10 percent of the total DNA content of a typical human cell. Hence, the hypothesis now most widely accepted postulates the existence in the human body of an enormous number of slightly different lymphocytes, each specialized for potential production of a different antibody.

Granulocytes and The Defense Mechanisms of the Human Machine

Granulocytes are the bacteria fighters of the blood. Derived from Hemocytoblasts through stages that include Myeloblasts, Progranulocytes and Myelocytes, these cells

eat, or Phagocytize (which is why they are also called Phagocytes) bacteria, other organisms such as viruses, and objects that have been identified as foreign by the immune system (see earlier description). The Phagocytes migrate in the blood based upon chemical substances liberated from infected tissue. This migration is called Chemotaxis. When they come into contact with identified foreign substances, they engulf the substances ("Phagein" = Greek, "To Eat" or ingest), and digest them with enzymes manufactured and secreted from their cellular lysosomes (granules).

There are two classes of Phagocytes: Macrophages, large mononucleated cells which ingest and dispose of dead body tissue, and, Microphages, smaller cells which ingest and dispose of bacteria and other smaller organisms. The corpuscles normally have a life span in the circulation of about 12 hours, and their numbers are greatly elevated in infectious disease.

Neutrophils are the most numerous of the leukocytes, accounting for 57 to 70% (2,000 to 6,000 per cubic millimeter of whole blood) of this class of blood cells. Derived from Myelocytes and Metamyelocytes, these cells appear in blood as Segmented Granulocytes ("Segs") and a small number (0 - 4%) of so-called "Band" Cells. The Neutrophils are phagocytic, functioning in the destruction of pathogenic micro-organisms and other foreign matter. They have a diameter of around 10 - 12 microns. At wound or

infected sites, the number of invading Neutrophils rises to a peak in 24 hours.

Eosinophils, derived also from Myelocytes and appearing in the blood as another class of segmented granulocytes, have about one tenth the total number per cubic millimeter of fluid as do Neutrophils. That is, one normally finds these from as few as 45 to as many as 480 per cubic mm, with 150-300 being a more common range (1-4% of the total white cell count). These leukocytes are involved in Phagocytosis of antigen-antibody complexes.

Basophils are the least common of the white blood cells, and, somewhat obscure in terms of their exact function. Derived from myelocytes as still a third form of plasma segmented granulocytes, these leukocytes account for only one-half to one percent of the total number of white cells, which translates to no more than about 100 cells per cubic millimeter of blood (often, basophils will be found in a sample of blood). Since the number of basophils is increased during the healing phase of inflammatory conditions, it is believed that these leukocytes are responsible for bringing anticoagulant substances to inflammed tissues. Their exact role in this process is, however, still uncertain.

Monocytes

Last, and somewhat least, of the Leukocytes are Monocytes, which are derived from bone marrow stem cells through a sequence of steps that include Monoblasts and Promonocytes. Having a diameter of 16-22 microns, these cells are the largest of all of the hematocytes, but they appear in the blood in very small numbers, only 200 to 800 per cubic millimeter of blood (3-8% of the total white cell count). Monocytes possess a relatively large amount of cytoplasm and a round or kidney-shaped nucleus. They are also a phagocytic cell, becoming transformed into macrophages after invading infected sites (where their numbers reach a peak in 48 hours). These leukocytes have a function related to antibody formation and bacterial identification, but their exact role in this process, as well as their more general involvement in the immune process is not clearly defined and is currently under intense investigation.

Platelets

Platelets (or Thrombocytes), as the name implies, are cells which have the shape of platelike discs, 0.5 to 2 or 3 microns in diameter (about half the size of erythrocytes). They appear in blood in concentrations ranging from 140,000 to 395,000 per cubic millimeter (140,000-395,000 for males, 214,000-360,000 for females). Having only one-tenth the

concentration of red blood cells, being only half their size, and occupying only about 0.3% by volume of the whole blood, these cells do not appear to be of much significance rheologically except in the clotting process (see below), when they clump together to form relatively large masses. That is to say, when blood vessel walls are injured, platelets collect at the site, sticking to the vessel wall, and release substances (in particular, 5-hydroxytryptamine), which attract more platelets and cause a local constriction of the arterial wall. This process is accelerated by Thrombin, an enzyme involved in blood clotting. The Platelets also release a substance (Adenosine Diphosphate) which causes all the platelets at the injured vessel wall site to adhere to themselves and to the vessel, forming a plug to prevent loss of further blood from the site. This is the role they play in hemostasis, i.e., the process of checking bleeding. After the completion of this process, which usually takes less then a minute, they release other substances (Platelet Factor 4 and Prostaglandins) which initiate the coagulation of the blood at this local site. This blood clot forms in the next ten minutes and acts as a permanent repair to the arterial wall until it can be replaced by cell ingrowth.

Platelets are irregularly-shaped cytoplasmic fragments of giant, multinucleated red bone marrow cells called Megakaryocytes, which, in turn, are derived from stem cells through a developmental sequence that includes

Megakaryoblasts and Promegakaryocytes. Their average circulatory life span is from less than four days to as much as 8 or 9 days, and they are capable of Ameboid movement. These hematocytes are rich in ATP and contain many of the same organelles normally found in mature cells.

Blood Coagulation

When blood begins to seep through an injured vessel an intracellular substance called Thromboplastin is released from the injured vascular tissue and from degenerating blood platelets. This substance initiates the clotting process by acting as a catalyst (the enzyme, Thrombokinase), to convert the plasma protein, Prothrombin, into Thrombin, in the presence of Calcium Ions. Thrombin then acts on the plasma protein, Fibrinogen, to convert it into Fibrin, in a polymerization process that also requires the presence of Calcium ions. The insoluble Fibrin is formed into a meshwork by being laid down as fine interlacing filaments in which are entrapped red and white blood cells and platelets, to form a coagulum, or "clot". This mechanism of clot formation, which is initiated by thromboplastin, is called "Extrinsic".

An alternate pathway for clot formation can be triggered by an "Intrinsic" mechanism which does not require contact with injured tissue. In this case, when blood

contacts certain surfaces (such as glass or the intimal subendothelial lining of blood vessels, which gets exposed to blood if the endothelial surface loses its integrity, or has a broken surface), a blood protein known as the Hageman Factor (or "Glass Factor", see below) is activated, and the clotting process then ensues as before. Normally, the entire lining (endothelium) of the cardiovascular system has the important function of inhibiting intravascular coagulation by preventing the adherence of the platelets which continuously bombard it. To accomplish this, the endothelial cells synthesize a compound called Prostacyclin, which, like the family of Prostaglandins to which it belongs, is derived from Arachidonic Acid. Prostacyclin is a strong inhibitor of platelet aggregation, as are some other natural anticoagulants -- the most important of which are heparin (a polysaccharide formed in the liver and the pericapillary mast cells), and Antithrombin III.

The clotting process is actually a cascading sequence of chemical reactions that involve at least thirteen known "factors": Factor I = Fibrinogen; Factor II = Prothrombin; Factor III = Thromboplastin, or, "Tissue Factor"; Factor IV = Calcium Ions; Factor V = A Protein Substance, Proaccelerin, which enhances the conversion of prothrombin to Thrombin; Factor VI = Accelerin (no longer believed to be involved in the clotting process); Factor VII = Preconvertin or serum prothrombin conversion accelerator (extrinsic activator of factor X); Factor VIII = Antihemophilic Factor

(Von Willebrand Factor, acts as a cofactor protein with Factor X); Factor IX = Plasma Thromboplastin Antecedent Component, or "Christmas" Factor (intrinsic activator of factor X); Factor X = Stuart Factor, or, Prower Factor (prothrombin converting enzyme); Factor XI = Plasma Thromboplastin Antecedent (in the presence of Calcium, this factor activates Factor IX); Factor XII = Hageman, or, Glass Factor (initiates the formation of active factor XI); and, Factor XIII = Fibrin Stabilizing Factor, or, Laki-Lorand Factor (activated by Thrombin). The coagulation factors are plasma proteins synthesized in the liver with the help of Vitamin K (especially needed for Prothrombin and Factors VII, IX, and X).

Clotting is retarded by cold, by smooth surfaces, by substances which combine with calcium (such as ethylenediamine tetraacetic acid, or, EDTA), by neutral salts (such as magnesium or sodium sulfate), and by certain substances of biological origin (such as hirudin, heparin, snake venoms, cystein, and dicoumarol). The clotting process is hastened by warming, by providing a rough surface, or by use of chemical substances such as adrenalin, thrombin, or thromboplastin.

BLOOD PLASMA

Removal of all hematocytes from blood by centrifugation

or other separating techniques leaves behind the aqueous (90% water by volume, 91% water by weight) suspending medium called plasma. Plasma is the fluid portion of the blood in which all the cellular elements are suspended. Seven to Eight Percent by weight of total plasma consists of the plasma proteins (8218 mg/102.4 grams, or 100 ml of plasma); another 0.7-0.9% by weight includes the electrolytes (745 mg per 100 ml plasma); some 0.5-0.6% consists of the carbohydrates (577 mg glucose and other sugars per 100 ml plasma); and the remainder is everything else (96 mg/100 ml Organic Acids, 79.5 mg/100 ml Nonprotein Nitrogenous Compounds, including primarily urea, uric acid, and creatinine; about 30 mg/100 ml free lipids; about 28 mg/100 ml plasma vitamins; 0.11 mg/100 ml hormones; a trace of enzymes; additional amino acids, dissolved gases, small lipid droplets, antibodies, and many other inorganic and organic molecules, all dissolved in water).

Plasma Proteins

The plasma proteins fall into three major fractions and one minor one. The major fractions include Albumin, the Globulins and Fibrinogen, and the minor one consists of Prothrombin. The globulins are further subdivided into alpha, beta, and gamma globulin, based upon how these proteins migrate in an electric field (i.e., under conditions of plasma protein electrophoresis). Each of the plasma proteins is large enough (see below) so that it will

not penetrate the capillary walls of the microcirculation. This produces an osmotic pressure across the capillary wall which tends to pull water into the blood, and it is commonly referred to as the Colloid Osmotic Pressure (COP) or the Oncotic Pressure. On the arterial side of a capillary, the COP is equalled, and exceeded, by the fluid dynamic hydrostatic pressure of the fluid entering the capillary, thus promoting mass exodus from the cardiovascular system. The hydrostatic pressure drop across the capillary, however, causes fluid pressure to fall below the COP on the venous side of the capillary, thus promoting mass influx into the cardiovascular system at this end. For more details about how these mechanisms operate in the physiologic system, see the report by Schneck (VPI-E-83-07).

Fibrinogen (Molecular Weight = 330,000; Normal Concentration in Blood = 0.15 to 0.3 grams per 100 ml of plasma, or about 4.0% of total protein), as already discussed, is a plasma protein that is involved in the clotting function of blood. The final step in coagulation is the conversion of fibrinogen to fibrin, which is the familiar gelatin substance of a blood clot.

Albumin (Molecular Weight = 69,000; Normal Concentration in Blood = 4.8 gms per 100 ml of plasma) plays an important role in osmotic regulation, i.e., it is present in plasma to a large extent to create the oncotic pressure. Although this is the primary function of Albumin, it is not

its only function. This plasma protein is also a transport molecule for many hormones and other chemical agents in the body, and it serves as well to regulate plasma volume and pH. Representing 45 to 60% of the total plasma protein fraction, it is by far the most abundant of all of the plasma proteins.

The Globulin class of proteins accounts for about 38% of total protein, and it is actually rather broad. It includes lipoproteins (protein molecules that are conjugated with lipids to account for about 6% of total protein), which assist in carrying blood fats, the whole category of proteins that are formed as part of the immune process (i.e., so-called Immunoglobulins which account for another 14% of total protein; see earlier discussion of white blood cells), additional transport molecules, Prothrombin, Free Globulin, and others. The immune properties of these globulins are a special function of the gamma fraction, i.e., the gamma globulins contain the antibodies responsible for identifying foreign organisms in the body. Hence, this fraction increases markedly in infectious diseases.

Lipoproteins are classified as very low density (VLDL, or Alpha-2-Globulins, or Alpha-2-Lipoproteins), Low Density (LDL, or Beta-Globulins or Beta-1-Lipoproteins), and High Density (HDL, or Alpha-1-Globulins, or Alpha-1-Lipoproteins). The Very Low Density Alpha-2-Globulins have a mass density less than 1.019 grams

per cubic centimeter, a molecular weight of 5-20 million, and a normal plasma concentration of 150-230 mg per 100 ml of plasma (they may account for 8-13% of total protein and about 15% of serum lipoprotein density fractions). The Low-Density Beta-Globulins have a mass density between 1.019 and 1.063 grams per cc, a molecular weight between 1.3 and 3.2 million, and a normal plasma concentration of 280-440 mg per 100 ml of plasma (they may account for 11 to 17% of total protein and about 30% of serum lipoprotein density fractions). The High-Density Alpha-1-Globulins are broken down into those with a mass density between 1.063 and 1.093 grams per cc (HDL-2) and those with a mass density between 1.093 and 1.149, to as high as 1.21 grams per cc (HDL-3). The latter have a molecular weight between 195,000 and 200,000, and a normal plasma concentration of 217-270 mg per 100 ml plasma. The former have a molecular weight of around 435,000 and a normal plasma concentration of 37-117 mg per 100 ml of plasma. Together, they can represent 5 to 8% of the total protein, and up to 12.5% of serum lipoprotein fractions. When not bound to lipids, Beta-Globulins, by themselves, have a molecular weight of around 90,000, but they rarely appear unbound in plasma (concentrations of the unbound form generally run well below 35 mg per 100 ml of plasma). Similarly, Alpha-Globulins, because of their mass transport function, are seldom found in free form circulating in plasma.

By far the most common of the Immunoglobulins is Gamma-

G-Immunoglobulin (IgG), which has a molecular weight of 156,000-161,000 and a plasma concentration in the range 1200-1800 mg/100 ml. Of lesser consequence are Gamma-A-Immunoglobulin (IgA, 100 mg/100 ml plasma); Gamma-M-Immunoglobulin (IgM, 75 mg/100 ml plasma); and Gamma-D-Immunoglobulin (IgD, from 30 on down to less than 0.3 milligrams per 100 milliliters of plasma).

Prothrombin is a stable protein of the alpha-2-globulin fraction and is normally present at a level of 20 mg per 100 ml of plasma. It is produced in the liver under the influence of vitamin K, and its role in the clotting process has already been discussed.

Other Components of Blood Plasma

Space does not permit, nor is it of any significant rheologic importance to discuss at length all of the remaining constituents of blood plasma. Indeed, there are many, many of them, and there is a wealth of available literature on the subject. The reader is thus referred to the references at the end of this report for further information. For the present, we shall simply describe some of the other plasma constituents rather briefly, with little or no detail concerning their specific functions.

Plasma Electrolytes and Trace Elements

As mentioned earlier, Plasma is a saline solution. The primary salt cations in this solution are:

Sodium (303-331 mg/100 ml serum = 132-148 meq./liter),

Potassium (13-18 mg/100 ml serum = 3.3-5.0 meq./liter),

Calcium (9-11 mg/100 ml serum = 4.5-5.5 meq./liter), and

Magnesium (1.68-2.88 mg/100 ml serum = 1.38-2.36 meq./l).

The primary Anions are:

Chlorine (350-370 mg/100 ml serum = 99-104 meq./liter),

Bicarbonate (55.4 vol. % = 27 meq./liter), and the buffering ions:

Phosphate (3.2-4.3 mg/100 ml serum = 2 meq./liter),

Sulfate (0.5-1.5 mg/100 ml serum = 1 meq./liter),

Citrate (2.5 mg/100 ml serum = 0.29 meq./liter), and

Ammonium (10.6-300 micrograms per 100 ml blood).

The normal total number of cations in plasma is 155 milliequivalents per liter (142 Sodium, 5 Potassium, 5 Calcium and 3 Magnesium). This is balanced electrically by 155 milliequivalents per liter of anions (104 Chlorine, 27 Bicarbonate, 2 Phosphate, 1 Sulfate, 5 Organic Acids, and 16 Protein). Organic acids consist of all those containing one

or more carboxyl groups (-COOH), and include Acetic Acid, Formic Acid, Lactic Acid (7.69 mg/100 ml = 0.85 meq./l), and all Fatty Acids (see later). They account for some 96 mg/100 ml of plasma, which, together with 745 mg/100 ml of plasma for the remaining electrolytes (excluding proteins), represents 0.8 to 0.9 percent by weight of the total dissolved compounds in plasma. Proteins have already been described above.

Trace Elements are organic elements normally found in minute quantities for a variety of metabolic purposes (see Schneck report Number VPI-E-83-42). They include virtually every element in the Periodic Table, e.g., Aluminum, Arsenic, Bromine, Boron (up to 1.3 micrograms per 100 ml blood from dietary sources, 0.75% appearing as boric acid), Cadmium, Cobalt, Copper (130 to 230 micrograms per 100 ml serum), Chromium, Fluorine, Iodine (Protein Bound, PBI, 3.5 to 8.0 micrograms per 100 ml serum), Iron (60 to 180 micrograms per 100 ml serum, although, including that found in red blood cells, there are 42 to 52 milligrams of total iron per 100 ml of whole blood = 7.5 to 9.3 meq. per liter), Lead (50 micrograms per 100 ml whole blood), Lithium, Manganese, Molybdenum, Nickel, Selenium, Silicon, Sulfur (Inorganic, 0.5 to 1.1 mg/100 ml or 0.31 to 0.69 meq/liter), Vanadium, Zinc, and other physiologically rare minerals.

Another major mineral that is found in blood in

addition to the major electrolytes and trace elements is Phosphorus, which exists in both an organic (18-29 mg/100 ml blood) and an Inorganic (3.2-4.3 mg/100 ml blood) form.

Nutrients and Vitamins

Dissolved Nutrients include the products of digestion as they are absorbed from the gastrointestinal tract, i.e., Free Fatty Acids from the digestion of fats, Amino Acids from the digestion of proteins, and Glucose and simple sugars from the digestion of Carbohydrates.

Free Fatty Acids are classified as Saturated (having only single bonds in their carbon chains) or Unsaturated (having one or more double or triple bonds in their carbon chains). The Saturated Fatty Acids include: Acetic, Butyric, Caproic, Caprylic, Capric, Lauric, Formic, Myristic, Palmitic, and Stearic Acid. The Unsaturated Fatty Acids include those of the Oleic Series (Oleic, Tiglic, Hypogeic, Palmitoleic, and Physetoleic Acids), and those of the Linoleic or Linolic Series (Linoleic, Linolenic, Clupanodonic, Arachidonic, Hydrocarpic, and Chaulmoogric Acids). The body's inability to manufacture Linoleic, Linolenic, and Arachidonic Acids has caused these to be labelled "Essential Unsaturated Fatty Acids" (Sometimes referred to as Vitamin F), in the sense that they must be ingested daily as at least one percent of the total caloric

intake.

Fatty Acids are insoluble in water. This would prevent their being absorbed from the intestines were it not for the action of bile salts on the fatty acids, to enable them to be absorbed. Lipids (fats) are packaged by the intestinal cell in the form of transport vehicles, namely, large lipoprotein complexes known as Chylomicrons. The Total Lipid (fat) concentrations in serum range from 467-694 mg% for men, to 424-656 mg% for women, and they are broken down further as described below.

Cylomicrons are spherical particles ranging from about 100 to 500 nanometers in diameter. They contain a core of neutral lipid (Triglycerides and Esterified Cholesterol) and are surrounded by a membranelike coat of protein and Phospholipid. Unesterified Cholesterol is also present in both the core and the outer coat. Triglycerides (combinations of glycerol with the three fatty acids: Stearic, Oleic and Palmitic) account for more than 90% of the weight of chylomicrons, proteins less than 1 per cent. Normal serum concentrations of Triglyceride range from 92-217 mg% (mg/100 ml) for Men and 0-169 mg% for Women. Phospholipids (Fatty Acids combined with Phosphorus and a Nitrogenous base, such as Lecithin) normally appear in male serum in concentrations ranging from 163 to 253 mg% (27-39% of total lipid), and in female serum in concentrations ranging from 170 to 285 mg% (33-46% of total lipid). And

Total Cholesterol is in the range 102-191 mg% for men and 90-250 mg% for women. This breaks down into an Esterified portion (72-165 mg%) and a Free portion (12-79 mg%).

Starches, Sugars and other Complex Carbohydrates are ultimately broken down by the digestive system into the simple sugar, Glucose, which appears in blood in concentrations ranging from 70 to 120 mg/100 ml blood (fasting). This range can have a greatly increased upper limit (hyperglycemia) in cases of Diabetes Mellitus, or after meals rich in carbohydrates (blood glucose levels can rise as high as 400 to 800 mg per 100 ml of blood), and it can have a greatly reduced lower limit (hypoglycemia) in pathologic conditions such as Addison's disease, where blood glucose levels fall to 50 mg/100 ml, or less.

Approximately 80 amino acids are found in nature, but only 22 are actually necessary for the proper growth and metabolism of humans. Of these, eleven are termed "essential", in the sense that they cannot be manufactured by the body but must be ingested daily in the food supplied to the organism. These are:

Histidine	IsoLeucine	Lysine
Methionine	Cysteine	Phenylalanine
Tyrosine	Threonine	Tryptophan
Valine		Leucine

There is still some controversy as to whether or not

Histidine, Cysteine and Tyrosine should be included in this list, so one sometimes hears of only eight essential amino acids. The eleven nonessential amino acids, which definitely can be produced by the body, are:

Alanine	Arginine	Aspartic Acid
Citrulline	Glycine	Glutamic Acid
Proline	Norleucine	Hydroxyglutamic Acid
Serine		Hydroxyproline

Amino Acids pass unchanged through the intestinal wall and portal vein into the blood, then through the liver into the general circulation, from which they are absorbed by the tissues of the body according to the specific amino acid needed by that tissue to make its own protein (c.f., VPI-E-83-42). Unused amino acids are converted into Urea, which appears in serum in quantities ranging from 25 to 52 mg per 100 ml (Blood Urea Nitrogen, BUN, range 8.9-15.2 mg per 100 ml. blood, or 6.36 to 10.8 milliequivalents per liter).

Blood Vitamins (Vital Amino Acids) can represent up to about 28 mg of dissolved compounds per 100 ml of plasma. They include: Vitamin A (as Carotenol, or Retinol, Preformed Vitamin A-1, 13 micrograms per 100 ml blood; as Carotene or Provitamin A-2, 120 micrograms per 100 ml blood); The B-Complex Vitamins: B-1 (Thiamine, 8.9 micrograms per 100 ml blood for males, 7.6 micrograms per 100 ml blood for females), B-2 (Riboflavin, 2.6-3.7 micrograms per 100 ml plasma), B-3 (Niacin, or Nicotinic

Acid, 0.6 mg per 100 ml blood), B-5 (Pantothenic Acid, 30 micrograms per 100 ml blood), B-6 (Pyridoxine, 36-44 micrograms per 100 ml blood), B-12 (Cobalamin, 200-800 micrograms per ml serum), Folic Acid (or Pteroylglutamic Acid, or Vitamin B-c, 3.4 micrograms per 100 ml blood), Biotin (1.23 micrograms per 100 ml blood), Para-amino Benzoic Acid (PABA, which is actually part of the Folic Acid Complex), Choline, and Inositol; Vitamin C (Ascorbic Acid, 0.62 mg/100 ml Blood), and associated Citrus Bioflavonoids (Vitamin P), Rutin and Hesperidin; Vitamin D (as Viosterol, D-2, 2.8 micrograms per 100 ml plasma), Vitamin E (as D-Alpha-Tocopherol, 0.9 to 1.9 mg/100 ml plasma), Vitamin E-Complex (as mixed Tocopherols, 0.71 to 0.79 mg per 100 ml plasma), and trace amounts of Vitamin K.

Miscellaneous Plasma Constituents

The list of constituents in blood plasma seems to go on endlessly, again emphasizing the importance of this "river of life" as a mass transport medium. Without even attempting to be exhaustive, we can add the following to the list to the extent that one may find the ingredients tabulated in clinical references (see References at the end of this report):

Acetone, 0.3-2.0 mg/100 ml serum;

Bilirubin (from the breakdown of red blood cells), up

to 1 mg%;

Creatine (from muscle tissue), 2.9-4.9 mg/100 ml blood;

Creatinine, 1-2 mg/100 ml blood;

Glycosamine, 60-90 mg/100 ml serum;

Hexosamine, 52.2-55.8 mg/100 ml serum;

Ketoglutamic Acid, 0.08-0.13 mg/100 ml blood;

Methemoglobin, 0.02-0.20 gms/100 ml blood;

Monoglucosamine Polysachharide, 110.8-117.2 mg/100 ml serum;

Mucoprotein, 11.2 mg/100 ml plasma;

Total Neuraminic Acid, 70.1-84.7 mg/100 ml serum;

Protein-Bound Carbohydrate (Polysaccharides), 103 mg/100 ml Plasma;

Pyruvic Acid, 0.73-1.45 mg/100 ml blood;

Uric Acid, 3.98-4.30 mg/100 ml blood = 0.471-0.509 meq/1 of blood; and so on, and so on, and so on, almost ad infinitum. All of these plasma constituents are in a homogeneous watery solution which has a density of about 1.035 grams per cubic centimeter, and a viscosity of some 1.2 - 1.3 centipoise at 37 degrees centigrade.

BLOOD SERUM

If blood is allowed to completely clot, and the clotted fluid is spun down at high speed in a centrifuge, such that virtually all of the hematocytes and the protein Fibrinogen

are removed, what remains behind is called Serum. Serum has a specific gravity of 1.022 - 1.028 and a viscosity of 1.04 centipoise at 37 degrees centigrade. This compares with 0.6947 centipoise for water at the same temperature, with a specific gravity for whole blood of 1.052 - 1.062, and with an apparent viscosity of whole blood in the range 3.5-5.4 (relative to water at the same temperature) at high rates of shear. Plasma viscosity relative to water at 37 degrees centigrade is 1.9 - 2.3.

APPENDIX B

Spearman Rank Correlation

The Spearman rank correlation coefficient is a descriptive statistic which reflects the degree of association between sample pairs of observations. Ranking is used for three reasons (1) because the quantitative measure is either not necessary to the information being conveyed, or is not accurate enough for more elaborate regression analysis; (2) because the information of interest is whether two different classifications of the same observations may be related, rather than what the actual functional relationship is, and, (3) because it is quick and simple (54).

The majority of information on the calculation of the coefficient of rank correlation was obtained from reference (55). The method of calculation involves the following procedure. First, a rank is assigned to each observation (e.g., value of total protein) in the particular variable set being considered. The lowest value is assigned the rank 1 and the highest N, where N is the total number of observations in the sample. Should two or more observations have the same value, they are given the same rank number, that number being the median between the rank which immediately preceded the tied observations and the rank which would have followed the last of these observations if they did not have identical values. The foregoing procedure is then repeated, this time for another set of observations, which, in the present study was the apparent viscosity at 230 sec^{-1} for each blood sample. We now have two sets of rank numbers for pairs of observations in the sample. The rank

difference, d, between corresponding paired ranks for each observation is calculated and squared. The sum Σd^2, for all observations is then computed and used to obtain a coefficient of rank correlation r_s, between the two variables -- in this example viscosity and total protein. The following formula is used to calculate r_s:

$$r_s = \frac{6\,\Sigma d^2}{N\,(N^2-1)}$$

A sample calculation follows:

1	2	3	4	5	6	7
Sample #	Total Protein	Protein Ranks same sample	Viscosity	Viscosity Ranks	d Col.3-Col.5	d^2 (Col.6^2)
1	5.9	2.5	4.7350	13	10.5	110.25
2	6.5	10.5	4.6850	12	1.5	2.25
3	6.6	14	5.3000	22.5 ┐	8.5	72.25
4	6.8	19	4.6133	10 |	9	81
5	7.3	24	5.5930 highest	25 |	1	1
6	5.9	2.5	4.6000	9 tied	6.5	42.25
7	6.9	21	4.7700	15 |	6	36
8	7.1 ┐ tied	22.5	4.7680	14 |	8.5	72.25
9	7.1 ┘	22.5	5.3000	22.5 ┘	0	0
10	6.5	10.5	4.9000	16	5.5	30.25
11	6.3	8	4.0400 lowest	1	7	49
12	6.0	4	4.2130	3	1	1
13	6.1	5	5.2350	21	16	256
14	6.6	14	4.5133	7	7	49
15	6.7	16.5	5.0000	18	1.5	2.25
16	6.2	6.5	4.9400	17	10.5	110.25
17	6.7	16.5	5.3933	24	7.5	56.25
18	6.2	6.5	4.6600	11	4.5	20.25
19	6.8	19	5.1700	20	1	1
20	5.7 lowest	1	4.3400	5	4	16
21	7.6 highest	25	5.1533	19	6	36
22	6.6	14	4.2600	4	10	100
23	6.5	10.5	4.1330	2	8.5	72.25
24	6.8	19	4.5267	8	11	121
25	6.5	10.5	4.5000	6	4.5	20.25

$$d^2 = 1358$$

$$N = 25$$

$$r_s = \frac{6\ \Sigma d^2}{N\ (N^2-1)}$$

$$= 1 - \frac{8148}{15600}$$

$$r_s = .4776$$

Probability values for the Spearman correlations (i.e., the confidence level for r_s) are obtained by treating $(N-2)^{\frac{1}{2}}\ r_s/(1-r^2)^{\frac{1}{2}}$ as coming from a t distribution with N-2 degrees of freedom, where r_s is the appropriate correlation.

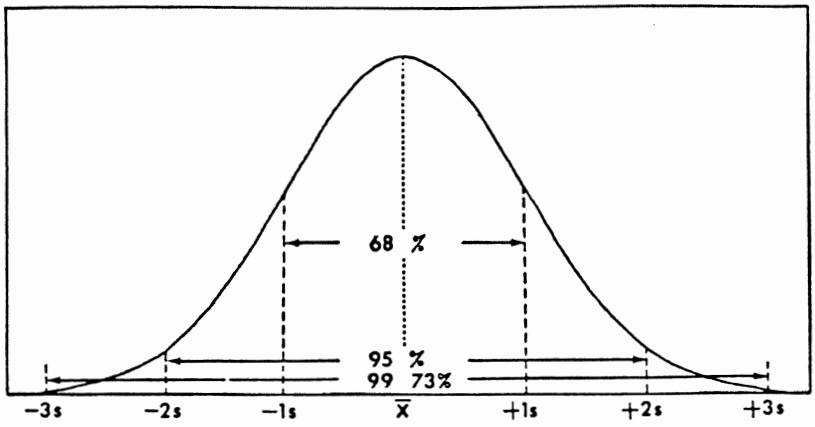
APPENDIX C

Quality Control in the Clinical Pathology Laboratory

The information contained in this appendix was obtained primarily from reference (53). The clinical pathology laboratory has two responsibilities to the physician, the first being to provide the physician with an estimate of the variation in the normal population and the second, to guarantee the reliability of each individual measurement. To express the normal values of the human population clearly, one measures a series of individuals in normal health. The complete expression of the normal population will consist of the average, showing the center of the normal distribution, and the standard deviation, which indicates the dispersion of the population about the average.

Normal Curve

(Normal distribution. Gaussian distribution.)
The statistical basis for quality control.



$$s = \frac{\Sigma(x-\bar{x})^2}{N-1}$$

s = standard deviation

$\bar{x}$ = mean of all values

x = individual observed value

N = number of observations

Laboratory results falling within the ± 2 s range are considered to be normal. It is necessary for each laboratory to measure its own normal values. It is known that because of many unknown variables, normal values from the literature cannot be assumed to be valid for any individual laboratory unless checked. When procedures are modified or changed, the normal values should be rechecked.

The second laboratory responsibility is to guarantee the reliability of every measurement. Three difficulties should be identified: First, no one method of analysis gives exactly the same result each time it is repeated. There is an inherent variability characteristic of each measurement procedure that cannot be avoided. Second, due to a variety of factors, <u>different</u> techniques which may be available to make the <u>same</u> measurement may not all yield the same normal ranges, thus reducing the absolute reliability of any given "normal" clinical value. Third, practical considerations limit the amount of sample available for measurement. Each sample is one of a population of possible samples that could have been taken from the subject. Quality control systems have been developed to guarantee reliability of one's procedures and at the same time require only limited blood samples. A

large number of identical samples from the same large pool are prepared and are frozen or lyophilized to preserve stability. Every batch of laboratory measurements is accompanied by one of the identical pool samples, the quality control serum. The variability of repeated analyses is measured under regular operating conditions, using the identical samples and is expressed in standard deviation units. If the quality control sample does not fall within the known limits of inherent variability, the measurements are rejected and the analyses are repeated. In this way each laboratory measurement is accompanied by a known sample, which undergoes exactly the same steps and conditions as the unknown samples.

In an effort to reduce the inherent variability of measurements, the laboratory should also concentrate on the areas of methodology and instrumentation. A method or an instrument that is highly reproducible on a single day may show considerable day-to-day variability over the period of a year. Therefore, it is important to evaluate new methods and new instruments for their inherent variability over a year's time in order to truly evaluate their acceptability in the field. The new instruments and new methods constantly being added in the clinical pathology laboratory tend to create more confusion and increase the variability of reported results both within the laboratory, itself, and when compared with results obtained in other laboratories.

Researchers who utilize the results reported by clinical laboratories should be aware of this variability between methodologies and instrumentation when making comparisons and developing conclusions

about a particular study. A high level of accuracy or reproducibility may not be attainable because of the limitations of the chemical procedures involved in obtaining information on blood chemistries. It should be pointed out, however, that the modern clinical laboratory provides the physician with valuable information of sufficiently high accuracy to enable him to assess the state of health of the patient. All laboratories also participate in external proficiency testing programs which allow them to monitor the accuracy and reproducibility of their results by comparing them with those of highly competent independent or state run laboratories.

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