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AN EVALUATION OF CENTRIFUGED vs ELECTRONICALLY DETERMINED HEMATOCRITS IN ASSESSING THE DEGREE OF POLYCYTHEMIA IN CYANOTIC CONGENITAL HEART DISEASE

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HEMATOCRITS IN ASSESSING THE DEGREE OF
POLYCYTHEMIA IN CYANOTIC CONGENITAL HEART DISEASE

by

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Secondary polycythemia is defined as an absolute erythrocytosis caused by an enhanced stimulation of red blood cell production. One of the most common methods of monitoring the degree of polycythemia is the measurement of the hematocrit. The hematocrit is the measurement of the volume of red blood cells expressed as a percentage of the volume of whole blood in a sample. This measurement is usually done using one of two methods: (1) the Coulter Model S® or (2) the centrifuged micro-hematocrit method. However, we have noted a significant difference in the hematocrit values determined by these two methods when the hematocrits exceed 54 per cent. As many as 10 hematocrit units of difference have been reported (1 hematocrit unit = 1 ml of packed erythrocytes/100 ml of whole blood).

Several questions arise: (1) does this variation in hematocrit (hct) values increase in proportion to the increase in hematocrit; (2) is there a relationship between the fibrinogen level and the amount of trapped plasma in the centrifuged micro-hct; (3) is there a relationship between the red blood cell count (RBC), mean corpuscular volume (MCV), and whole blood viscosity; (4) which measurement is a better indicator of the degree of polycythemia and the increase in viscosity.

To answer these questions data from the Coulter Model S®, centrifuged micro-hct, whole blood viscosity and fibrinogen determination were evaluated. With these data it was planned to determine if it would be possible (1) to con-
struct a nomogram establishing the relationship between the micro-hct and Coulter S hct greater than 50 per cent for the conversion of one value to the other, and (2) to assess the degree of polycythemia and the need for phlebotomy by the use of each measurement.
LITERATURE REVIEW
SECONDARY POLYCYTHEMIA

A significant increase in total red cell mass may occur in any situation which results in tissue hypoxia. This lack of oxygen leads to an increase in the level of erythropoietin, a humoral substance that is believed to be primarily responsible for the regulation of erythropoiesis.

In secondary polycythemia the increase in the red cell mass is often accompanied by a decrease in plasma volume. The excessive production of erythrocytes may lead to an elevation of the red blood cell mass sufficient enough to produce alterations of the viscosity of the blood, thereby increasing the danger of thrombosis (4).

CARDIAC MALFORMATION AND SECONDARY POLYCYTHEMIA

Any cardiac malformation which results in a high grade veno-arterial shunt may produce secondary polycythemia (77, 78, 79, 80). Due to the mixing of venous and arterial blood, poorly oxygenated arterial blood reaches the peripheral tissues. As a result, it is thought that an increase in erythropoietin occurs. Subsequently, the increased red blood cell count that results increases the oxygen carrying capacity of the blood. According to Kontras (6), the increased red cell count serves as a compensatory mechanism for decreased oxygenation until the hematocrit levels are greater than 70 per cent. When this occurs, the value of the increased oxygen carrying capacity is offset by the increased blood viscosity.
OTHER CAUSES OF SECONDARY POLYCYTHEMIA

Secondary polycythemia may result from many hypoxic stimuli such as decreased atmospheric pressure, impaired pulmonary ventilation or the presence of certain abnormal hemoglobins (methemoglobin or a hemoglobin variant with an increased oxygen affinity).

VISCOSITY AND SECONDARY POLYCYTHEMIA

Dintenfass (29) showed that if the viscosity of blood were tested at high shear rates (high flow velocities), in the absence of aggregation of the red cells, blood viscosity was approximately a semi-logarithmic function of the hematocrit reading.

A description of several factors that influence viscosity was given by Charm, et al (30). Some of these factors were the shape and elasticity of the red cell, changes in its rigidity, deformability and internal viscosity. These were listed as erythrocytic factors. A separate category of plasmatic factors was made to discuss the molecular aspect of viscosity.

Erythrocytic Factors

The internal viscosity of the erythrocyte has been calculated to range between 1 and 6 centipoise (24, 41). The centipoise is a unit of viscosity derived by dividing the shear stress (dyne/cm²) by the shear rate. The estimate
given by Dintenfass includes cell membrane effects as well as hemoglobin viscosity. The internal viscosity of the erythrocyte was found by Dintenfass (24) to become significant at hematocrit values of 40 per cent and to be of major importance at values of 70 per cent and above. A pronounced increase in blood viscosity can therefore be caused by crenation of the red cells or by hypoxia, both resulting in an increase in the internal red cell viscosity. The mechanism for the increased viscosity associated with crenation is the reduction in volume without a compensatory decrease in the hemoglobin content of the cell. In the case of hypoxia, significant changes in the viscosity are noted when the arterial oxygen saturation is 66-75 per cent or less. At this saturation the pH is decreased resulting in an increased red cell membrane rigidity.

According to Kurland and Charm (30, 54), cell rigidity is related to the characteristics of the cell contents as well as the cell membrane. The cell membrane is capable of moving around the cell contents. The evidence for this lies in the fact that packed cells possess a viscosity much lower than would be expected (24). At a low pH the hemoglobin becomes ionized. In this case, the viscosity of the cell contents, i.e., hemoglobin, is the controlling viscosity factor (33, 27).

Bircher (28) states that this movement of the cell membrane around the contents is the mechanism by which red cells adapt to flow. In pathological states, deform-
bility is reduced due to either membrane defects or hemoglobin abnormality. Abnormal molecular interactions with reciprocal binding of cellular constituents represent the basic mechanism responsible for increased cell rigidity. Intracellular reduced glutathione reduces the interactions and is the primary protective agent against loss of cell deformability.

Jandl, et al (46) found that a lowered intracellular ATP level allows the formation of calcium bridges between cell constituents causing increased cell rigidity.

According to Bircher (28) the degree of red cell aggregation as well as the size of the aggregate depend on the flow velocity (shear rate) and on the intrinsic properties of blood. The mechanism of aggregation is dependent upon the interaction of blood proteins with the red cell membrane. The presence of aggregated red cells interferes with streamline flow by decreasing the development of shear planes. This causes an increase in the whole blood viscosity and a severe handicap to the circulation (24, 25, 26).

Plasmatic Factors

Chien, et al (16) showed that there was a correlation between the molecular weight of plasma proteins and their ability to cause aggregation of red blood cells. The heavier proteins, such as macroglobulins (molecular weight = 1,000,000) and fibrinogen (molecular weight = 325,000) are the strongest aggregation inducers. Conversely, Wells (25, 28) noted
that albumin tends to decrease red cell aggregation, possibly because of its strong negative charge. Of the proteins normally found in plasma, fibrinogen, which has a neutral charge and is a much larger molecule than albumin, has the greatest influence in promoting red cell aggregation. Both Charm (28) and Wells (17) stated that as the fibrinogen level exceeds the upper limits of normal (500 mg/dl), a significant increase in red cell aggregation begins. However, it was demonstrated by both authors that macroglobulins were the strongest inducers of aggregation.

VISCOSITY AND ANTICOAGULANTS

Anticoagulants were grouped into two categories by Rosenblum (22): (1) those which shrink erythrocytes (citrate and oxalate) and (2) those having no effect on erythrocytes size or shape if in the correct concentration and if examined immediately (heparin, ethylene-diamino-tetra-acetic acid (EDTA), and acid citrate dextrose (ACD)). However, EDTA may cause significant swelling of the red cells if the mixture is left at room temperature for 24 hours. In addition, if the concentration of EDTA is too high, red cell shrinkage may result. Although cells shrink in citrate, they do not in ACD according to Galluzzi, et al (45).

The first group of anticoagulants increases viscosity eventhough citrate reduces the hematocrit; while those in the second group have no effect if used in the proper concentration and time limit (within 24 hours if refrigerated
at 4°C). Brittin, et al (81) stated that after storage for 24 hours at 4°C no significant difference was observed in the hemoglobin, hematocrit, red cell count, size, shape, or content determined by the Coulter Model S® or by conventional methods. However, when these same measurements were made on duplicate samples stored at room temperature for 24 hours significant swelling of the red cells was seen.

Blood in ACD can be maintained for two days at 4°C with no change in viscosity. However, the viscosity of blood in potassium oxalate changes in two or three hours. Galluzzi, et al (45) went on to say that blood in "balanced" oxalate, i.e., ammonium oxalate and potassium oxalate mixture, EDTA, or heparin may also be stable for one to two days at 4°C.

FACTORS AFFECTING THE CENTRIFUGED vs ELECTRONICALLY DETERMINED HEMATOCRITS

The Effect of Anticoagulants

The type of anticoagulant used in blood collection affects the centrifuged micro-hct and the mean corpuscular volume (MCV). Although salts of EDTA provide excellent anticoagulants for hematologic determinations, high concentrations of EDTA (greater than 2 mg/ml whole blood) produce errors in the hematocrit determined by the micro-centrifuged method (73). This elevated EDTA concentration causes shrinkage of the red cells. This error may be seen in incompletely filled evacuated tubes containing a fixed amount of anticoagulant.
Lampasso (73) reported that the error of the hematocrit may reach 5 per cent below the true value when a Vacutainer® tube designed to draw 7 ml of blood is filled with 2 ml, and it may reach 10 per cent if the tube contains only 1 ml. He observed a progressive decrease in hematocrit values as the concentration of EDTA increased. Reliable micro-hct estimations cannot be performed when the concentration of EDTA exceeds 2 mg/ml of whole blood.

Brittin, et al (70) showed the advantage of using the Coulter Model S® over the centrifuged micro-hct method. The Coulter S® eliminated the errors in hcts produced by excessive EDTA. This was done by making a 1:50,000 dilution in an isotonic medium. This 1:50,000 dilution in an isotonic medium restored the shrunken red blood cells to their initial size (70, 74).

A comparison of the effect of anticoagulant concentration on centrifuged and electronically determined hcts was made by Ferro (72). In his study three methods of measuring hcts were examined: (1) micro-centrifuged hct, (2) macro-centrifuged hct, and (3) Coulter Counter Model F® methods. Prior to determining the hct with the Model F® a 1:50,000 dilution of blood in an isotonic solution was made. The concentration of EDTA used in this evaluation ranged from 1.3 to 9.0 mg per ml of whole blood. When the latter concentration of EDTA was used, it decreased the micro- and macro-hcts by an average of 4 hct units. These hct units were defined as the equivalence of 1 ml packed erythrocytes/
100 ml whole blood. These same specimens were concurrently checked for hct values by electronic computation using the Coulter Counter Model P®. No effect from excessive amounts of EDTA was observed. In a comparable study by Brittin, et al (71) no effect on the computed hct using the Coulter Model S® was observed.

The data presented confirmed the fact that the concentration of anticoagulant must be properly controlled to avoid decreases in packed cell volume by micro- and macro-centrifuged hematocrits. In addition, the usefulness of the Coulter Counter electronic technique for hematocrit estimation was supported. The maximum difference in values for hematocrits by the three methods using the proper proportion of anticoagulant and whole blood (1.3 mg EDTA/ 7 ml whole blood) was 1 hct unit. The hematocrits examined ranged from 17 to 54 per cent (72).

Other Factors Affecting Centrifuged Hematocrits

Dacie and Lewis (50) stated that plasma proteins, red blood cell count, size, and morphology all affect the micro-hct. According to Williams, et al (2) the differences in hematocrit values are dependent upon the amount of trapped plasma which may range from 2 to 8.5 per cent, which in turn, is affected by the relative centrifugal force employed and the duration of centrifugation.
Factors Affecting the Electronically Determined Hematocrits

Significant errors in the mean corpuscular volume (MCV) and consequently, the hematocrit will occur with the Coulter Counter Model S® when the leukocyte count is markedly increased. In addition, it was pointed out by several authors (2, 47, 48, 49, 50, 51) that as the number of cells counted is increased, there is an increased chance that two or more cells will be sensed as one by the detecting device. Therefore, techniques for minimizing or correcting for coincidence error have been a requirement of electronic cell counting devices.
METHODS AND MATERIALS
SELECTION OF DONORS

It has been noted that the number, morphology and hemoglobin content of the red blood cells can affect their aggregation tendencies. In addition, plasma proteins, especially fibrinogen, tend to influence aggregation. When the hematocrit exceeds 50 per cent, there is an exponential increase in the whole blood viscosity accompanies by an increase in the amount of trapped plasma in the centrifuged micro-hct sample. This trapped plasma results in a 4 to 10 per cent increase in the hct values determined by the micro-hct method. Because cyanotic congenital heart disease (CCHD) children frequently have elevated hematocrits, they were selected as the primary donor group for study (with the permission of the Pediatric Cardiology Department of the Medical College of Virginia Hospitals). These donors had had varying amounts of therapy.

Since there was a limited number of CCHD children available for study, it was thought that a simulated condition of secondary polycythemia could be produced by the following method: (1) centrifugation (1800 rpm for 10 minutes) of randomly selected monlipemic, nonhemolyzed blood; (2) after centrifugation the plasma could be removed until the desired micro-hct was obtained. However, prior to centrifugation an initial micro-hct and Coulter S(S) evaluation was made. Only samples with normal hemoglobin (hgb), hematocrit (hct), red blood cell counts (RBC), and red cell indices (MCV, MCH, MCHC) were selected for study.
It was noted that adults were the primary donors in this group. However, no literature reviewed indicated that there was any significant difference between the red blood cells of adults as compared to those of children except to reflect the differences in reticulocyte counts. Normally about 0.5 to 1.5 per cent of all erythrocytes in adults are reticulocytes. Normal values at birth range from 2.5 to 6.5 per cent, falling to normal adult level by the end of the second week.

In a text by Miale (1) it was noted that the degree of reticulocytosis was proportional to erythropoietic activity. It was stated that significant increases in the reticulocyte count were observed in response to increases in erythropoietin. Dintenfass (27) found no significant difference between the viscosity values obtained comparing fetal and adult bloods, that had been adjusted to the same micro-hct despite the differences in reticulocyte counts. He found that the pH of the bloods had more effect on the viscosity. Furthermore, it was shown that the acid-base values of infants and children are more variable than those of adults (3).

Another group of donors was selected for the study of the effects of elevated fibrinogen levels on the difference between the micro- and Coulter S hemocrits. The blood from any patient who had had a fibrinogen determination and Coulter S evaluation performed was selected for study. The fibrinogen level and initial Coulter S data were recorded. The same centrifugation and micro-hct adjustment procedures
were done as previously described. The micro-hct adjustment was followed by a Coulter S\textsuperscript{®} measurement of the hematocrit.

**TESTS PERFORMED**

**Micro-hematocrit Centrifugation Method**

The volume of the red cells occupied in whole blood was determined by subjecting the blood to sufficient centrifugal force to pack the cells in as small a volume as possible. This measurement was expressed as a percentage derived by the ratio of the volume of packed RBC's (nl)/100 ml of whole blood. The amount of plasma remaining in the packed cells varies somewhat with the method used, but is usually about 1 to 4 per cent for micro-hcts less than 50 per cent. For the standard micro-hct method capillary tubes by Clay-Adams\textsuperscript{®} were used. They were 75 mm in length with an internal diameter of about 1mm. No anticoagulant coating the interior of the capillary tubes was used. The EDTA anticoagulated blood was allowed to enter the tube by capillary action, leaving at least 15 mm unfilled. The tube was then sealed by the use of a plastic seal. After centrifugation (Adams Autocrit Centrifuge\textsuperscript{®} by Clay-Adams) at 12,000 rpm for 5 minutes the packed cell volume was measured using a reading device. Micro-hcts greater than 50 per cent were centrifuged an additional 5 minutes at 12,000 rpm to determine if further packing of the red cells would result.
Coulter Model S® Hematocrit Method

By use of the Coulter Model S® one is able to make six measurements relating to the red blood cell. These measurements are as follows: RBC count, hemoglobin (Hgb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and Mean corpuscular hemoglobin concentration (MCHC). In addition one may measure the leukocyte count. By the use of a pneumatic and vacuum system approximately 1 ml of whole blood was aspirated. 44.7 lambda of this blood were initially diluted 1:224 with 10 ml of an isotonic solution. A second dilution (1:50,000) was made from a 44.7 lambda sample from the first dilution and mixing with another 10 ml of isotonic solution. By means of a vacuum RBC suspension was forced to flow through three apertures of specific dimensions. The sample was drawn through all three aperture tubes simultaneously for a period of 4 seconds. In the chamber with the RBC suspension two apertures were used for measuring the size of each cell passing through. The values obtained were then averaged. Each aperture tube had an internal electrode, and there was a common electrode in the bath acting as a ground. As the cells passed through two of the three apertures, they, being nonconductors, displaced the electrolyte, thus changing the resistance between the two electrodes, producing a voltage pulse magnitude proportional to the volume of the cell.

The voltage pulses were amplified and displayed on an
oscilloscope screen as vertical spikes. The number of spikes represented the number of cells passing through the aperture. All three apertures were used in enumerating the RBCs. The three values obtained were then averaged. The hematocrit was computed by the product of the MCV and the RBC count (1, 47, 51).

One may also count capillary blood using the Model S. 4.4.7 lambda of capillary blood was diluted with 10 ml of isotonic diluent, the whole blood 1:224 dilution switch was turned to the 1:224 position and blood was aspirated through the capillary blood aspirator. The results of both procedures (capillary and whole blood) were provided in the form of print-outs.

Since the Model S was limited by the number of digits it could print out, RBC counts greater than 9.99 million/mm$^3$ had to be diluted to a measurable RBC range. When this problem was encountered with a few of the samples from the CCHD children, a 1:2 dilution of an aliquot of the patient's blood was made with the isotonic solution, and the sample was then run as the whole blood would have been. The printed out result was then multiplied by 2. Duplicate determinations were performed and averaged.

**Viscosity Studies**

The Brookfield cone-plate viscometer was used in these experiments. The principle of the cone-plate viscometer involves the rotation of a flat cone upon a plane surface (the
plate) at different selected speeds of rotation (different shear rates). A torque measuring device connected to the driving mechanism to a vertical spindle from which the cone was suspended. A 2 ml sample of fluid between the cone and plate offered a resistance to the rotation of the cone and developed a torque to a degree that was a function of the shear stress in the fluid. Knowing the geometric constants of the cone, and observing the rate of rotation and the torque, one could determine separately the shear stress (dyne-cm\(^2\)) and the shear rate (number of revolutions per minute) in the liquid. By changing the shear rate a series of values of the shearing stress could be obtained. By plotting these values against one another the rheological characteristics of the fluid could then have been defined directly in terms of a shear stress-shear rate diagram.

The following rheological experiments using EDTA anticoagulated blood were carried out: (1) the viscosity was measured on the pre- and post-phlebotomy samples and on a few mid-phlebotomy samples; (2) the viscosity of randomly selected bloods with adjusted Coulter S\(^2\) hematocrits of 45 ± 5 per cent was measured. Blood viscometry was done at various shear rates of 212 sec\(^{-1}\) (60 rpm), 106 sec\(^{-1}\) (30 rpm) and 42 sec\(^{-1}\) (12 rpm). At least 3 determinations of shear stress were made at each shear rate setting. All measurements were made at 37°C. The accuracy of the model used was guaranteed to have been within ± 1% whatever the shear rate employed. By definition, the viscosity, \(n\), was
shear stress divided by shear rate in dyne/cm^2 or poise. The result multiplied by 100 gave the more commonly used unit of centipoise (cp).

To evaluate the relationship between the viscosity and the hematological data obtained from the micro-hct and the Coulter S® graphs were constructed. The Coulter S® hct and micro-hct were compared to the normal viscosity curve established by using EDTA anticoagulated blood reconstituted by each patient's own plasma. Individual whole blood samples anticoagulated with EDTA were centrifuged at 1800 rpm for 10 minutes. The plasma and RBCs were separated and re-mixed to produce micro-hcts of 20, 40, 60 and 80 per cents. (See Table I)

**Fibrinogen Determination-Fibrometer Method**

The fibrinogen method chosen was the one currently in use at the Medical College of Virginia. This procedure was based on an automated thrombin time using the fibrometer. The thrombin converted fibrinogen to fibrin, the time taken for the reaction depending on the amount of fibrinogen present.

A pre- and post-phlebotomy aliquot of blood was mixed with citrate (1 part 3.8% citrate to 9 parts blood). The anticoagulated sample was centrifuged at 1800 rpm for 10 minutes and the plasma removed. The randomly selected specimens were treated in the same manner. A 1:10 dilution of patient's plasma was made with Veronal buffer.
TABLE I

Viscosity Data for Reconstituted Normal Human Blood Measured at Various Levels of Hematocrit and Shear Rate

<table>
<thead>
<tr>
<th></th>
<th>RPM</th>
<th>Sec^-1</th>
<th>VISCOSITY (cp) at 37°C</th>
<th>± 2 Std. Deviations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma</strong></td>
<td>60</td>
<td>212</td>
<td>1.4</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>106</td>
<td>1.5</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>42</td>
<td>1.5</td>
<td>0.28</td>
</tr>
<tr>
<td><strong>Hct-20%</strong></td>
<td>60</td>
<td>212</td>
<td>2.5</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>106</td>
<td>2.6</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>42</td>
<td>2.8</td>
<td>0.54</td>
</tr>
<tr>
<td><strong>Hct-40%</strong></td>
<td>60</td>
<td>212</td>
<td>3.8</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>106</td>
<td>4.4</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>42</td>
<td>5.3</td>
<td>1.24</td>
</tr>
<tr>
<td><strong>Hct-60%</strong></td>
<td>60</td>
<td>212</td>
<td>6.5</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>106</td>
<td>7.2</td>
<td>0.96</td>
</tr>
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<td></td>
<td>12</td>
<td>42</td>
<td>8.8</td>
<td>0.96</td>
</tr>
<tr>
<td><strong>Hct-80%</strong></td>
<td>60</td>
<td>212</td>
<td>---</td>
<td>---</td>
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<td></td>
<td>30</td>
<td>106</td>
<td>14.4</td>
<td>0.82</td>
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<tr>
<td></td>
<td>12</td>
<td>42</td>
<td>21.7</td>
<td>1.20</td>
</tr>
</tbody>
</table>
0.2 ml of diluted plasma was incubated at 37°C for 2 minutes. The timing was started with the simultaneous addition of 0.1 ml thrombin reagent. The duplicate results checked within 0.5 seconds. The clotting times for each specimen were averaged. The thrombin times of purified fibrinogen of known concentrations were used to construct a standard curve. The thrombin times of each patient were compared to this standard curve. The normal fibrinogen range using this method was 170-410 mg/dl.

Collection and Storage of Blood

The minimum amount of blood needed for hct and viscosity testing was 3 ml anticoagulated with EDTA. From the donors with CCHD the amount of venous blood withdrawn ranged from 250 to 460 ml during the phlebotomy with plasma exchange (15-20 ml/kg body weight). The methods of blood collection were either by venipuncture using a Vacutainer® system or by an I.V.-butterfly infusion set® with a stop-cock system. Usually during the phlebotomy an initial 7 ml sample was taken followed by 7 ml aliquot samples from each subsequent 30 to 50 ml of blood removed.

Viscosity studies were performed once weekly. Some samples required storage at 4°C for 24 to 72 hours before viscosity studies were performed. Other samples had viscosity measurements performed within 2 to 4 hours of the phlebotomy (see results). The exchange plasma anticoagulated with ACD which does not affect the RBC or viscosity was used for
adjusting the micro-hct values from the phlebotomized donors. 10 to 15 ml of the exchange plasma were saved for use in adjusting the final blood sample to a normal micro-hct range (42 ± 6%).

**Preparation of Test Samples**

The ACD plasma used in the phlebotomy and exchange as stated before was used to dilute the final phlebotomy sample to a normal micro-hct range. The EDTA blood from the random patients was centrifuged at 1800 rpm for 10 minutes. The plasma was removed and the hematocrits were adjusted to a level greater than 50 percent. Since temperature was critical in viscosity determinations, the refrigerated specimens were allowed to reach room temperature prior to testing. Furthermore, each sample was thoroughly mixed before any testing was done. This eliminated any red cell aggregates caused by the lowered temperature and settling of the blood. As stated earlier all viscosity measurements were made after the sample temperature reached 37°C. This temperature was maintained by a circulating 37°C water jacket incorporated in the structure of the viscometer.
RESULTS
This is a comparative study of the Coulter Model S® hematocrit vs the centrifuged micro-hematocrit methods to determine the relative value of each method in assessing polycythemia in cyanotic congenital heart disease children. The accepted treatment for polycythemia observed in these children is phlebotomy with plasma exchange (15-20 ml/kg body weight). This procedure is usually done when the micro-hematocrit exceeds 65 per cent.

Table II summarizes the clinical and hematological data on the CCHD children. The ages of these children ranged from 7 months to 8 years. No other known disease was present except for hgb S-A found in T. N. Due to transfers from the hospital the charts and diagnoses of the type of the type of CCHD of two of the children could not be obtained.

With the exception of S.® all subjects had normocytic (MCV-86±5) normochromic (MCH - 29±3; MCHC - 31±4) red blood cells. S.®'s red cells were microcytic and hypochromic. This was probably due to long standing therapy which had produced an iron deficient state caused by the rapid production and removal of the red cells (by phlebotomy) after his hematocrit reached 65 per cent.

The phlebotomy data indicate the average micro-hct was 67 per cent which was approximately 1.7 times the normal value. Centrifugation for an additional 5 minutes at 12,000 rpm caused no further packing of the red cells. The hematocrit units of difference were calculated by subtracting the Model S® hematocrit value (S) from the micro-
hematocrit (micro-hct) value. Ideally there would have been no difference between the two values.

The initial viscosity (cp) was about 2.4 times the normal level in all of these children who had, as mentioned previously, had varying amounts of therapy for their conditions.
TABLE II
Pre-Phlebotomy Clinical and Hematological Data.

<table>
<thead>
<tr>
<th>Donor</th>
<th>S.P.</th>
<th>S.P.</th>
<th>A.H.</th>
<th>T.T.</th>
<th>T.N.</th>
<th>M.S.</th>
<th>S.B.</th>
</tr>
</thead>
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<td>7 mos.</td>
<td>4 yrs.</td>
<td>8 yrs.</td>
<td>4 yrs.</td>
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<td>D-transposition of great vessels, sub-pulmonary stenosis, common atrium, common ventricle Patent Ductus Arteriosus</td>
<td>Tricuspid Atresia with Blalock Shunt</td>
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</table>

*--- See Methods and Materials.
HEMATOCRIT STUDIES

The hematological data obtained from each specimen collected during the phlebotomy and plasma exchange are presented in Table III. Following the phlebotomy an 8.4 to 21.1 per cent reduction of the RBC count, micro- and Coulter S hematocrits was observed. The phlebotomies were accompanied by a reduction in the difference between the two hematocrit values. The pre-phlebotomy differences ranged from 2.6 to 10.0 hematocrit units; while the post-phlebotomy differences ranged from 0.9 to 8.0 hematocrit units. Additional centrifugation did not result in any change in the micro-hematocrit.
### TABLE III

**Phlebotomy Data**

<table>
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<tr>
<th>Donor</th>
<th>Total Volume Removed</th>
<th>Total Plasma Replaced</th>
<th>Micro-Hct</th>
<th>Model S'Hct</th>
<th>RBC</th>
<th>Micro-S Hct Units</th>
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<td>RBC</td>
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</tr>
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<td>-----------------------</td>
<td>-----------</td>
<td>-------------</td>
<td>-----</td>
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</tr>
<tr>
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<td>52.9</td>
<td>5.91</td>
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</table>
Figure 1 shows a comparison of the micro-hct and the Coulter S hematocrits of each phlebotomy subject. The ratio of micro-hct to the Coulter S hct yielded an average value of 1.08. Ideally the ratio would have yielded a value of 1.00, thereby indicating no difference between the values obtained using both methods. However, theoretically it was possible to obtain a micro-hct smaller than the Coulter S hct. In this case the ratio would have been less than 1.00. 95.5 per cent of all the measurements fell within ±2 standard deviations of the mean ratio value.
Figure 1

Microhematocrit vs. Coulter S® Hematocrit

- **Donors = Phlebotomy Subjects**
- **Mean Ratio of Micro-hct (108)**
- **± 2 Standard Deviations**
Since only a limited number of CCHD donors was available for study, it was thought that a condition similar to that observed in secondary polycythemia could be created by the removal of plasma from randomly selected normal bloods prior to testing (See Methods and Materials). The samples were selected regardless to the age or diagnosis of the donor. The only prerequisites were the presence of normal RBC counts, hgb, hct and red cell indices.

Figure 2 shows a comparison of the mean ratio of micro-hct to Coulter S® hct in the random donors and in the CCHD donors. In the random donors this ratio was found to have a value of 1.10.

The statistical analysis of these data is presented in Table IV. A standard deviation of ± 0.05 was calculated for the mean ratios of both the CCHD children and the random donors. Analysis by the t-test gave a value of 2.063 indicating a significant difference between the two populations.

Each blood sample from the phlebotomized donor was treated as a new and unique specimen since treatment (plasma exchange) had occurred prior to the withdrawal of each blood sample. Therefore, it was as if a new patient were being examined. The difference between the two populations suggests that there is a unique feature common to the erythrocytes of the CCHD donors examined that cannot be duplicated by the simple concentration of normal erythrocytes.
Figure 2
A comparison of the mean ratio of micro-Hct of the random and CCHD samples.
TABLE IV

Statistical Analysis of the 2 Populations Examined

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<th>Source</th>
<th>Number of Samples</th>
<th>Number of Donors</th>
<th>Mean Micro-hct</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>St. Dev.</th>
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<td>0.0023</td>
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<td>45</td>
<td>1.10</td>
<td>0.1090</td>
<td>0.0025</td>
<td>.0.05</td>
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</table>

\[ t = -2.063 \]
To assess the accuracy of the averaged value of the ratio of micro-hct to the Coulter S\textsuperscript{E} hematocrit (1.08) as a factor by which the observed Coulter S\textsuperscript{E} hct could by converted to the micro-hct, the data in Table V were compiled and evaluated. This factor was found to be 0.925 for the conversion of the micro-hct to the corresponding Coulter S\textsuperscript{E} hematocrit. However, the Coulter S\textsuperscript{E} hematocrit had to be multiplied by 1.08 to obtain the corresponding micro-hct. These factors were found to be reliable for Coulter S\textsuperscript{E} hcts equal to or exceeding 50 per cent.

The observed differences (observed micro-hct minus the observed Coulter S\textsuperscript{E} hct = d\textsubscript{o}) and the calculated differences (observed micro-hct minus the calculated Coulter S\textsuperscript{E} hct = d\textsubscript{c}) are shown in Table V. In addition, it was found that 98.2 per cent of all the observed Coulter S\textsuperscript{E} hcts (S\textsubscript{o}) fell within ±2 standard deviations of the mean calculated Coulter S\textsuperscript{E} hematocrits (S\textsubscript{c}).
<table>
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<th>Micro-Hct</th>
<th>S&lt;sub&gt;o&lt;/sub&gt;</th>
<th>Mean</th>
<th>S&lt;sub&gt;c&lt;/sub&gt; ±2 SD</th>
<th>d&lt;sub&gt;c&lt;/sub&gt;</th>
<th>Mean</th>
<th>d&lt;sub&gt;c&lt;/sub&gt; ±2 SD</th>
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<td>48.1</td>
<td>42.2-53.0</td>
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<td>3.9</td>
<td>-1.0-7.8</td>
</tr>
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</table>
In an effort to simplify the conversion of micro-hct to Coulter S\(^6\) hct, Figure 3 was constructed. The object was to obtain an accurate estimate of the Model S\(^6\) hct. The micro-hct observed minus the calculated hematocrit units of difference would yield the estimated Coulter S\(^6\) hematocrit. To calculate the mean difference the following procedure was used:

1. The average value of the ratio of micro-hct was determined and found to equal 0.925 or the reciprocal of 1.08.

2. The Coulter S\(^6\) hct was calculated by multiplying the observed micro-hct by 0.925.

3. The calculated Coulter S\(^6\) hct was then subtracted from the observed micro-hct, thus yielding the calculated mean difference between the micro- and Coulter S\(^6\) hematocrits.

For example, at an observed micro-hct of 60 per cent one would expect the average calculated difference between the hct values of the two methods to be 4.5 hct units. All calculated and observed values were based on the blood from the phlebotomized CCHD donors.
FIGURE 3

A COMPARISON OF THE MEAN CALCULATED DIFFERENCES WITH THE OBSERVED MICROHEMATOCRITS
The day, time and number of samples for viscosity determinations were regulated. Therefore, many of the samples were refrigerated at 4°C until the viscosities could be determined. It is common practice to assay samples within 8 hours of collection. Yet, from the data shown in Table VI there seemed to be no correlation between the age of the refrigerated samples (up to 72 hours) and the viscosity.

The viscosities were measured at 3 different shear rates (rpm). The results were as expected. Since the bloods examined were very viscous, the more consistent readings were obtained at the lower shear rate of 12 rpm. The maximum scale readings obtained at shear rates of 30 and 60 rpms were 10.28 and 5.14 centipoise (cp) respectively. The highest reading at 12 rpm using the Wells-Brookfield microviscometer was 25.70 cp.
**TABLE VI**

**Viscosity Data**

<table>
<thead>
<tr>
<th>Donor</th>
<th>Micro-Hct</th>
<th>Model S-Hct</th>
<th>(d_o)</th>
<th>Viscosity (cp) at 12rpm</th>
<th>Viscosity (cp) at 30rpm</th>
<th>Viscosity (cp) at 60rpm</th>
<th>Viscosity Sample Age</th>
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<td>S. P.</td>
<td>67</td>
<td>57.0</td>
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<td>9.5</td>
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<td>8.5</td>
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</tr>
<tr>
<td></td>
<td>57</td>
<td>48.5</td>
<td>8.5</td>
<td>9.3</td>
<td>7.0</td>
<td>4.9</td>
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<td>8.0</td>
<td>8.2</td>
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<td>13.5</td>
<td>9.9</td>
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<td>55.2</td>
<td>6.8</td>
<td>10.2</td>
<td>7.7</td>
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<td>3.8</td>
<td>9.3</td>
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<td>4.9</td>
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<td></td>
<td>54</td>
<td>51.0</td>
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<td>8.3</td>
<td>6.2</td>
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<td>T. N.</td>
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<td>3.0</td>
<td>12.1</td>
<td>9.6</td>
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<td>8.6</td>
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<td></td>
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<td>7.5</td>
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<td></td>
<td>58</td>
<td>57.1</td>
<td>0.9</td>
<td>9.1</td>
<td>7.3</td>
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<td>10.1</td>
<td>8.5</td>
<td>---</td>
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<td>7.2</td>
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<td>2.9</td>
<td>8.2</td>
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<tr>
<td>(Dil)</td>
<td>49</td>
<td>48.1</td>
<td>0.9</td>
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<td>5.8</td>
<td>12.9</td>
<td>---</td>
<td>---</td>
<td>48 hours</td>
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<td>1.9</td>
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<tr>
<td>(Dil)</td>
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<td>3.0</td>
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<td>6.1</td>
<td>4.2</td>
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<td>4.3</td>
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<td>8.6</td>
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<td>50 hours</td>
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<td>48 hours</td>
</tr>
<tr>
<td>(conc.)</td>
<td>72</td>
<td>63.9</td>
<td>8.1</td>
<td>14.4</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Donor</td>
<td>Hct</td>
<td>Micro-&lt;sup&gt;3&lt;/sup&gt;Hct</td>
<td>Hct</td>
<td>Model</td>
<td>Viscosity (cp) at 12rpm</td>
<td>Viscosity (cp) at 30rpm</td>
<td>Viscosity (cp) at 60rpm</td>
</tr>
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<td>-------</td>
<td>-----</td>
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<td>------------------------</td>
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<td>------------------------</td>
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<tr>
<td>J. H. (conc.)</td>
<td>72</td>
<td>60.6</td>
<td>11.4</td>
<td>14.8</td>
<td>---</td>
<td>---</td>
<td>48 hours</td>
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<tr>
<td>J. R. (conc.)</td>
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<td>56.5</td>
<td>3.5</td>
<td>10.7</td>
<td>8.7</td>
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<tr>
<td>M. C. (conc.)</td>
<td>61</td>
<td>56.4</td>
<td>4.6</td>
<td>12.4</td>
<td>9.8</td>
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<td>24 hours</td>
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<tr>
<td>R. W. (conc.)</td>
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<td>6.5</td>
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<td>44</td>
<td>42.5</td>
<td>1.5</td>
<td>6.7</td>
<td>5.2</td>
<td>4.7</td>
<td>24 hours</td>
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</table>
Figures 4 and 5 show a comparison of the normal hct vs viscosity (at 12 rpm) curve and the observed micro- and Coulter S hematocrits vs viscosity from the CCHD and random donors. 94.4 per cent of all of the micro-hcts vs viscosity points fell within ± 2 standard deviations of the normal curve constructed from the values given in Table I. However, only 38.9 per cent of the Coulter S hct vs viscosity points fell within this same region. It must be remembered that the normal curve for the viscometer used was constructed using hematocrits determined by the micro-method.
COULTER'S HEMATOCRIT vs VISCOSITY NORMAL CURVE

FIGURE 5

- RANDOM SAMPLES
- CCHD SAMPLES
- MEAN (NORMAL CURVE)
- ± 2 STANDARD DEVIATIONS

HEMATOCRIT UNITS (%)

VISCOSITY AT 12 RPM (CP)
A comparison of the whole blood viscosity and the micro-
and Model S® hematocrits of the phlebotomy subjects was
shown in Figure 6. It was found that a linear relation-
dship existed between the Model S® hematocrit and the whole
blood viscosity. However, as mentioned in the Literature
Review, there was a linear relationship between the micro-
hematocrit until a hematocrit value of 50 per cent was
reached. After the micro-hematocrit level exceeded 50 per
cent, the relationship became exponential.

When comparing the viscosities at numerically equivalent
micro- and Model S® hematocrits one notes that for hematocrits
between 25 and 50 per cent the Model S® corresponds to higher
whole blood viscosities. However, the corresponding micro-
hematocrits result in lower whole blood viscosities. Yet
one must remember that the Model S® hct values greater than
50 per cent correspond to micro-hct values that on an aver-
age are 1.08 times as large. For example, a Model S® hct
of 55 per cent is equivalent to a micro-hct of approximately
60 per cent.

Curve B determined by the Model S® hcts and viscosity
offers minimal assistance in indicating clinically when phle-
botomy therapy is needed. Curve A appears to be of more
value clinically since the critical point of the exponential
increase in whole blood viscosity can be seen. Therefore,
curve A may be more useful in indicating the need for phle-
botomy therapy.
FIGURE 6

A COMPARISON OF THE MICRO- AND MODEL S® HEMATOCRIT
AND VISCOSITY IN THE PHLEBOTOMY SUBJECTS

HEMATOCRIT (%)

VISCOITY AT 12 RPM (cp)
The limited data presented in Figure 7 permit an evaluation of the effect of viscosity on the difference between the micro- and Coulter \( ^\text{R} \) hematocrits. No direct correlation could be seen in the differences in hematocrit units between the two methods and the increase of whole blood viscosity. Additional samples must be examined before definite conclusions can be drawn.
Fig. 7

The effect of viscosity on the difference between micro- and Coulter's hematocrits.
Charm (18) and Wells (17) reported that there was a correlation between the aggregating tendencies of red cells and the fibrinogen level. The differences between the micro- and Coulter S hcts were examined to determine whether the cause of these differences was due to elevated fibrinogen levels. However, no such correlation could be established in this study. The amount of trapped plasma in the centrifuged micro-hct seemed more related to the hematocrit level than to the fibrinogen level (Figure 8).
FIGURE 8

THE EFFECT OF FIBRINOGEN ON THE DIFFERENCE BETWEEN THE MICRO- AND COULTER'S HEMATOCRITS
MEAN CORPUSCULAR VOLUME AND RED CELL COUNT vs VISCOITY

The Coulter $S^3$ hct is calculated from the product of the MCV and the red cell count. The study was extended to the examination of the relation of the number of red cells and the MCV to the viscosity (Figure 9). The data collected showed that there was a direct (exponential) relationship between the number of red cells and whole blood viscosity. In addition, it was found that there was an inverse relationship between the size of the red cell and the number of red cells required to produce a specific viscosity.
Figure 9

Erythrocyte Count vs Viscosity
DISCUSSION
There is a marked difference between the Coulter S® and micro-hematocrit values in children with cyanotic congenital heart disease. A comparison of the hematocrit values obtained using these methods showed that a difference as high as 10.2 hematocrit units may exist.

Monitoring the hematocrit is very important in the clinical determination of the degree of polycythemia in these children. An increase in the packed cell volume determined by either method was accompanied by an increase in the whole blood viscosity. However, the data showed that the micro-hematocrit value gave a more accurate indication of the degree of polycythemia. In addition, the increase in the micro-hematocrit was shown to more closely parallel the increase in whole blood viscosity.

The decreased accuracy of the Coulter S® hematocrit in clinically assessing polycythemia and increased viscosity was probably due to the 1:50,000 dilution of the whole blood eliminating the effects of the factors that influenced red cell packing and viscosity (70).

Since only 6 cyanotic congenital heart disease children were obtained for phlebotomies, another donor group was selected (see Methods and Materials). It was found that a significant difference existed between the blood cells of the normal donors and those of the CCHD donors. Concentrations and dilutions did not produce comparable conditions in either population. Therefore, the aggregating and packing tendencies of the red cells from each population were different.
Several explanations for this difference exist. According to Dintenfass (29) the aggregation and packing tendencies of erythrocytes are influenced by the rigidity of the cells, which in turn, is related to the cell contents as well as the cell membrane. Jacobs (33) showed that the cell membrane is capable of moving around the cell content. The evidence for this lies in the fact that the packed cells possess a viscosity much lower than would be expected.

It has been suggested that microcytic hypochromic red cells differ from normal red cells in their rheological properties. In this case, the viscosity of the cell contents, i.e., hemoglobin, is the controlling viscosity factor. In two studies by Dintenfass (24, 41) the internal viscosity of the normal red cell was examined and found to range between 1 and 6 centipoise. This estimate included the cell membrane effects as well as hemoglobin viscosity. An assessment of hypochromia and microcytosis should be made to determine the affect each might have on whole blood viscosity.

It was thought that storage might have had an adverse effect on the integrity of the red cell membrane, thereby affecting the viscosity. However, insufficient data were available to ascertain the effect of storage on the viscosity of the blood samples. Whole blood was suitable for red cell counts, hemoglobin and hematocrit determinations for 24 to 48 hours if stored at 4°C (1). Therefore, with these measurements unchanged by storage one would expect no significant change in viscosity.
As indicated in Table VI some samples did not have the viscosity determination performed until 72 hours after the phlebotomy. It was noted that in one patient who had had two phlebotomies, there was no difference in the whole blood viscosity of the blood samples with comparable micro-hematocrits even though the ages of the samples were 2 and 72 hours. Yet, despite the equivalent micro-hematocrits the Coulter S hematocrits differed by 5.4 units. Furthermore, the red blood cell counts differed by as much as 1,280 million/\text{mm}^3.\text{.}

One major question was raised: what events occurred to change the packing characteristics of the erythrocytes in this patient? It was shown that the differences between the micro-hct and Coulter S hct changed from 8.3 units after the first phlebotomy to 3.4 units after the second phlebotomy six months later. No medication had been administered prior to either phlebotomy. Both phlebotomies were performed in the same manner with the micro-hct and Coulter S hct determinations done within thirty minutes of the sample withdrawals. The only difference observed in the patient during the phlebotomies was the fact that during the first phlebotomy the patient was relatively calm. Yet, during the second phlebotomy the patient was very upset and tended to hyperventilate. Could the hyperventilation have been sufficient enough to enhance the oxygenation of the circulating red blood cell aggregates, thereby changing the venous pH from acid to alkaline?
Oxygenation of "sludge" causes a decrease in the number of red cell aggregates being formed. This explanation was supported by the data obtained by Dintenfass (27). The range of pH chosen for his study was 6.9 to 7.4. The lower limit corresponds to blood pH observed in babies with acute respiratory distress syndrome. Actually the localized blood pH can be seen lower in a number of conditions in which trapped red cells and localized stasis exist.

His study utilized both adult and fetal blood. The fetal blood was obtained from the cord and placenta immediately after delivery. Adult blood was drawn by venipuncture. In the first series, the metabolic pH changes were induced by additions of sodium hydroxide, hydrochloric acid and bicarbonate solutions in isotonic concentrations. Packed cells were prepared by centrifugation, pH adjusted, and then hematocrits adjusted to the required ones. Blood pH was measured with a radiometer pH-meter. It was shown that a decrease of pH led to a pronounced increase of viscosity at all rates of shear. It was also shown that at high hematocrits the viscosity elevation at low pH could be attributed both to an increase in the rigidity of the red blood cells and to their aggregation. These results were common to both fetal and adult bloods. A pronounced decrease in the filterability of blood with the fall of the pH was observed.

There was a further effect to consider that was mentioned, namely, that, due to an increase in the rigidity of the red blood cells, an apparent increase of hematocrit took
A fall in pH of blood by 0.5 units was accompanied by a rise in hematocrit (micro-method) of about 15 per cent. The effect of the pH was attributed to the increase in volume of the red cells and a change of their shape to spherical, the swelling being due to the entry of anions as the pH decreased in accordance with equilibria with the degree of ionization of hemoglobin. Human red cells do not act as perfect osmometers and resist swelling. The effects of tonicity on the viscosity of the red cell were due to the fact that the internal viscosity of the red cell increases during crenation while swelling of the red cells in the hypotonic systems lead to higher tension in the red cell membrane. Both these effects resulted in an increased viscosity and looser packing of the red blood cells.

Since the 1:50,000 dilution in an isotonic medium restores the red blood cells to their original size (70) and the rigidity of the cells have no effect on the measurement, the Coulter S hematocrit would not be elevated. Therefore, the degree of polycythemia and increased viscosity would not be reflected accurately by the Coulter S hematocrit.

However, other data provided by the Coulter S might be used to assess the degree of polycythemia and increased viscosity. As stated previously there is an inverse relationship between the mean corpuscular volume and the number of red cell required to produce a specific viscosity. A graph such as Figure 9 that incorporates the MCV, erythrocyte count and whole blood viscosity could prove useful in esti-
mating the viscosity in patients being monitored. In this instance the red cell count would be indicated as more valuable than the Coulter $S$ hematocrit in monitoring the degree of polycythemia in cyanotic congenital heart disease children.

Perhaps venous pH and red cell filterability in conjunction with whole blood viscosity, micro-hematocrits and red cell counts might give a more accurate delineation of the patient's circulatory status and need for phlebotomy. In addition, the determination of glutathione and ATP levels might give indications of the degree of rigidity of the cells which in turn influence the viscosity of the blood. All of these factors influence the assessment of the degree of polycythemia and whole blood viscosity.
SUMMARY AND CONCLUSIONS
Monitoring the hematocrit is very important in the clinical evaluation of the degree of polycythemia and the need for phlebotomy therapy. We have noted significant differences between the Coulter S® and micro-hematocrit values. These hematocrit values differed by as many as 10.2 hematocrit units, with the Coulter Model S® yielding the lower value. The difference between the hematocrit values of the two methods was virtually unaffected by the fibrinogen concentration.

The red blood cell count with the MCV proved to be of more value than the Coulter S® hematocrit in assessing the degree of polycythemia and increased viscosity in cyanotic congenital heart defect children. There was an inverse relationship between the MCV and the number of these red cells required to produce a particular viscosity.

Simple dilutions and concentrations of normal blood did not result in equivalent ratio values of micro-hct to Coulter S® hct when compared to the values obtained using the blood from CCHD donors. This indicates that the differences observed were not simple dilution or concentration phenomena.

Finally, the data in this study suggest that the micro-hematocrit is a more reliable and accurate measure of the degree of polycythemia; but that other parameters may be measured and used concurrently to determine the need for phlebotomy therapy.
REFERENCES


distress associated with high hematocrit reading.

14. Maurer, H. M., McCue, C. M., Caul, J. and Still, W. J.: 
Impairment in platelet Aggregation in congenital heart

15. Dintenfass, L.: Some observations on the viscosity of
pathological human blood plasma. Thrombosis et Diathesis

16. Chien, S., Usami, S., Taylor, H., Liniberg, J.S. and
Cregeren, M.: The effects of hematocrit and plasma
protein on human blood rheology at low shear rates.

17. Wells, R. E., Merrill, E. W., and Gabelnick, H.: Shear
rate dependence of viscosity of blood: Interaction of

18. Charm, S. E. and Wong, B. L.: Shear degradation of
Fibrinogen in the circulation. Science. New York,

blood viscosity, hematocrit, and serum lipid levels in
normal subjects and patients with coronary heart disease.

20. Merrill, E. W., Gilliland, E. F., Lee, T. S., and Salzman,
E. W.: Blood Rheology: Effect of Fibrinogen deduced

21. Pruzanski, W. and Watt, J. C.: Serum Viscosity and
Hy perviscosity Syndrome in IgG Multiple Myeloma. Annals

blood viscosity in macroglobulinemia mice. The Journal
of Lab. and Clin. Med. vol 71, no. 2, pp. 201-211.
Feb. 1968.

23. Lindsley, H., Teller, D., Noonan, B., Peterson, M. and
Mannik, M.: Hy perviscosity Syndrome in Multiple Myeloma
—A reversible, Concentration-dependent Aggregation of
the Myeloma Protein. The Amer. Jour. of Med. vol. 54,

24. Dintenfass, L.: Blood viscosity internal fluidity of
the red cell, dynamic coagulation and the critical capil-
lary radius as factors in physiology and pathology or


