# A CLINICAL AND TECHNICAL EVALUATION OF PLASMA VISCOSITY COMPARED TO THE ERYTHROCYTE SEDIMENTATION RATE TEST

by

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A THESIS

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# INTRODUCTION

## A. STATEMENT OF THE PROBLEM

The Plasma Viscosity Test was originally developed to gain an understanding of the rheology of human blood. Rheology is the study of deformation and flow of matter (23). From these earlier studies developed a new field of rheology called haemorheology. As early as 1938, reports of the importance of blood viscosity, as it is related to disease and health, began to appear. Plasma viscosity has held a key position throughout the studies of haemorheology, in that plasma is the continuous medium in which the formed elements of blood are suspended. The three formed elements of blood are the erythrocyte or red blood cell, the leukocyte or white blood cell and the thrombocyte or platelet (24). The complexity of plasma led to the discovery of many variables which influenced the development of methods and instruments designed to measure the viscosity of plasma. The Ostwald Viscometer gained wide popularity as an instrument due to its simple construction and cost, but the methods used to operate this apparatus were highly variable. These variables resulted in the lack of standardization of results and a great deal of confusion (12). The manual methods of the Ostwald Viscometer employed required a large amount of technologist involvement and time. In 1963, Dr. John Harkness developed a semi-automated capillary viscometer for use in the clinical laboratory setting (11).

It is the intent of this study to evaluate the usefulness of the Harkness Capillary Viscometer in the clinical hematology laboratory.

Normal healthy individuals, as well as patients who suffered a wide variety of diseases, were studied and their plasma viscosities correlated with the Erythrocyte Sedimentation Rate, as well as with their clinical status.

It is clear, from review of the literature, that a standardized, economical and clinically applicable method for the measurement of plasma viscosity is becoming a necessary part of diagnostic and therapeutic haemorheology.

## B. THE ERYTHROCYTE SEDIMENTATION RATE (ESR)

The Erythrocyte Sedimentation Rate is a measurement performed in most clinical hematology laboratories. The basis of this test is to observe a tube of anticoagulated whole blood held in a vertical position over a given period of time. Erythrocytes will fall out of the suspension under the influences of gravity, and the rate of sedimentation of the red blood cells is recorded as the ESR.

Studies of Erythrocyte Sedimentation Rates began in the eighteenth and nineteenth centuries, but did not gain recognition until 1918 when Fahraeus was able to convince practitioners of the usefulness of this test as a diagnostic tool (6, 10). His work showed that the erythrocytes of many patients settled more rapidly than did those in normal blood. His studies on the blood of pregnant women are acclaimed as bringing the ESR into clinical acceptance (6, 10).

Essentially, rapid sedimentation of erythrocytes was associated with disease whereas slower sedimentation was a feature of health. Intensive research was started to clarify the factors which influenced the Erythrocyte Sedimentation Rate. With the onset of this research emerged a variety of methodologies to perform the ESR. Generally,

the two most frequently accepted methods are those of Wintrobe and Westergren (6).

The Wintrobe method uses a glass tube which is 110 millimeters (mm) in length and has an internal diameter of 2.8 mm. The tube is calibrated in millimeters from 0 - 100 over the lower 100 mm of the tube. Anticoagulated whole blood is used to fill the tube. A rack is used to hold the tubes in a vertical position at room temperature. The distance in millimeters that the red cells have settled in sixty minutes is the rate of sedimentation (2, 4).

The Westergren methodology employs a 300 mm tube with an internal diameter of 2.8 mm. The tube is calibrated in 2 mm increments over the lower 200 mm of length. The anticoagulated whole blood is diluted four parts blood to one part of a 3.8% solution of sodium citrate prior to filling the tube. The tube is then placed in a vertical rack for one hour and read in the same manner as the Wintrobe ESR (2).

From this research it was shown that procedural variations had marked effects on the ESR. The length and internal diameter of the tube, as well as the cleanliness of the glass effected results. Varying the vertical position of the tube will result in marked changes in the results. Temperature was also shown to affect the results, as did vibrations from other sources such as centrifuges and electrical motors (4, 6). In any rate measurement, the time element is also critical. The procedures of Wintrobe and Westergren attempted to standardize and control these variables.

The factors influencing the ESR which have clinical significance can be categorized as: a.) the size of the erythrocyte; b.) the degree or ability of the red cells to aggregate (rouleaux formation); c.) the

hematocrit or packed cell volume; and d.) the viscosity of the plasma (6).

The size of the erythrocyte can best be seen to influence the sedimentation rate by looking at the Stokes equation for the sedimentation velocity of a single isolated sphere (26).

$$V_s = \frac{2g\ (d_s - d_f)r^2}{9\eta}$$

In this equation $V_s$ is the sedimentation velocity of a single sphere, $g$ is acceleration of gravity, $d_s$ and $d_f$ are the densities of the suspended particle and of the fluid respectively, $\eta$ is the viscosity of the fluid and $r$ is the radius of the falling sphere (6, 26). Ponder in 1948 suggested that a term $ac$ be substituted for the $r^2$ factor in light of the discoid shape of red blood cells, where $a$ is the radius of the disc and $c$ is its thickness. However, one can still see that the size or radius of the red cell is directly proportional to the velocity of its sedimentation under these conditions.

The ability of red cells to aggregate or to form rouleaux complexes can be seen to affect the sedimentation velocity in much the same manner. The larger the aggregate, the larger its radius becomes and the faster it will settle. However, the mechanisms involved in the aggregation of red cells have not been clearly defined (6). It is known that asymmetric macromolecules (primarily proteins) will influence the aggregation of red cells (2). Fahraeus was able to show that fibrinogen had the most pronounced effect of the plasma proteins on red cell aggregation. Fibrinogen was followed by the globulins and finally by albumin which had the least effect.

The hematocrit influences on the ESR are the most frequently discussed variable. Hematocrit is an expression of the concentration of red blood cells on a percentage scale. This variable is again

related to the preceding one in that the concentration of red cells will affect the ability of red cells to interact and form aggregates (6, 10). Also related to concentration is the suspension stability of red cells. Surrounding red cells is the negative electrical field known as the zeta-potential. This field is due, at least in part, to the sialic acid residues associated with red cell membranes (5).

This negative electrical field is thought to repel one red cell from another and thereby act to hold the red cells in the suspension. It can be seen that this suspension stability relies on the interaction of one red cell with another. Concentration or hematocrit will then influence the suspension stability as well as the aggregating ability of the red cells. Several mathematical models have been proposed to describe the sedimentation of human whole blood. In all of these models, the concentration of red cells is a critical factor, and must be maintained as a constant in their application (15, 22).

The viscosity of the suspension fluid will affect the sedimentation velocity inversely as can be seen in the Stokes equation. However, the relationship between red blood cells and plasma in human blood is not nearly so straightforward (6, 10). Increases in plasma viscosity of human blood are generally associated with increases in the ESR. This obvious deviation from Stokes formula, for and isolated falling sphere, is related to red cell aggregation. Fahraeus pointed to the various plasma proteins as influencing red cell aggregation. These same proteins cause changes in the plasma viscosity. The nature of their influence will be discussed in a later section of this thesis

Evaluations of the clinical usefulness of the ESR were made in a variety of disease states. The ESR was accepted as a useful screening

test for the presence of infection, particularly chronic infection and as a tool to follow the progress of inflammatory processes (2).

Correlations of disease states and degrees of abnormality were hampered largely by the methods employed (1, 2). Results obtained by the Wintrobe method did not correlate well with the Westergren results. Charts used for hematocrit corrections were available for the Wintrobe method but not for the Westergren method. Others have proposed correction charts for plasma viscosity as well as temperature variations (6). However, these aids were not always used, nor were they always accepted as being correct. These issues have led to heated controversy concerning these two methodologies.

Recently, a new method has been proposed to replace the Wintrobe and Westergren procedures. This procedure is called the Zeta Sedimentation Ratio (ZSR). In this method the hematocrit is incorporated in the calculation of the test results. Essentially, the procedure uses a glass tube which is 75 mm in length and 2 mm internal diameter. The tube is partially filled with anticoagulated blood and one end is sealed closed. The tube is placed vertically in a centrifugal device. The tubes are then subjected to a horizontal centrifugal force of approximately 7 to 8 g for four 45-second periods. At the end of each 45-second period the tube is rotated 180 degrees about its vertical axis. The red cells and aggregates traverse the tube due to the centrifugal force and settle due to gravitational force. The tube is removed from the centrifuge and placed in a reading device where the degree of sedimentation is noted. This procedure is not, however, without its problems.

Dr. Brian S. Bull (1) wrote an excellent article in 1975, listing the advantages and disadvantages of the Wintrobe, Westergren and ZSR

procedures. Generally, he found the Wintrobe method was best used when slight elevations of the ESR were seen and when hematocrit corrections were needed. The ZSR had essentially the same attributes with the added advantage of the hematocrit inherent in the method. Both methods were poor in conditions of high elevations of hematocrit and ESR. Under these conditions he recommends the Westergren method (1, 2). To further complicate the problem, each method has its own set of normal ranges. For the Wintrobe method the normal ranges are 0 - 9 mm/hour for males and 0 - 20 mm/hour for females. The normal ranges for the Westergren procedure are 0 - 15 mm/hour for males and 0 - 20 mm/hour for females. The ZSR normals are 40-51% for both sexes. These variations in units as well as ranges have led to added confusion.

A problem not addressed by Bull (1) is the lack of quality control material. Commercial material is not available and laboratory reagents have not been acceptable for controls in Wintrobe, Westergren, or ZSR procedures. Now that standardization of methods and reagents has become a major issue in clinical laboratory medicine, a procedure which utilizes easily obtainable control material is a preferred method, if other aspects are comparable. The usage of control materials allows the laboratory to insure that the variables affecting the test are uniform throughout the procedure as well as from one test to another. Controlled tests allow clinicians to compare one set of results with that of another with more confidence knowing the results were obtained under similar conditions.

The ESR is an inexpensive screening test for a wide variety of diseases. It has been plagued by a host of problems ranging from selection of procedure to variations in normal ranges. Hematocrit corrections

have not been uniformly available or used. A test superior to the ESR would give the same clinical information as the ESR, without being affected by sex or hematocrit. The Plasma Viscosity Determination fulfills these requirements.

## C. THE PLASMA VISCOSITY DETERMINATION

The flow of a fluid through a tube or pipe has a very characteristic pattern. The fluid in the center of the pipe flows faster than the fluid off-center. The difference in these flow rates is defined as a velocity gradient (23, 26). The concept of a velocity gradient is important in understanding the term, viscosity. We can define viscosity as the resistance to shear flow. Shear flow is always associated with a velocity gradient. To overcome the resistance to shear flow, a force is required. This force is frequently called shear stress. The following formula relates shear stress to a velocity gradient.

$$\text{Viscosity} = \frac{\text{Shear Stress}}{\text{Velocity Gradient}}$$

The units in viscosity determinations will involve force per unit area and a velocity gradient. The unit of viscosity dealt with in this paper is the poise. A poise is dyne-seconds/centimeters$^2$. This unit of viscosity is termed an absolute expression of viscosity. Absolute is used to denote that the measurement is a determination of the shear-flow viscosity. Kinematic viscosity pertains to flow measurements, as opposed to force measurements, and is defined as the absolute viscosity divided by the density of the fluid. The unit of kinematic viscosity is a centimeters$^2$/second called a stoke (18, 25).

This paper deals only with absolute viscosity in which the units are the poise. The range of viscosities of various fluids is wide. In the study of plasma viscosity, a very narrow range of values are encountered which are of a relatively low viscosity. Therefore, it is customary to use the centipoise or one-hundredth of a poise when measuring plasma viscosity (18).

There exists a wide variety of instruments to measure viscosities. They can be classified as rotational viscometers, transverse-flow viscometers, oscillation-type viscometers, filtration-type instruments, falling-ball viscometers and capillary viscometers (7, 25). In this paper the falling-ball viscometer and capillary viscometer are discussed.

The ESR as previously described is essentially a falling-ball viscometer, although a poor one, at best. Normally, a falling-ball viscometer uses a high density ball which is allowed to fall through a tube filled with fluid. The time for the ball to pass through a given distance is recorded. The following formula is used to describe the viscosity measured in a falling-ball viscometer.

$$\text{Viscosity} = \frac{4\ tr^2g\ (d/d' - 1)\ (1 - r/R)^{2.25}}{18s}$$

This viscosity is a kinematic measurement where $d$ and $d'$ are densities of the ball and fluid respectively, $r$ and $R$ are the radius of the ball and tube, $s$ is the distance over which the time, $t$, for the ball to fall is recorded in seconds (18.25). It is easy to see that the ESR and falling-ball viscometers are, in fact, related in principle. However, the ESR has poor control over the radius of the falling spheres (red blood cells and red cell aggregates). The point is that the ESR is essentially a falling-ball type of viscometric measurement.

A capillary viscometer can be mathematically described by Poiseuille's Law which states:

$$\text{Viscosity} = \frac{\pi R^4 P t}{8LV}$$

Here R is the radius of the capillary, L is its length, V is the volume of fluid flowing in time, t. P is the pressure difference between the ends of the capillary tube (18).

The Ostwald Viscometer mentioned earlier is a capillary viscometer. It was the forerunner to the instrument used in this study. The semi-automated Harkness Capillary Viscometer is a modification of the basic Ostwald instrument (18, 25). The Harkness Capillary Viscometer employs a capillary tube with an internal diameter of 0.3 millimeters (mm) and 20 centimeters (cm) in length. The pressure difference is supplied by a column of mercury with a driving head of 75 mm. Coulter Electronics of Harpenden, Hertfordshire, England produces the instrument and glassware with a specified rate of shear being 660 $sec^{-1}$ (11). This instrument was employed in this paper for plasma viscosity results.

There are numerous environmental and physical factors which have effects on the measurement of plasma viscosity which are not included in Poiseuille's Law.

In the design of this instrument, errors for meniscal resistance have been minimized by flaring the ends of the capillary tube (16), thereby allowing a large amount of flow through the capillary with a small amount of movement of the fluid meniscus. The capillary of the Harkness instrument is made from glass and is, therfore, subject to adherence of materials to its surface. Changes in surface tension between the glass and fluid will have an impact on the flow rate (19). The surface tension changes can be counteracted by frequent cleaning of

the glass as well as frequent standardization. Detection of these changes by standardization and controls will allow for correction of the problem. The Harkness Capillary Viscometer can be cleaned and standardized with a minimum of effort.

Temperature is a factor which also influences viscosity measurements. Very small changes in temperature can, in fact, have large effects on the viscosity. It was shown by Harkness and Whittington (13) that plasma viscosity over a range of 15 - 35° C changed approximately 65% inversely with temperature. Temperature exerts its influence through proteins, lipids and electrolytes found in plasma as shown by Reis and Kirmaier (21). Therefore, it is important that viscometers have highly controlled temperature environments. The Harkness viscometer is contained in a water bath that is maintained at 25° C ± 0.05° C. This is important since an error of 0.3° C can result in changes of 1% in the plasma viscosity (11).

In the study of fluid viscosity, solutions are generally classified as being Newtonian or non-Newtonian. Newtonian fluids, when subjected to shear flow or force, undergo flow which is directly proportional to the applied force. The ratio of the flow rate to the force is a constant for a Newtonian fluid. In non-Newtonian solutions or liquids this ratio of flow to force is not constant (23). Most plasmas at high shear rates are nearly Newtonian (7); however, plasmas at low shear rates or shear velocity show non-Newtonian behavior. This problem is partially alleviated by using a relatively high range of shear rates (approximately 660 $sec^{-1}$) in the Harkness Viscometer. Dintenfass says: "Most normal plasmas are nearly Newtonian, and capillary viscometry would be suitable. Perhaps the most efficient instrument available is a Coulter Automatic

Viscometer designed by Harkness (1963)" (7). This in the instrument used in this study.

The components that make up plasma are quite numerous. However, plasma is essentially an aqueous solution of salts, which contains small amounts of proteins. Proteins have by far the largest effect on the viscosity of plasma (8, 17, 24). The molecular nature of various groups of proteins allows for further classification as to which types of proteins have more affect than others (27, 28). Hardwicke and Squire were able to show that the largest effects were produced by fibrinogen, less by alpha and globulins and least by albumin (10). Interestingly, Fahraeus showed these same proteins, in the same order, are the factors that affect red cell aggregation in the ESR (6, 10).

Viscosity changes caused by other components of plasma have not been studied in as great a detail as proteins. Lipid materials and their effect on plasma viscosity have not been clearly defined. In one study on cholesterol levels in dogs, cholesterol did show an effect on whole blood viscosity but not on plasma viscosity (20). Very few studies of the effects of other lipids exist. Somer reports: "The effects of other non-protein components of plasma have been reported to be unimportant in regard to viscosity, or if they slightly modify the viscosity, this action possibly is mediated through changes in the proteins" (27). Essentially, the plasma viscosity measurement is a screening test for variations in plasma proteins. The larger the protein is and the more its shape departs from a sphere, the more pronounced its effect on the viscosity (28). Therefore, fibrinogen has a larger effect than globulins, which have a larger effect than albumin (12).

Any disease process which would then alter plasma protein concentration of configuration could then be tested for by variations in plasma viscosity. Excellent correlation between disease states and plasma viscosity have been documented by Harkness (12), Eastham and Morgan (8) and Somer (27). Clinicians today rarely use the plasma viscosity determination as a diagnostic aid. The reason may well be due to the tortuous historical course of the plasma viscosity methods.

The first reports of viscosity measurements on human material appeared early in the 1900's. Many papers were written but were heavily criticized due to the inaccuracy of the instruments used. During these early years, confusion was added when investigators did not state if serum or plasma had been used in their studies. It was during these early years that Fahraeus was able to show the usefulness of the new ESR. As a result, plasma viscosity remained a research measurement. By 1940 many researchers began to find significant correlation of plasma and serum viscosity with disease states. The major problem which plagued these researchers was the lack of standard techniques. Harkness very beautifully describes this portion of history: "Unfortunately, while reaching identical conclusions and giving unanimous support for the new test, no agreement was reached as to the type of viscometer to be used, the optimum temperature for measurement, the anticoagulant, or the range of normal values, and such was the resulting chaos that no others adopted the test which quietly faded into oblivion." (12).

It was from the rubble of this oblivion that Harkness designed the first semi-automated instrument and attempted to standardize the formentioned variables (11). This thesis study was designed to corroborate the performance levels of this instrument as demonstrated

previously by Harkness and his staff in England (12). A normal range study of plasma viscosity in Portland, Oregon was developed and the results compared to those published by Harkness. Correlation studies of plasma viscosity and ESR were designed to evaluate the clinical usefulness of results obtained with this instrument. In addition, the feasibility of a quality control program was studied using laboratory prepared control materials.

# METHODS AND MATERIALS

## A. REAGENTS

### 1. Purified water

All water used for reagents in this project was obtained from a Culligan Reverse Osmosis System.

### 2. 3.6% (w/v) Sodium Chloride

Thirty six grams of Mallinckrodt Analytical Reagent Grade Sodium Chloride (NaCl) crystals were added per one liter of purified water. One ml of a 1% (w/v) sodium azide in 0.9% (w/v) NaCl solution was added per liter of 3.6% NaCl to prevent the growth of fungi.

Eighteen liters of this reagent were prepared for each lot. To check for potential errors in the production of these large volumes, sodium and chloride ion measurements were performed. This reagent is used as the standard calibrating reagent for the Coulter Harkness Viscometer. The viscosity of 3.6% NaCl at $25^{o}$ C is 0.944 cp according to the Instrument Manual supplied with this instrument.

### 3. Sucrose solutions

A series of solutions was prepared by adding non-dried Mallinckrodt Analytical Reagent Grade Sucrose to purified water. The approximate concentrations of the solutions were 10 grams (gm)/100 ml purified water ($H_2O$), 20 gm/100 ml $H_2O$, 30 gm/100 ml $H_2O$ and 40 gm/100 ml $H_2O$.

The concentrations of these solutions were not a critical factor for this study. The sucrose solutions were used as control materials to check for instrument drift and to insure the quality of the 3.6% NaCl

standard on a day to day basis. These solutiosn were dispensed in 2 ml aliquots and frozen for storage.

4. 1% Sodium Azide in 0.9% Sodium Chloride

One gram of Matheson, Coleman & Bell Reagent Grade Sodium Azide was dissolved in 100 ml of a 0.9% NaCl solution.

This reagent was used to prevent fungal growth in the stored 3.6% NaCl standard solution. It was also used in the specimen preparation and storage study of this thesis.

5. Cleaning solutions

a. Chromic Acid Solution was prepared by mixing equal volumes of sulfuric acid and saturated aqueous potassium dichromate. This reagent was primarily used to clean the glassware of the viscometer. It was also used to unplug an occluded capillary tube when other attempts had failed

b. 40% (w/v) Sodium Hydroxide was prepared by dissolving forty grams of sodium hydroxide in 100 ml of purified water. This reagent was primarily used for routine cleaning of the capillary tube as recommended in the Instruction Manual for the Coulter Harkness Viscometer.

## B. INSTRUMENTATION

1. Coulter Harkness Viscometer

This instrument is designed to measure the viscosity of fluids by capillary tube technique. The instrument uses a capillary tube that is 0.3 mm in internal diameter and 20 cm in length connected to a modified Coulter mercury manometer to supply a constant head of pressure. An electrical timing device is employed to measure the time it takes for

the mercury meniscus to move between the two electrodes. A vacuum is supplied by a small electrical pump connected to the instrument by a waste collection bottle. A glass tank filled with water serves as a temperature control environment. The temperature of the water is maintained at $25^{o}$ C $\pm$ $0.50^{o}$ C by a heating - mixing thermostat. The viscosity results obtained are partially a function of this particular viscometer and are therefore referred to as "apparent viscosity" results (11). To compare apparent viscosity results of one instrument to another, it is necessary to know the average rate of shear of the instruments. The rate of shear is related to the length and bore of the capillary as well as the driving pressure. The average rate of shear used in this instrument is 660 $sec^{-1}$ (11).

2. Coulter Zetafuge

This instrument is a vertical centrifuge used to measure the sedimentation rate of blood samples. The instrument centrifuges ZSR collection tubes (75 mm in length and 2 mm internal diameter) in a vertical position at a centrifugal force of approximately 7 to 8 g (gravitational acceleration). The specimen collection tubes are rotated 180 degrees every forty-five seconds throughout the 180 second instrument cycle.

3. Dupont Instruments, Sorvall General Laboratory Centrifuge

This instrument was used to centrifuge whole blood specimens for plasma harvest. The collection tubes are spun horizontally at 2600 rpm (1,240 g) for 10 minutes to obtain platelet-poor plasma.

4. Coulter Counter Model S

This automated, electronic, particle counter was used to obtain hematocrit readings as well as white blood cell counts, red blood cell

counts, hemoglobin values, mean corpuscular hemoglobins, mean corpuscular volumes and mean corpuscular hemoglobin concentrations.

The hematocrit is calculated in this instrument by multiplication of the red blood cell count by the mean cell volume. The other hematological paramters were recorded but not used in the correlation studies of this thesis.

5. Instrument Laboratories, Inc., IL 443 Flame Photometer

This instrument was used for sodium determination on the 3.6% sodium chloride solution.

6. Radiometer CMT 10 Chloride Titrator

This instrument was used for chloride ion concentrations of the 3.6% sodium chloride solution.

7. Orion Research Instruments, Analog pH Meter, Model 301

This instrument was used for plasma pH determinations.

C. PROCEDURES

1. Specimen collection

Whole blood specimens were obtained from the anticubital vein using 5 ml Vacutainers (a product of Becton-Dickenson) containing 1.5 mg of ethylenediaminetetra-acetic acid (EDTA) per ml of whole blood as an anticoagulant. Standard phlebotomy technique was employed. The Vacutainers were inverted several times immediately after collection to insure adequate anticoagulation by the EDTA.

2. Filtration of the 3.6% Sodium Chloride Solution

The 3.6% sodium chloride solution was filtered through a Gelman Instrument Company's Pleated Membrane Capsule with a membrane pore size of 0.2 microns (product number 21206). The unfiltered solution was placed in a clean reservoir. Rubber tubing was used to connect the

reservior with the filter capsule. The capsule was further connected to a five liter vacuum flask by rubber tubing. Negative pressure was supplied by a water flow aspirator. Large quantities of 3.6% NaCl solution may be prepared in advance and stored in plastic Isoton cubes (Coulter Diagnostics) which will hold 18 liters. One ml of a 1% sodium azide in 0.9% NaCl solution was added to each liter of filtered reagent to prevent fungal growth. This reagent is used as the standard in the Harkness Viscometer to which unknown viscosities are compared. It is, therefore, important to insure the concentration is correct and the solution is particle free.

3. Hematocrit

The hematocrit was calculated by the Coulter Counter Model S electronic cell counter. The procedure used was that recommended in the "Instruction and Service Manual for the Model S Coulter Counter," 4th preliminary edition, Coulter Electronics, Hialeah, Florida 33010. Occasionally, when sample size was critically small, the hematocrit was performed using the microhematocrit centrifuge made by Phillips-Drucker Company, and read on the International Micro-Capillary Reader made by International Equipment Company. The procedure used was that recommended in International Equipment Company Bulletin C, 1957, "International Hematocrit."

4. Erythrocyte Sedimentation Rate (ESR)

The Westergren ESR procedure was used employing the Ulster Scientific Incorporated disposable Dispette system. The procedural reference was product enclosure entitled "Disposable Dispettes for ESR," Ulster Scientific Inc., Highland, New York.

5. Zeta Sedimentation Rate

The ZSR was performed using the Coulter Zetafuge following the procedure recommended in the product manual entitled "The Zeta Sedimentation Ratio" (Coulter product number 361-246), Curtin Matheson Scientific, Inc., 177 Andover Park West, Tukwilla, Washington 98188.

6. Sodium Determinations

Sodium measurements were performed on the Instrument Laboratories Inc., IL443 Flame Photometer as recommended in the product enclosure, "Instructions for IL443 Flame Photometer," Instrument Laboratories Inc., 113 Hartwell Avenue, Lexington, Massachusetts 02173. The 3.6% NaCl was diluted one part 3.6% NaCl with two parts purified water using one ml and two ml volumetric pipettes. This dilution is to bring the sodium ion concentration within the range normally measured by this instrument.

7. Chloride Determinations

The chloride ion concentrations were measured using a Radiometer, CMT 10 Chloride Titrator using the procedure recommended in "The Instruction Manual - CMT 10 Chloride Titrator," Radiometer A. S, Copenhagen NV, Denmark. The same dilution prepared for the sodium determination was also used here to lower the chloride ion concentration within the normal operational range of this instrument.

8. pH Determinations

The pH of plasma was determined using the Orion Research Instruments Analog pH meter, Model 301 by following the instructions provided in the instrument enclosure.

9. Plasma Viscosity

The "Instruction Manual for Coulter Harkness Viscometer" first

the adjustment knob on the face of the instrument. Following adjustments for the mercury, the 3.6% NaCl was removed and discarded from the specimen cup. As soon as air began to enter the capillary tube, a one ml aliquot of 3.6% NaCl was added to the specimen cup. This allowed a small air bubble to pass through the capillary.

After the bubble had passed through the capillary, the timing device was reset to zero and tap A then closed.

The mercury began to return to a resting position, allowed by flow of the solution in the capillary tube. When the mercury reached the first timing electrode, the timing device started recording. The timing period stopped when the mercury had reached the second electrode. This time period is referred to as the flow time. The flow times for five consecutive one ml aliquots of 3.6% NaCl were recorded. A reproducibility of $\pm$ 0.1 seconds was desired for the five determinations. If this was not obtained, the capillary tube was removed and cleaned with 40% sodium hydroxide following the recommended procedure on page 24 of the Instrument Manual.

When the desired reproducibility was obtained the five flow times were averaged. This average flow time for 3.6% NaCl is referred to as the calibration flow time.

The quality control materials were then analyzed following the same technique. However, duplicate flow times were recorded for each solution (not five). Each solution was separated from the previous solution by a small air bubble in the capillary. Following the quality control material, an aliquot of 3.6% NaCl

was used to rinse the capillary and sample cup. The results of the quality control determination were compared with results recorded on previous days to check for instrument drift and quality of the calibrating standard.

If no substantial changes were observed, the test plasmas were analysed and duplicate flow times recorded for each. A reproducibility of ± 0.5 seconds was desired for plasma specimens. If this was not obtained, the capillary was again cleaned and a calibration flow time recalculated. Each plasma specimen was followed by a 3.6% NaCl aliquot to rinse the capillary and to check for instrument variations.

Following the daily run, the instrument was cleaned using the recommended procedure in the Instrument Manual.

D. CALCULATIONS

1. Plasma Viscosity

The calibration flow time was calculated by averaging the five 3.6% NaCl flow times measured at the beginning of the run. The duplicate flow times for the test plasma specimens and quality control materials were then averaged.

An instrument calibration factor was then calculated using the following formula:

$$\text{Instrument Calibration Factor} = \frac{\text{Viscosity of Calibration Fluid (3.6\% NaCl)}}{\text{Calibration Flow Time}}$$

The instrument calibration factor was then multiplied by each of the averaged plasma and control material flow times to obtain their viscosities.

An example of a typical calculation follows:

Flow time of 3.6% NaCl: 23.86 seconds

Flow time of EDTA Plasma: 42.71 seconds

$$\text{Instrument Calibration Factor} = \frac{0.944 \text{ cp}}{23.86 \text{ sec.}} = 0.0396$$

Plasma viscosity = 42.71 seconds X 0.0396 = 1.69 cp.

2. Zeta Sedimentation Ratio

The ZSR is calculated by dividing the patient's hematocrit by the Zetacrit and multiplying by 100. The Zetacrit is a percentage expression of the degree of sedimentation following the vertical centrifugation. A typical example follows:

Zetacrit = 80%

Hematocrit = 40%

$$\text{ZSR} = \frac{\text{Hematocrit}}{\text{Zetacrit}} \text{ X } 100 = \frac{40}{80} \text{ X } 100 = 50\%$$

3. Statistics

The following statistical calculations were used to describe the experimental results

a. Measures of Central Tendency

The median and mean are used in this paper as measures of the central tendency. The median is defined as the middle score in a set of ordered scores. The mean ($\bar{X}$) is the sum of raw scores divided by the number of scores.

b. Measures of Variability

The measures of variability are used to describe how the data varies about its mean. The normal range is defined as the mean plus or minus two standard deviations (S. D.).

To calculate the the standard deviation the following formula was used:

$$S.D. = \sqrt{\frac{\Sigma X^2 - (\Sigma X)^2/N}{N}}$$

S.D. = standard deviation

X = the raw score

$\Sigma X$ = the sum of the raw scores

$\Sigma X^2$ = the sum of the squared raw scores

$(\Sigma X)^2$ = the sum of the raw scores squared

N = the number of scores.

The standard error of the mean is used to describe the variability of the data about the mean of the sampling distribution. The following formula was used to calculate the standard error of the mean.

$$S_{\overline{X}} = \sqrt{\frac{\Sigma X^2 - (\Sigma X)^2/N}{N(N-1)}}$$

$S_{\overline{X}}$ = standard error of the mean

$\Sigma X^2$ = the sum of the squared raw scores

$(\Sigma X)^2$ = the sum of the raw scores squared

N = the number of raw scores

The confidence interval is a band of scores which has a reasonable surety of encompassing the population mean. It was calculated by the following formula:

$$\overline{X} - zS_{\overline{X}} \leq \mu \leq \overline{X} + zS_{\overline{X}}$$

$\overline{X}$ = sample mean

$S_{\overline{X}}$ = standard error of the mean

$\mu$ = the population mean

z = a confidence value obtained from a standard normal curve table, i.e., 95% confidence value equals a Z of 1.96.

A coefficient of variation was used to denote inherent variabliity characteristic of the Harkness Viscometer as well as the reagents used in its operation. The following formula was used:

$$C.V. = \frac{S.D.}{\overline{X}}$$

C.V. = coefficient of variation

S.D. = standard deviation

$\overline{X}$ = the sample mean

c. Testing Hypotheses about Means

This statistic is used to determine whether the mean of a particular sample differs significantly from the population mean. For this statistic the null hypotheses states that the sample mean equals the population mean ($\overline{X} = \mu$) and the alternative hypothesis states that the sample mean does not equal the population mean ($\overline{X} \neq \mu$). The alpha level or probability level chosen was ($P \leq 0.05$). The level has a table $\underline{z}$ score of $\pm$ 1.96. The following formula is used to evaluate the hypotheses.

$$\underline{z} \text{ calculated} = \frac{\overline{X} - \mu}{S.D./\sqrt{N}}$$

The directive rule used states that if the $\underline{z}$ calculated is greater than the table value of $\underline{z}$ ($\pm$1.96) then the null hypotheses is rejected and the alternative hypotheses is accepted.

d. Correlation Coefficients.

Two correlation coefficients were used in this paper. The first is Pearson's r or Pearson's product moment correlation coefficient. This test is used to correlate two sets of variables which are linearly

related and have least interval scale data. The following formula was used:

$$r = \frac{N\Sigma XY - X(\Sigma Y)}{\sqrt{\{N\Sigma X^2 - (\Sigma X)^2\}\{N\Sigma Y^2 - (\Sigma Y)^2\}}}$$

X and Y are the two sets of variables.

The second correlation coefficient used was Spearman's rank order correlation coefficient or rho ($\rho$). This test correlates two sets of rank ordered data. The following formula was used:

$$\rho = 1 - \frac{6\Sigma D^2}{N(N^2-1)}$$

$\rho$ = Spearman's rank order correlation coefficient

D = the difference in a pair of ranks

e. Testing the Hypothesis about Correlations

For Pearson's r, I used the null hypotheses that $r = 0$ and an alternative hypotheses that $r \neq 0$. The alpha level chosen was 0.05 or $P \leq 0.05$) which has a table z score of $\pm$ 1.96. In this data, $N \geq 30$, so the following formula was used:

$$\underline{z} \text{ calculated} = r\sqrt{N-1}$$

The directive rule used stated that if the $\underline{z}$ calculated exceeds the table z score, the null hypotheses is rejected.

For Spearman's rank order correlation coefficient ($\rho$), the following formula was used:

$$t = \frac{\rho\sqrt{N-2}}{\sqrt{1 - \rho^2}}$$

Here, a t-test must be used to test the null hypothesis instead of the z statistic. The t-test table value for ($P \leq 0.05$) is $\pm$12.7. The same null and alternative hypotheses were used as in Pearson's r as well as the same directive rule.

f. Coefficient of Determination

This statistic is used to evaluate the predictability of Pearson's r. It is calculated simply by squaring r. If the $r^2$ value is greater than 0.50, then the correlation is useful, if linear regression analysis is being considered.

4. Concentration Tests for the 3.6% NaCl Solution

The following formula was used to calculate the correct sodium and chloride ion concentrations for the 3.6% NaCl solution:

$$\text{Correction concentration (milliequivalents/liter)} = \frac{\text{milligram/100 ml X 10}}{\frac{\text{gram molecular weight}}{\text{valence of the ion}}}$$

E. QUALITY CONTROL MATERIALS STUDY

The following reagents were studied as potential quality control materials.

1. Purified water

Purified water obtained from a Culligan Reverse Osmosis System was used as a viscosity control reagent. The viscosity of water at $25^{\circ}$ C is 0.8904 cp.*

2. Pooled EDTA Plasma

100 ml of pooled EDTA plasma was obtained by pooling all EDTA plasma from the routine hematology laboratory specimens at the end of the day. The plasma was mixed and dispensed in 2 ml aliquots and frozen. Before using, the pooled plasma was warmed to room temperature and mixed and recentrifuged to remove any precipitated matter.

3. Sucrose Solutions

Sucrose Solutions were prepared in approximate concentrations of 10, 20, 30, and 40% (grams/100 ml distilled water.) The solutions were dispensed in 2 ml aliquots and frozen. The solutions were warmed to room temperature and mixed by inversion before usage.

*Handbook of Chemistry and Physics, 52nd ed., Chemical Rubber Co., Cleveland, Ohio, 1971, p. f-36.

F. SPECIMEN PREPARATION AND STORAGE

Harkness discusses the effects of anticoagulants on plasma viscosity and recommends that EDTA, in concentrations of 1-2 milligrams (mg) per ml of whole blood, be used (12). The majority of blood collected for the routine hematology laboratory is collected in Vacutainers containing 1.5 mg of EDTA per one ml of whole blood. In this light, we elected to follow the recommendations of Harkness for the anticoagulant.

Harkness, in the same article, discusses the preparation and storage of specimens (12). A study was designed to investigate which of several methods would best suit our needs. A 300 ml quantity of whole blood was collected from a healthy volunteer by using a Fenwall EDTA Platelet-Pack (Code number JP-1) containing 30 ml of an EDTA solution, equivalent to 1.5 mg of EDTA per ml of whole blood (this product is no longer commercially available), by standard blood collection technique. The anticoagulated blood was dispensed in 3 ml aliquots and stored in Vacutainer tubes containing no further anticoagulant. The specimens were divided into eleven categories containing seven tubes each. The eleven categories are as follows: 1.) whole blood stored at room temperature; 2.) whole blood stored at $4^{o}$ C; 3.) whole blood stored at room temperature and centrifuged without stoppers; 5.) whole blood stored at room temperature with one drop of 1% azide in 0.9% sodium chloride solution added; 6.) whole blood stored at $4^{o}$ C with one drop of a 1% sodium azide in 0.9% sodium chloride solution added; 7.) whole blood mixed to resuspend red cells every 24 hours; 8.) EDTA plasma stored at room temperature; 9.) EDTA plasma stored at $4^{o}$ C; 10.) EDTA plasma stored at room temperature with 1 drop of a 1% sodium azide in 0.9% sodium chloride solution added; and 11.) EDTA plasma stored at $4^{o}$ C with one drop of a 1%

sodium azide in 0.9% sodium chloride solution added. One aliquot from each category was selected each day and analyzed for plasma viscosity. This study continued for seven days. The specimens stored at $4^{o}$ C were allowed to reach room temperature prior to testing. The plasma specimens were mixed and recentrifuged to remove precipitated material before testing.

The variables of refrigeration and the addition of sodium azide were to control potential contamination of specimens with microorganisms. Centrifugation in unstoppered tubes was added to investigate evaporation effects due to centrifugation. The mixing variable was added to evaluate the effect of mailing whole blood specimens. This was done in response to requests for plasma viscosity determinations received from other laboratories.

G. INSTRUMENT WARM-UP STUDY

To evaluate how long the Harkness Viscometer requires to stabilize at $25^{o}$ C, the following experiment was designed. EDTA plasma was added to the specimen cup of the instrument prior to turning the switch on. The instrument was then turned on and the flow time of the pooled plasma recorded. Immediately, the plasma was replaced with 3.6% sodium chloride and the flow time for it recorded. Alternating plasma and 3.6% NaCl flow times were recorded every 2-3 minutes for a total of 30 minutes. The temperature of the water bath was recorded every two minutes.

H. INSTRUMENT REPRODUCIBILITY STUDY

To evaluate the reproducibility of the Harkness Viscometer, 25 consecutive flow times on both pooled EDTA plasma and 3.6% NaCl were measured during a one day period. The mean, standard deviation, and coefficient of variation were calculated for each reagent.

I. pH STUDY

To evaluate the effects pH might have on plasma viscosity, the following study was used. Pooled EDTA plasma was dispensed in 2 ml aliquots into seven different tubes. The following amounts of 0.1 Normal (N) sodium hydroxide, 0.1 N hydrochloric acid and purified water were added to the 2 ml aliquots using a Pasteur pipette: 1.) 12 drops of 0.1 N sodium hydroxide; 2.) 8 drops of 0.1 N sodium hydroxide and 4 drops of water; 3.) 4 drops of 0.1 N sodium hydroxide and 8 drops of water; 4.) 12 drops of water; 5.) 4 drops of 0.1 N hydrochloric acid and 8 drops wtaer; 6.) 8 drops of 0.1 N hydrochloric acid and 4 drops water; and 7.) 12 drops of hydrochloric acid. The tubes were mixed by inversion and the pH of the plasma in each tube determined. The plasma viscosity of each tube was then measured. A graph was then constructed from the data.

J. SUBJECTS FOR THE NORMAL POPULATION STUDY

Individuals were selected for the normal population study at random from the blood donor population, seen during a three day period at the American Red Cross Blood Center; 4200 S. W. Corbett, Portland, Oregon. Five ml Vacutainer tubes (Becton-Dickenson) containing 1.5 mg of EDTA per ml of whole blood were used for specimen collection. The donor population of the American Red Cross is selected using the criterion set down by the American Association of Blood Banks (AABB).

The mean, standard deviation, standard error of the mean, normal range and confidence interval were calculated on the resulting data. Statistical evaluation of this population by age and sex was also conducted.

## K. SUBJECTS AND METHODS FOR THE CLINICAL CORRELATION STUDY

Patients for the clinical correlation study were obtained by their physicians ordering an ESR from the routine hematology laboratory. The phlebotomy team was instructed to draw two 5 ml EDTA Vacutainers (Becton-Dickinson) on all patients being drawn for ESR determinations. One tube was submitted to the routine hematology laboratory for ESR and hematocrit determinations. The other tube was for plasma viscosity and ZSR determinations. The patient's hematocrit, ESR and plasma viscosity results were recorded, as well as the patient's name, birthdate and hospital number. a scatterplot of 700 determinations using ESR and plasma viscosity was prepared. From the identification data, the patient's medical records were then requested.

Most of the requests for ESR tests came from four different clinics and examples of the types of patients tested from each clinic, follows.

| Clinic | Patients |
|---|---|
| Immunology | Systemic Lupus Erythematosis, Acute and Chronic Rheumatoid Arthritis Collagen-Vascular disorders and Auto-Immune disorders. |
| Obstetrics-Gynecology | Acute and Chronic Pelvic Inflamatory Diseases |
| Hematology | Lymphomas and Paraproteinemias |
| General and Orthopedic Surgery | Acute and Chronic Inflamatory Disorders and Post-Operative cases |

The medical records were given to Dr. Kirt V. Sheth or Dr. Vasant Joshi of the University of Oregon Health Sciences Center, Department of Clinical Pathology, for evaluation of the patients's clinical status.

It was felt that the clinical impressions of the patients could be

divided into four broad categories of rank order to facilitate analysis of the clinical data. The following is a list of the ranks and an example of medical histories characteristic of each rank.

| Rank | History |
|---|---|
| 0 | Clinically, no fever, edema or swelling. No microbiological culture studies ordered. Final diagnosis and treatment not suggestive of any tissue destruction, i.e., Psychosomatic or Psychological disorders. |
| 1 | Clinically, pain swelling or constitutional symptoms of a moderate degree. Analgesic, antibiotic or chemotherapy given. Further follow up showing no acute inflammation, i.e., Rheumatoid Arthritis, Chronic Obstructive Pulmonary Disease, Osteomyelitis, Chronic Cholecystitis or quiescent phase of Systemic Lupus Erythematosis. |
| 2 | Clinically, the presence of an acute inflammatory process and/or tissue destruction. Antibiotics and/or analgesics given. Positive microbiological cultures., i.e.,Acute Pelvic Inflammatory Disease, resolving pneumonia, Active Systemic Lupus Erythematosis and Active Rheumatoid Arthritis. |
| 3 | Clinically, fever, increased pulse rate, septicemia (by positive microbiological cultures) and/or extensive tissue trauma, i.e., 3-4 days post-operative for major surgery, Acute phase of pneumonia, Acute Cholecystitis, and Massive trauma of auto accidents. |

The preceding rank order system was designed to categorize clinical abnormalities in order of their effect on plasma proteins. The rank order system was developed by Dr. K. V. Sheth and myself after searching the literature for a precedent. However, none could be found. After studying the medical record, the patient's clinical status was assigned to a rank without knowledge of the plasma viscosity or ESR results. 121 individual results were removed from the study population because of unavailable medical records or incomplete clinical information.

The remaining 579 results comprised the basis of the clinical correlation study.

The following rank order system for the plasma viscosity and ESR results was designed.

| Plasma Viscosity | ESR |
|---|---|
| 0 = 1.47 - 1.76 centipoise (cp) | 0 = 0-20 mm/hour |
| 1 = 1.77 - 1.87 cp | 1 = 21-50 mm/hour |
| 2 = 1.88 - 2.00 cp | 2 = 51-80 mm/hour |
| 3 = 2.01 and greater cp | 3 = 81 and greater mm/hour |

The ranks were arbitrarily developed based on reports in the literature comparing the degree of abnormality with the test results (2, 12).

A subpopulation of 46 patients was chosen at random from the clinical study. ZSR, ESR, plasma viscosity and hematocrits were determined on this subpopulation for correlation statistics. This was done to evaluate the nature of the correlations for the total study population. Following this study, the total group of 579 patients was evaluated for Pearson's r and Spearman's rank order correlation coefficients using the ESR and plasma viscosity results.

The total group (N=579) was screened for anemia and erythrocytosis using the criterion of a normal hematocrit (39.6 $\pm$ 3.2%).* Pearson's r was then calculated on this normal hematocrit group (N=327).

The statistics were then evaluated for significance using the appropriate calculation.

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*Normal hematocrit values were calculated by the University of Oregon Health Sciences Center, Department of Clinical Pathology, Clinical Hematology Laboratory, using blood obtained from 200 American Red Cross Blood donors, age 17-75 years in May 1976.

# RESULTS

## A. INSTRUMENT WARM-UP STUDY

The time necessary for the Harkness Viscometer to reach a stable temperature of 25° C can be seen on graph number 1, page 57 . The graph shows the flow times of pooled EDTA plasma and 3.6% NaCl in seconds without converstion to centipose units.

The instrument water bath was capable of reaching 25° C in approximately 10 minutes. The flow times of pooled EDTA plasma and 3.6% NaCl show an inverse relationship to the increasing temperature during the initial 10 minute period. The temperature of the water bath was stable at 25° C following the 10 minute warm-up period. The flow times of the pooled EDTA plasma and 3.6% NaCl appear to stabilize at approximately 20 minutes.

## B. INSTRUMENT REPRODUCIBILITY STUDY

The results of the reproducibility study are summarized in table 1.

TABLE 1

REPRODUCIBILITY* STUDY OF THE COULTER HARKNESS VISCOMETER

University of Oregon Heatlh Sciences Center
Portland, Oregon 1977

| | Mean | Std. Deviation | Coef. Variation |
|---|---|---|---|
| 3.6% Sodium Chloride | 23.47 sec | 0.04 sec | 0.17% |
| Plasma (Pooled EDTA) | 43.34 sec | 0.29 sec | 0.65% |
| | 1.78 cp | 0.01 cp | 0.56% |

*Calculated on 25 repeat determinations.

This table shows the mean, standard deviation and coefficient of variation for both 3.6% NaCl and pooled EDTA plasma. These statistics were calculated from 25 consecutive repeat determinations on each solution. The data shows a reproducibility of $\pm$0.01 cp for the pooled EDTA plasma. The coefficients of variation for both 3.6% NaCl and pooled EDTA plasma were less than 0.7%

C. SPECIMEN PREPARATION AND STORAGE

The data collected for the specimen preparation and storage study may be seen on graph number 2, page 59, and table number 2, page 37.

Graph number 2 shows the eleven categories of specimens tested. Each category was tested daily for a seven day period. The plasma viscosities were plotted for each category and day. Viscosities in the range of 1.41 - 1.46 cp were encountered. (See page 59.)

Table 2 shows the plasma viscosity fluctuation ranges for each category over the seven day period. Whole blood mixed daily shows a fluctuation range of 0.05 cp. The fluctuation ranges for the other ten categories were within 0.03 $\pm$ 0.01 cp.

During this study no macroscopic contamination of microorganisms was noted in any of the test specimens. Centrifugation without stoppers did not show an appreciable evaporation effect. Mixing whole blood daily had the most pronounced effect on plasma viscosity in this study.

TABLE 2

SPECIMEN PREPARATION AND STORAGE STUDY FOR PLASMA VISCOSITY DETERMINATIONS ON THE COULTER HARKNESS VISCOMETER

University of Oregon Health Sciences Center
Portland, Oregon 1977

SPECIMEN PREPARATION AND STORAGE

| Type of Specimen | Range |
|---|---|
| Whole blood at room temperature | 0.03 cp (1.41 - 1.44) |
| Whole blood at 4° C | 0.04 cp (1.41 - 1.45) |
| Whole blood at room temperature centrifuged opened | 0.04 cp (1.41 - 1.45) |
| Whole blood at 4° C centrifuged opened | 0.04 cp (1.41 - 1.45) |
| Whole blood with azide at room temperature | 0.03 cp (1.41 - 1.44) |
| Whole blood with azide at 4° C | 0.02 cp (1.41 - 1.43) |
| Whole blood mixed daily | 0.05 cp (1.41 - 1.46) |
| Plasma (EDTA) at room temperature | 0.04 cp (1.41 - 1.45) |
| Plasma (EDTA) at 4° C | 0.03 cp (1.41 - 1.45) |
| Plasma (EDTA) with azide at room temperature | 0.02 cp (1.41 - 1.43) |
| Plasma (EDTA) with azide at 4° C | 0.02 cp (1.41 - 1.43) |

D. pH STUDY

The results of the pH study may be seen on graph number 3, page 61. The graph shows a decreasing viscosity of 1.71 cp to 1.67 cp (0.04 cp) in the range of pH 7.2 to pH 7.8. With an instrument reproducibility of ±0.01 cp, the graph probably represents a monotonic decrease in viscosity from pH 6.8 to pH 8.0

E. CONCENTRATION TEST ON 3.6% SODIUM CHLORIDE SOLUTION

The calculated value for the correct concentration of the sodium and chloride ions in a dilution of one part 3.6% NaCl with two parts purified water was 205 milliequivalents per liter (meq/l). The measured values on the five 18 liter lots of 3.6% NaCl solution prepared may be seen on table number 3.

TABLE 3

CONCENTRATION TESTS ON THE 3.6% SODIUM CHLORIDE SOLUTION

FOR THE COULTER HARKNESS VISCOMETER

Univeristy of Oregon Health Sciences Center
Portland, Oregon 1977

| Date | Sodium ion* | Chloride ion* |
|---|---|---|
| 1/3/76 | 205 meq/1 | 204 meq/1 |
| 5/27/76 | 204 meq/1 | 205 meq/1 |
| 6/3/76 | 205 meq/1 | 202 meq/1 |
| 10/15/76 | 206 meq/1 | 203 meq/1 |
| 3/30/77 | 206 meq/1 | 202 meq/1 |

*Results were obtained on a dilution of one part sodium chloride solution with two parts purified water.

Sodium ion concentrations of 204-206 meq/1 and chloride ion concentrations of 202-205 meq/1 were obtained. These results were measured in a dilution of one part 3.6% NaCl to two parts purified water using volumetric pipettes to prepare the dilutions.

F. QUALITY CONTROL MATERIALS STUDY

The data and associated statistics obtained on the quality control materials may be seen on table number 4.

TABLE 4

QUALITY CONTROL MATERIAL STUDY

FOR THE COULTER HARKNESS VISCOMETER

University of Oregon Health Sciences Center
Portland, Oregon 1977

| | N | Mean | Std. Deviation | Coef. Variation |
|---|---|---|---|---|
| Dist. $H_2O$ | 30 | 0.897 cp | 0.009 | 1% |
| Approx. 10% sucrose | 14 | 1.174 cp | 0.006 | 1% |
| Approx. 20% sucrose | 15 | 1.616 cp | 0.011 | 1% |
| Approx. 30% sucrose | 14 | 2.353 cp | 0.016 | 1% |
| Approx. 40% sucrose | 14 | 3.669 cp | 0.034 | 1% |
| Pooled frozen plasma | 14 | 1.767 cp | 0.030 | 4% |

The number of determinations, the mean, standard deviations and coefficients of variation are shown for each reagent. The tested viscosity of purified water was 0.897 cp, the published viscosity of purified water at $25^{\circ}$ C is 0.8904 cp as previously stated. The

viscosities of the sucrose solutions range from 1.174 to 3.669 cp. The viscosity of the pooled frozen plasma was 1.767 cp. The coefficients of variation for purified water and the sucrose solution were 1% and for pooled frozen plasma 4%.

G. NORMAL POPULATION STUDY

The results of the normal population study may be seen on graph number 4, page 63. The study was based on 254 individuals from the American Red Cross Blood Center donor population in Portland, Oregon

The 95% confidence interval for the population mean ($\mu$) of this data is 1.61 - 1.63 centipoise units. The mean for the normal population was 1.62 cp. A slight negative skewness can be seen in the distribution (median is 1.60 cp and mean is 1.62 cp). The normal range (mean $\pm$ 2 Standard Deviation) is 1.48 - 1.76 cp.

By subdividing the normal population by age and sex, the statistics on table number 5, page 41, were seen.

The tests of the hypotheses about the means for the subdivisions by age and sex did not differ significantly ($P \leq 0.05$) from the population mean ($\mu$).

TABLE 5

PLASMA VISCOSITY NORMAL POPULATION SUBDIVISION BY AGE AND SEX

University of Oregon Heath Sciences Center
Portland, Oregon 1977

| Age (in years) | Sex | Plasma Viscosity Mean Value (cp units) | 1 S. D. |
|---|---|---|---|
| less than 20 | M (3) | 1.61 | 0.06 |
| | F (4) | 1.59 | 0.02 |
| 20-29 | M (42) | 1.61 | 0.06 |
| | F (44) | 1.60 | 0.08 |
| 30-39 | M (42) | 1.62 | 0.07 |
| | F (24) | 1.63 | 0.10 |
| 40-49 | M (35) | 1.62 | 0.07 |
| | F (15) | 1.59 | 0.08 |
| 50-59 | M (23) | 1.62 | 0.08 |
| | F (12) | 1.64 | 0.06 |
| 60-69 | M (5) | 1.67 | 0.03 |
| | F (5) | 1.62 | 0.11 |

M = male
F = female
(N) = number of raw scores
S.D. = Standard Deviation

H. CLINICAL CORRELATION STUDY

The clinical study was composed of two parts. The first consisted of a small subpopulation (46 patients, and the second study was performed on the larger population (579 patients).

The results of the small subpopulation study may be seen on table 6.

TABLE 6

SUBPOPULATION CORRLEATION COEFFICIENTS OF THE

CLINICAL CORRELATION STUDY ON PLASMA VISCOSITY DETERMINATION

Univeristy of Oregon Health Sciences Center
Portland, Oregon 1977

Erythrocyte Sedimentation Rate to Zeta Sedimentation Ratio:
$r = 0.798$

Plasma Viscosity to non anemic* Erythrocyte Sedimentation:
$r = 0.759$

Plasma Viscosity to Erythrocyte Sedimentation Rate:
$r = 0.718$

Plasma Viscosity to Zeta Sedimentation Ratio:
$r = 0.658$

Plasma Viscosity to Hematocrit:
$r = 0.068$

*Non anemic is used to denote a normal hematocrit ($39.6 \pm 3.2\%$)

Tests of the hypotheses about the correlation coefficients showed all correlation coefficients listed as being statistically ($P \leq 0.05$) different from zero. The correlations are arranged in descending order with the highest correlation seen with ESR to ZSR ($r = 0.798$) and the least correlation with Plasma Viscosity to Hematocrit ($r = 0.068$).

For the second portion of this study, a scatter plot of the raw data may be seen on graph number 5, page 65. This scatter plot contains three different symbols (·, x, and ■). These symbols were used to denote the number of data coordinates that fell on a particular point. The symbol (·) represents a single plot of an ESR and Plasma Viscosity determination on a patient specimen. The (x) symbolized two different patient specimens or two repeat patient specimens which had identical ESR and Plasma Viscosity values. The (■) represents three different patient specimens or three repeat patient specimens which had identical ESR and Plasma Viscosity values. From this scatter plot some degree of linearity was felt to exist.

The correlation statistics for the study population can be seen on table 7. Tests of the hypotheses about the correlations showed the results differed statistically ($P \leq 0.05$) from zero. This was true for both Pearson's r and Spearman's rank order correlation coefficients.

Pearson's r correlation studies show a higher degree of correlation of non-anemic ERS to Plasma Viscosity ($r = 0.76$)) than with the total study population ESR to Plasma Viscosity ($r = 0.71$). The coefficients of determination were both higher than $r^2 = 0.50$. This represents the minimal required correlation if linear regression analysis is being considered. Further linear regression analysis was not performed on this data.

Spearman's Rank Order Correlation Coefficients used for the clinic data analysis were different for the two systems. The correlation for plasma viscosity to clinical impression was ($\rho = 0.626$) higher than the correlation of ESR to the clinical impression ($\rho = 0.587$). (Refer to table 7, page 44.)

TABLE 7

CLINICAL CORRELATION STUDY OF THE PLASMA VISCOSITY DETERMINATION AND ERYTHROCYTE SEDIMENTATION RATES

University of Oregon Health Sciences Center
Portland, Oregon 1977

Pearson's r Correlation Coefficient

Non anemic ESR* to Plasma Viscosity: $r = 0.76$ (N=327)

ESR to Plasma Viscosity: $r = 0.71$ (N=579)

Coefficients of Determination

Non anemic ESR* to Plasma Viscosity: $r^2 = 0.58$

ESR to Plasma Viscosity: $r^2 = 0.51$

Spearman's Rank Order Correlation Coefficient: (N = 579)

Plasma Viscosity to Clinical Evaluation: $\rho = 0.626$

ESR to Clinical Evaluation: $\rho = 0.587$

*Non anemic patients were defined as having hematocrits in the range of $39.6 \pm 3.2\%$.

Table 8 shows the frequency distribution of the ranks for both systems studied.

TABLE 8

FREQUENCY DISTRIBUTION OF RANKS FOR SPEARMAN'S RANK ORDER CORRELATION COEFFICIENT (ρ) STUDY

University of Oregon Health Sciences Center
Portland, Oregon 1977

CLINICAL IMPRESSION RANKS

| Plasma Viscosity Ranks | 0 | 1 | 2 | 3 |
|---|---|---|---|---|
| 0 | 216 | 103 | 6 | 0 |
| 1 | 40 | 78 | 6 | 0 |
| 2 | 3 | 66 | 9 | 0 |
| 3 | 3 | 33 | 15 | 1 |

N=579
ρ=0.626

CLINICAL IMPRESSION RANKS

| ESR Ranks | 0 | 1 | 2 | 3 |
|---|---|---|---|---|
| 0 | 188 | 77 | 3 | 0 |
| 1 | 55 | 95 | 11 | 0 |
| 2 | 12 | 72 | 10 | 0 |
| 3 | 7 | 37 | 11 | 1 |

N=579
ρ=0.587

This table shows the frequency of each pair of ranks for both systems studied. The main frequency for each column was then used to score the ranks and calculate the correlation coefficients (ρ).

# DISCUSSION

## A. INSTRUMENT WARM-UP STUDY

The importance of temperature control in rheological studies has been strongly stressed. Harkness has shown that changes of $1^{o}$ C can result in 2 to 3% error in plasma viscosity measurements (12). The Coulter Harkness Viscometer operates at $25^{o}$ C $\pm$ $0.05^{o}$ C. This temperature is slightly above the ambient temperature of most laboratories. The ambient temperature of our laboratory is generally between 20 to $22^{o}$ C. The small increase in temperature required to reach $25^{o}$ C can be accomplished with little energy input. It was for this reason that Harkness chose $25^{o}$ C as the operational temperature for this viscometer. If the ambient temperature should exceed $25^{o}$ C, a cooling mechanism would be required for temperature control.

Harkness nor the Instrument Manual chose to give the time element necessary for the viscometer to stabilize at $25^{o}$ C. This choice may have been based on the variety of ambient temperatures which exist for different laboratories. In our laboratory setting, the water bath temperature reached $25^{o}$ C in about ten minutes but the flow times of test fluids stabilized at twenty minutes: this data may be seen on graph #1, page 57. The most probable reason for this ten minute lag period is thermal equilibration of the glassware with the water bath. Once at $25^{o}$ C the temperature of the water bath was very stable. It must be stressed that following the twenty minute warm-up period the temperature of the water bath must be checked. If the temperature is not correct, adjustments are required.

This initial warm-up period has the advantage of allowing the tech-

nologist time to organize the daily work. Such things as centrifugation of specimens, organization of a work sheet, and maintenance procedures can be performed during this time.

Our experience did not include measurements of plasma viscosity during the night shift operation of the laboratory. Most requests for plasma viscosity were made during the day shift and performed during the day shift. The instrument was switched off at the end of the day shift to conserve electrical energy as well as to help control instrument ware.

B. REPRODUCIBILITY STUDY

Part of a clinical laboratory's responsibility is to provide the clinical staff with a range of results considered normal and abnormal for each analysis it performs. The point at which one range divides the other is difficult to define. A factor which plays a key role in this discussion is the degree of error associated with the instrument used in the analysis. Harkness suggests that the Coulter Harkness Viscometer has an instrument error of 1% or less. This degree of error is consistant with a reproducibility of $\pm$ 0.01 cp (12). Eastham and Morgan (8) studied this variable using normal plasma (1.695 cp) and verified the earlier claims of Harkness.

In this thesis the reproducibility of the Harkness Viscometer was studied using slightly abnormal plasma (1.78 cp). The findings of this study as shown in table 1, page 35, are identical to those previously published by both Harkness (12) and the Eastham and Morgan study (8).

C. SPECIMEN PREPARATION AND STORAGE

Harkness discusses the usage of various anticoagulants in measuring plasma viscosity. He points out that no anticoagulant would be ideal

but, due to clotting and removal of fibrinogen, an anticoagulant is needed. He recommends the salts of EDTA in concentrations of 1 to 2 mg/ml of whole blood. This anticoagulant yields plasma viscosity values closest to those of plasma containing no anticoagulant (12). This recommendation was accepted for this thesis. The majority of specimens collected for the clinical hematology laboratory are collected in EDTA (1.5 mg/ml of whole blood). This situation permitted the plasma viscosity to be added to a battery of tests ordered on one specimen of blood.

Harkness states that plasma viscosity specimens collected in EDTA are stable for seven days. The data here supports this claim, as seen in table 2, page 37, if care is taken not to resuspend settled red blood cells of whole blood specimens. This has greatest significance when mailing of specimens is being considered. It was felt that plasma specimens stored in sterile containers were best for mailing. Sterility of specimen tubes was added due to experiences with contamination. On two occasions, prior to this study of specimen storage, contamination of plasma with fungal organisms was seen. As a result, refrigeration and sodium azide were added as variables in an attempt to control this problem during the study. However, no contamination was observed in any specimens of this study. This may be related to using Vacutainer tubes for specimen storage. The manufacture of Vacutainers (Becton-Dickenson) does not guarantee the sterility of the tubes but sufficient sterility may have been present in the tubes. Refrigeration is not recommended to control contamination due to potential loss of cryoprecipatable proteins.

The last variable studied was centrifugation without stoppers. Harkness claims this may be a source of error due to evaporation effects (12). The data of this study did not substantiate this claim. However, the ease

of centrifuging Vacutainers with stoppers in place warrants conformity to Harkness's recommendation.

D. pH STUDY

Changes in pH of protein solutions will change the viscosity of the solution (17). These changes are the result of complex mechanisms. The most likely vehicle for this change is associated with charged groups on protein molecules. Changes in charged groups may alter the structure of a protein which in turn may influence molecular flexibility (17, 27). In this study pH changes of 6.8 to 8.0 resulted in a decrease of 0.04 cp in the plasma viscosity. This can be seen on graph #3, page 61. Changes in plasma pH may alter the viscosity of plasma without change in protein concentration. The clinical implications of plasma viscosity changes in the range of 0.04 cp or less should be viewed in light of the acid-base states of the patient.

Harkness does not discuss pH as a variable affecting plasma viscosity (11, 12). He does say, however, that changes of 0.05 cp and greater be considered clinically significant. The effect of pH on plasma viscosity becomes less significant when considering only changes of 0.05 cp and greater but should not be considered negated.

E. CONCENTRATION TEST FOR 3.6% NaCl

Preparation of large lots (18 liters) of the 3.6% NaCl standard was desirable due to the filtration process used in the study. Sodium and chloride ion measurements were used to detect error in the preparation of this reagent. From the results, seen in table 3, page 38, it was felt that adequate reagent preparation technique had been used.

F. QUALITY CONTROL MATERIALS STUDY

Control materials for the ZSR and ESR procedures are not yet available.

This is generally felt to be related to inadequate methods for preservation of red cells. This situation does not affect control materials for plasma viscosity measurements.

Several materials were evaluated as potential control materials. The results of this study are summarized in table #4, page 39. It was found that purified water obtained from a Culligan Reverse Osmosis System served well as a reference control of the 3.6% NaCl standard. Sucrose solutions in various concentrations were well suited to observe viscosities covering the ranges of normal and abnormal. The concentrations of the sucrose solutions were not exactly determined for this study because they were being studied as controls and not standards. This is not to imply that exact concentrations could not be used. Exact concentrations would provide for a duality of function, one to serve as a reference controls for the standard and secondly to serve as controls for instrument drift.

Care was taken during this study to maintain the performance level of the instrument as high as possible. This was done to evaluate the reproducibility of these reagents. The low coefficients of variation suggest the reproducibility of the sucrose solutions is excellent. Therefore, when changes in the viscosity of these reagents occur, instrument drift should be investigated.

Frozen pooled plasma had a larger coefficient of variation which may reflect the loss of cryoprecipitable materials. It was therefore felt that the sucrose solutions were preferred as control materials.

G. NORMAL POPULATION STUDY

The normal population studies of Harnkess were conducted in England. His data shows a mean of 1.64 and a standard deviation of 0.052 cp (12). Harkness gives his normal range as 1.50 - 1.72 cp but does not state his

criterion for a normal range. In this thesis a normal range is defined as the mean ± 2 standard deviation. The statistics for this study can be seen on graph #4, page 63. The normal range established from this data is 1.48 - 1.76 cp. The mean is 1.62 and the standard deviation is 0.07 cp. It is felt that this data correlates well with that published by Harkness.

Breakdown of the data by age and sex did not reveal any significant variation from the population mean. A single normal range then applies to the adult population. This is in contrast to the normal range for the ESR procedure which is influenced by sex.

H. CLINICAL CORRELATION STUDY

The ESR is generally accepted as being an index of health or disease. Multiple factors have been shown to influence ESR values. Plasma proteins are one of these factors which have clinical signifance (10). These same proteins have also been shown to influence plasma viscosity (10, 27). These tests are affected by proteins not only due to concentration but also the interaction of one protein with another (17). Quantitative protein studies have not been capable of supplying information about the nature of these interactions. Therefore no quantitative measurement as of yet will serve as a reference method for both procedures.

The first portion of this study attempts to demonstrate a correlation of factors affecting both the ESR and plasma viscosity. A study population was collected and a subpopulation extracted and analysed using Pearson's r. Highest correlation was found between ZSR and ESR results (r = 0.798). This may be related to red cell aggregation being the indicator system for both tests. Correlation of plasma viscosity to ESR was quite good (r = 0.718)

but improved when hematocrit was restricted to a narrow range ($r = 0.759$). This is a reflection of hematocrit influence on ESR as stated previously. ZSR and plasma viscosity correlations were somewhat lower ($r = 0.658$). The exact nature of this observation was not investigated since the main thrust of this thesis was to compare plasma viscosity to ESR. The correlation of hematocrit ot plasma viscosity was essentially zero ($r = 0.068$) which substantiates the findings of Harkness (12). Pearson's r was also calculated for plasma viscosity and ESR in the total study population. The results were identical to those of the subpopulation statistics. The overall conclusion of these correlations was that a relationship of the factors affecting both tests appears to be somewhat linear and in a positive direction.

Clinical impression data was then correlated with the ESR and plasma viscosity results by using Spearman's Rank Order Correlation Coefficient. Better correlation ($P \leq 0.05$) was seen between plasma viscosity and the clincial impression ($\rho = 0.626$) than was seen for ESR and clinical impression ($\rho = 0.587$). However it must be stated that both of these correlation values are lower than ideal. The low nature of the values is no doubt related to the rank order system used. It is very difficult to form a system which will assign numerical value to the clinical impressions extracted from medical records. The system used here provided pleasing statistics but other systems may prove to be more meaningful. The data obtained in this study does however, reflect the opinions of Harkness (12) as well as those of Eastham and Morgan (10). Their opinion is that plasma viscosity is a superior test, for clinical application, than is the ESR.

# SUMMARY AND CONCLUSIONS

I feel that the Harkness Viscometer has a place in the clinical laboratory setting. It has been shown that plasma viscosity is an excellent screening test for small changes in plasma proteins, as seen in tissue destructive processes.

The technical error of the instrument is less than 1%. Specimen requirements are minimal (1 ml of EDTA plasma). Simple preparation techniques and a common anticoagulant are advantages of this procedure. The specimens, when stored at room temperature, are stable for one week; therefore, small hospitals, clinics and laboratories would easily be able to mail specimens. Quality control programs can be inexpensively implemented, not only on a local level, but on a national level as well. The normal range of plasma viscosity is narrow, the same for both sexes, and independent of age in the adult population studied.

The clincial information obtained from plasma viscosity determinations using the Harkness Viscometer is at least equally applicable as the information obtained from the Westergren ESR in the setting of this study, Correlation studies support the concept that protein factors affecting plasma viscosity are the same factors affecting the Westergren ESR.

The plasma viscosity results obtained from the Coulter Harkness Viscometer were technically superior and clinically better than those obtained by the Westergren Erythrocyte Sedimentation Rate method.

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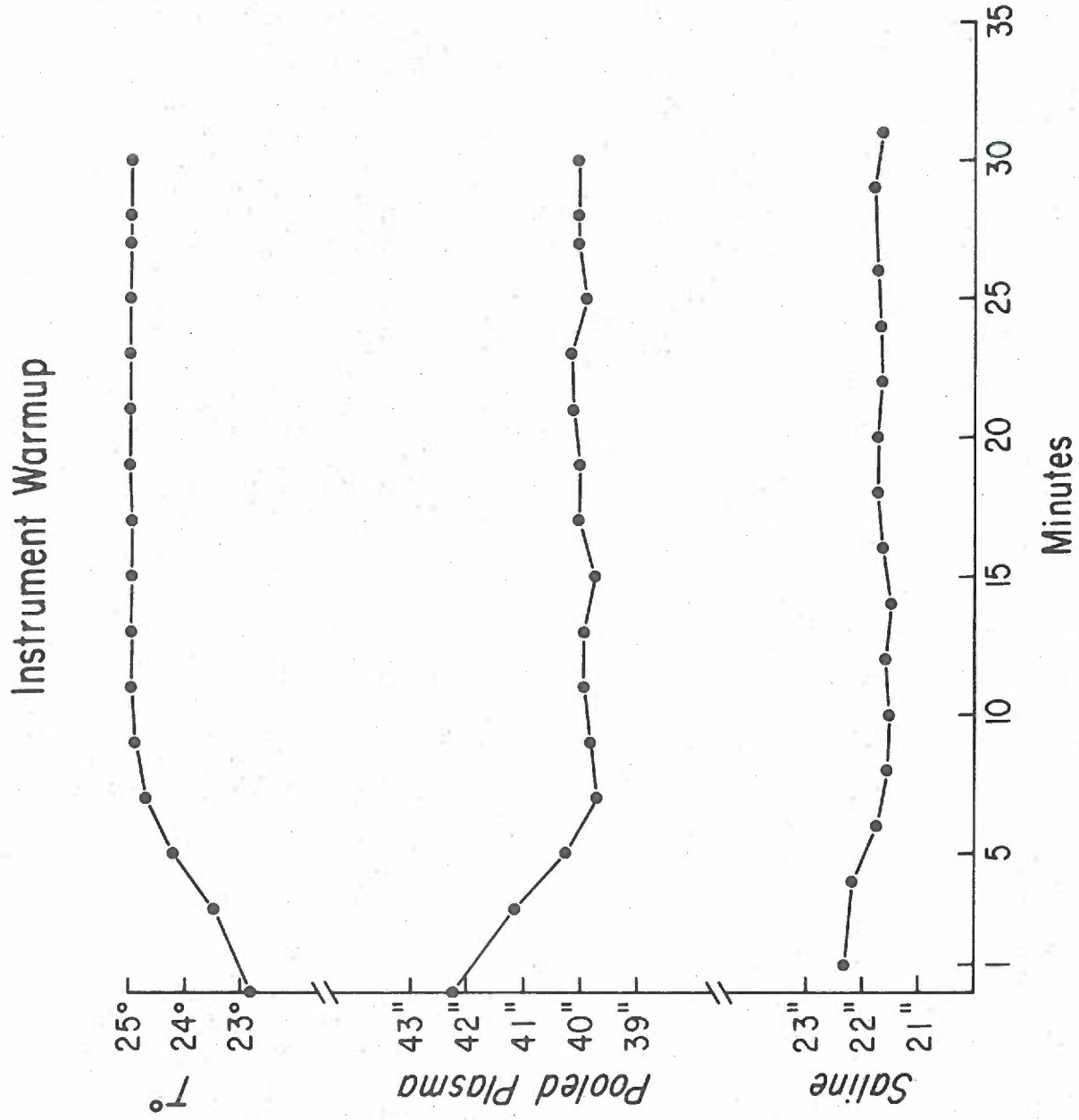
GRAPH #1

Instrument warmup study showing change in temperature ($T^{o}$), Pooled EDTA plasma viscosity (Pooled Plasma) in seconds, and 3.6% Sodium Chloride Solution viscosity (Saline) in seconds.

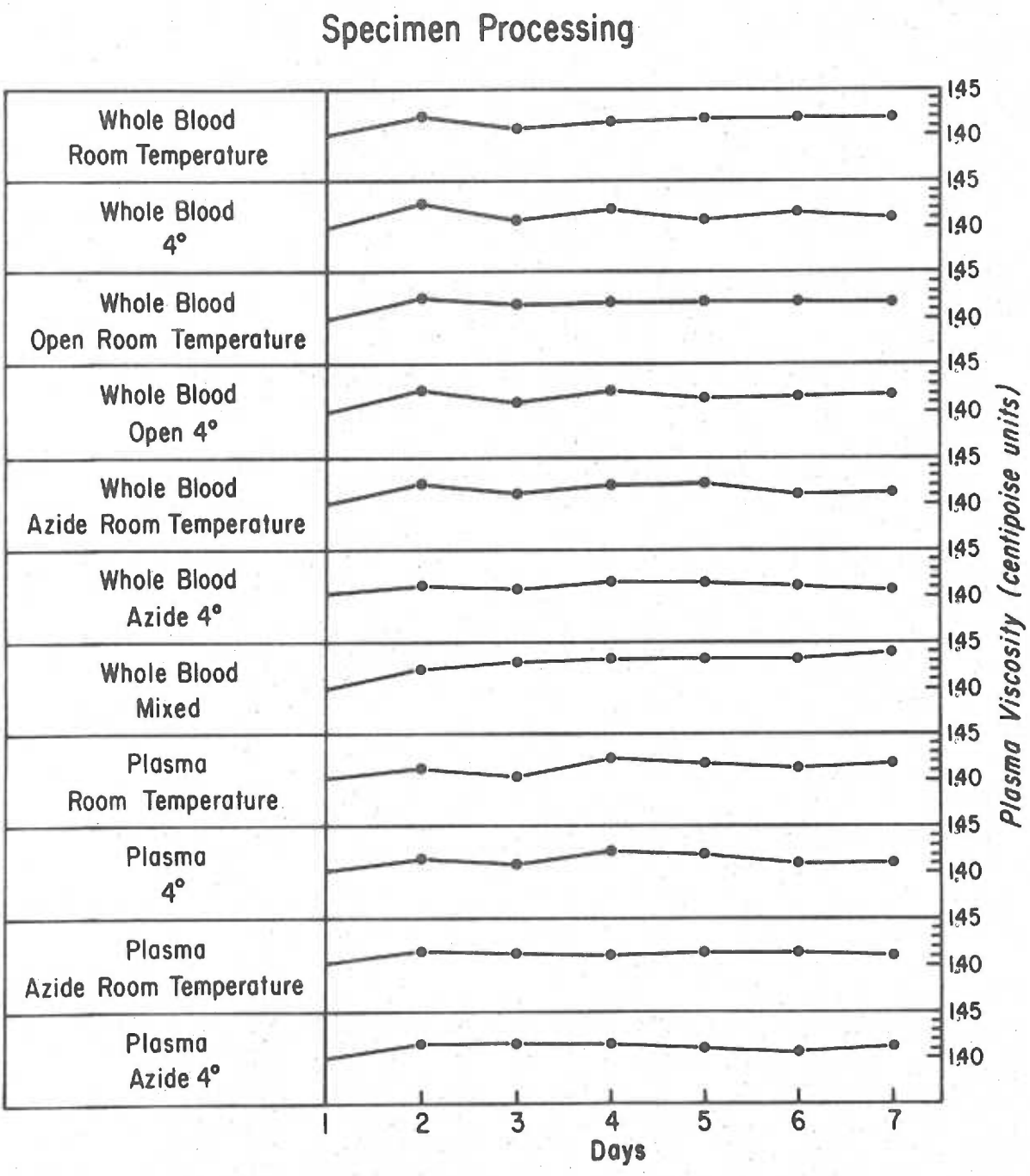
University of Oregon Health Sciences Center, Portland, Oregon 1977


Instrument Warmup
T°
25°
24°
23°
Pooled Plasma
43"
42"
41"
40"
39"
Saline
23"
22"
21"
1
5
10
15
20
25
30
35
Minutes

GRAPH #2

Specimen preparation and storage data using the Coulter Harkness Viscometer.

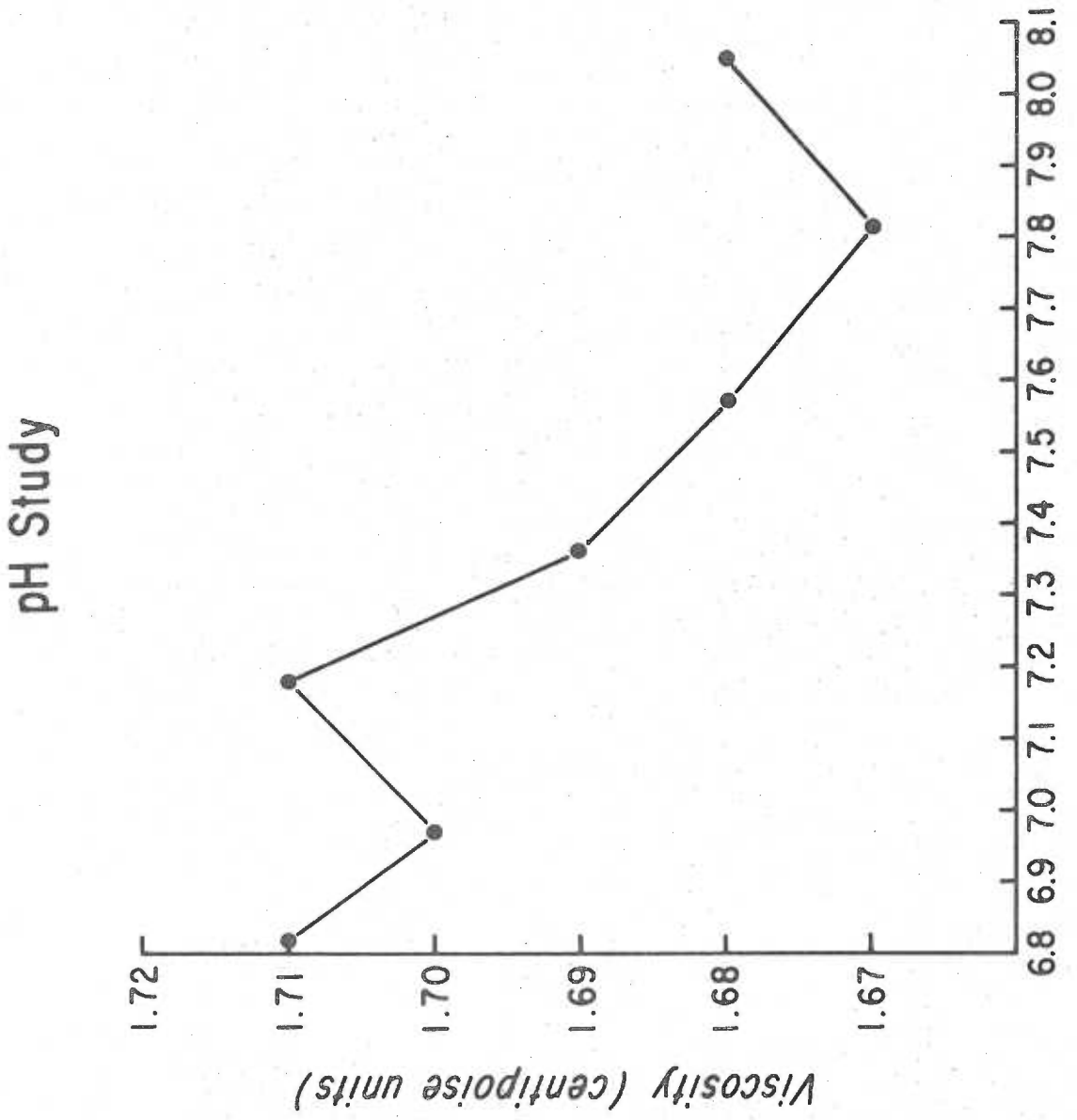
University of Oregon Health Sciences Center, Portland, Oregon 1977


Specimen Processing
Whole Blood
Room Temperature
Whole Blood
4°
Whole Blood
Open Room Temperature
Whole Blood
Open 4°
Whole Blood
Azide Room Temperature
Whole Blood
Azide 4°
Whole Blood
Mixed
Plasma
Room Temperature
Plasma
4°
Plasma
Azide Room Temperature
Plasma
Azide 4°
145
140
1
2
3
4
5
6
7
Days
Plasma Viscosity (centipoise units)

GRAPH #3

A graph showing the effects of EDTA plasma pH on plasma viscosity using the Coulter Harkness Viscometer.

University of Oregon Health Sciences Center, Portland, Oregon 1977


pH Study
Viscosity (centipoise units)
1.72
1.71
1.70
1.69
1.68
1.67
6.8 6.9 7.0 7.1 7.2 7.3 7.4 7.5 7.6 7.7 7.8 7.9 8.0 8.1
pH

GRAPH #4

Frequency Histogram of the Normal Population Study with accompanying statistics.

University of Oregon Health Sciences Center, Portland, Oregon 1977

LEGEND: $\overline{X}$ = mean

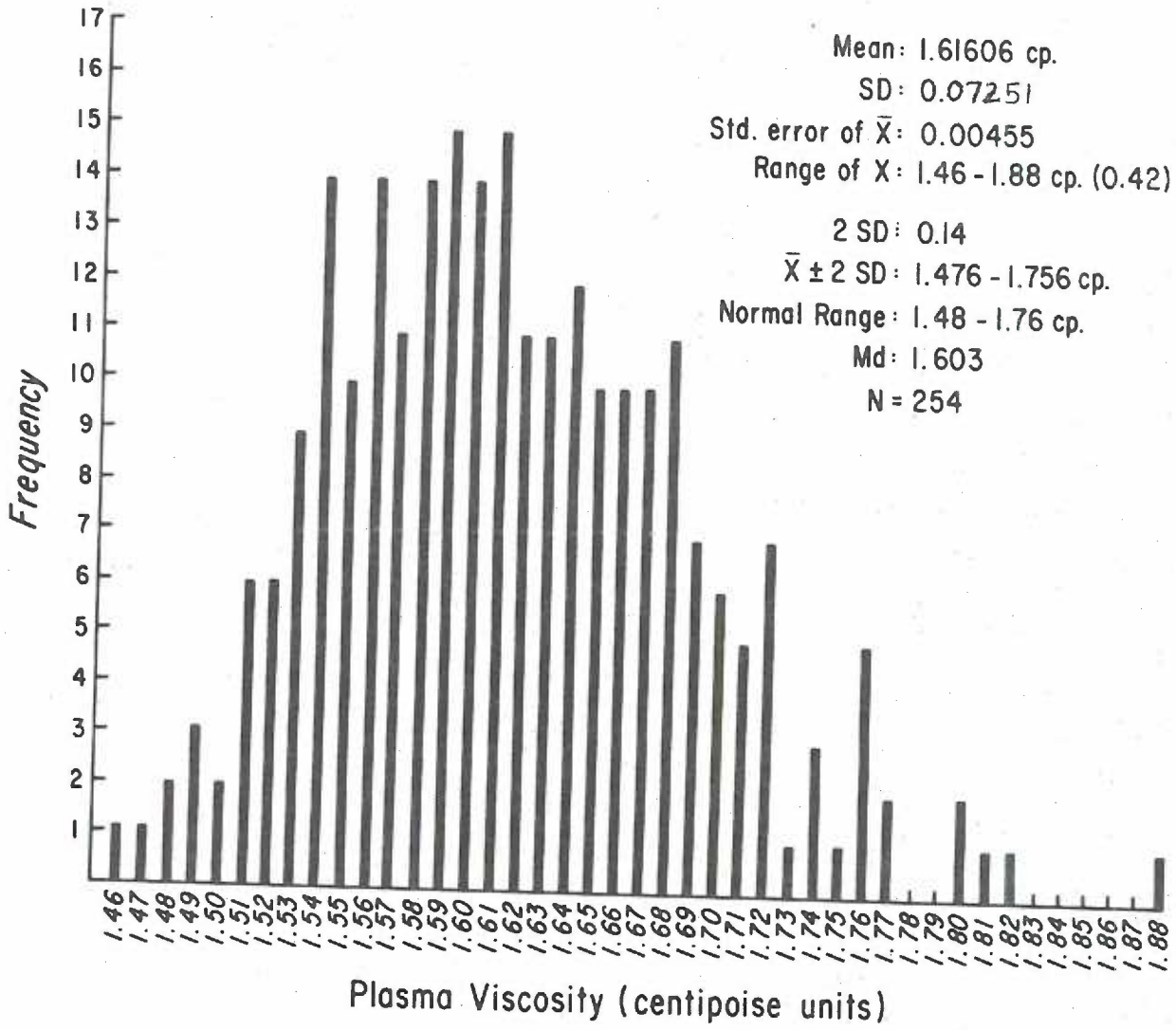
S.D. = Standard Deviation

Md = Median

N = number of raw scores

Normal Range = $\overline{X} \pm 2$ S.D.

## Frequency Histogram of Normal Population



GRAPH #5

Scatterplot of EDTA plasma viscosity versus Westergren Erythrocyte Sedimentation Rates (ESR) for 700 determinations.

University of Oregon Health Sciences Center, Portland, Oregon 1977

• = single data point

x = duplicate data points

■ = triplicate data points

## Scatter-plot of 700 Plasma Viscosity/ESR Determinations

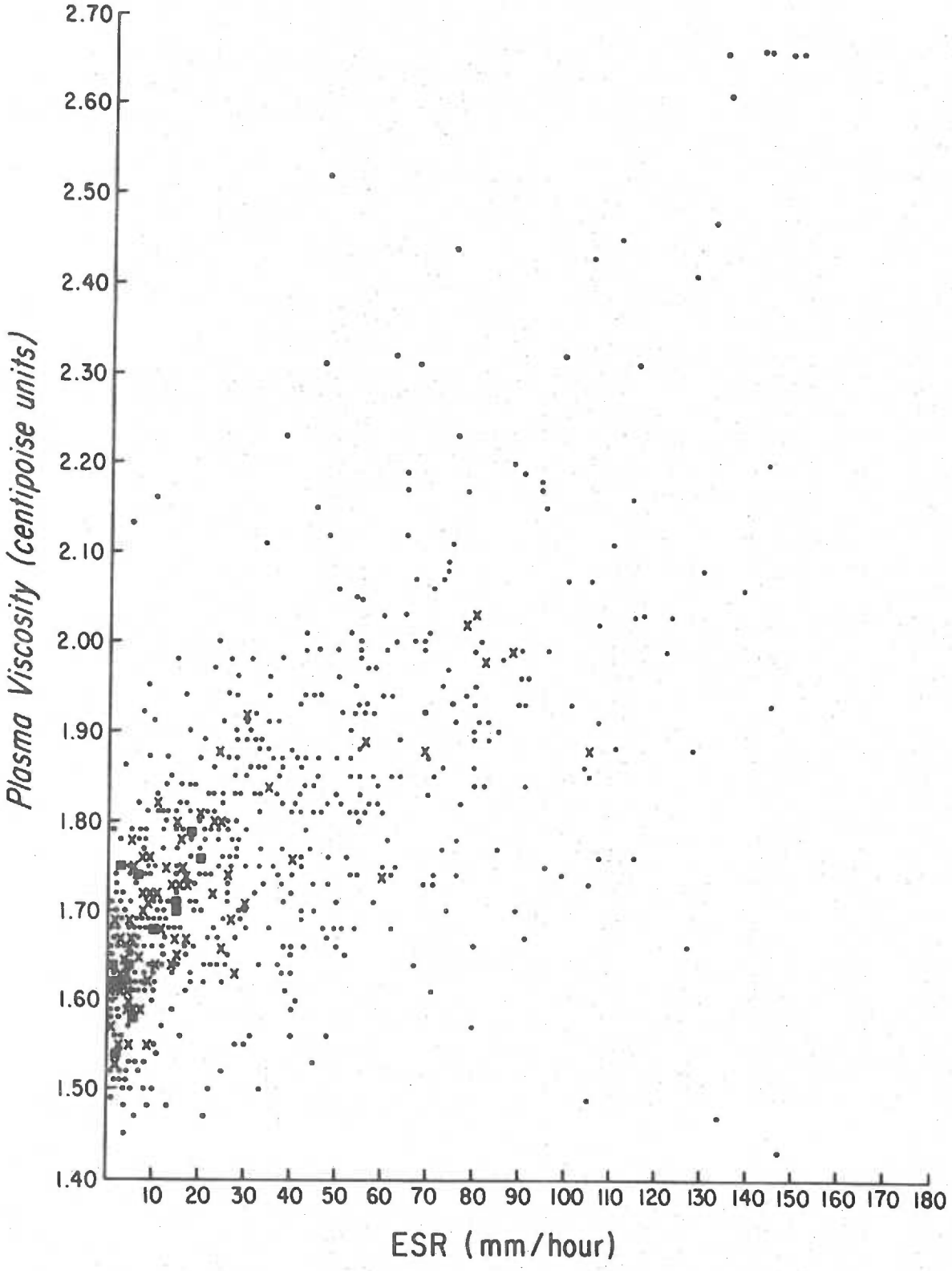
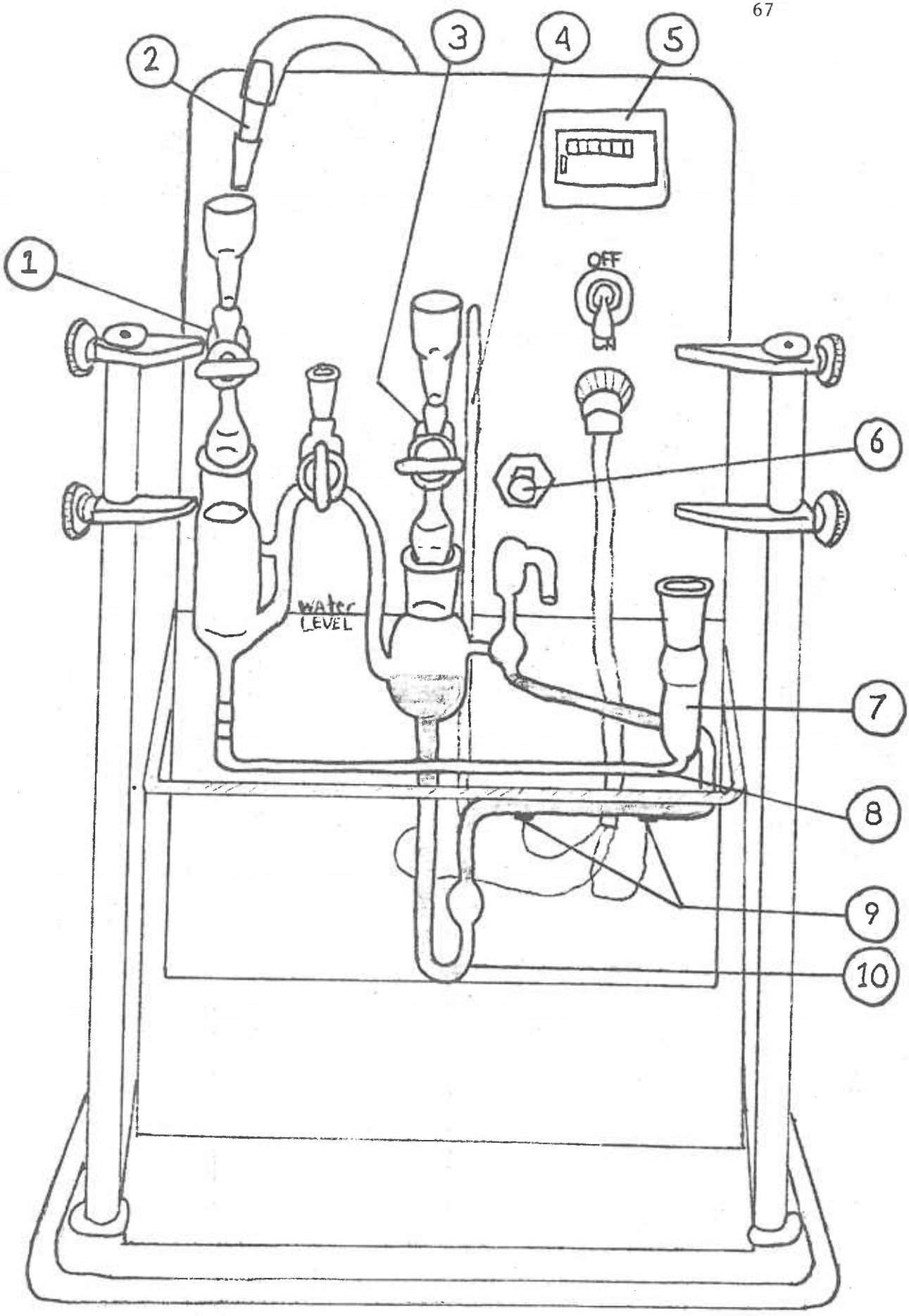


DIAGRAM #1

INSTRUMENT DIAGRAM OF THE

COULTER HARKNESS VISCOMETER

1. Tap A
2. Vacuum supply line
3. Tab C
4. $27^{o}$ C thermometer
5. Electrical timer
6. Mercury adjustment screw
7. Sample cup
8. Capillary tube (internal diameter of 0.3 mm)
9. Timing electrodes
10. Mercury manometer (shaded area represents mercury)


1
2
3
4
5
6
7
8
9
10
OFF
water
LEVEL