AN ABSTRACT OF THE THESIS OF

Jessica Andrea Filosa for the Master of Science Degree

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Title: Effects of acetylsalicylic acid on blood viscosity in healthy rats, *Rattus norvegicus*

Abstract approved: David K. Saunders

For many years acetylsalicylic acid (ASA) has been used as an analgesic, anti-inflammatory and antipyretic agent. In the past decade, the use of ASA has increased because it is thought to be beneficial for the cardiovascular system. Acetylsalicylic acid has been shown to reduce the risk of death from cardiovascular-related disorders such as myocardial infarction, unstable angina, and stroke. The purpose of this study was to determine the effects of ASA on blood viscosity in healthy rats, *Rattus norvegicus*. The effects of ASA were investigated at different concentrations (40 and 80 mg/kg body weight (bw) per day for one week) and for different time intervals (40 mg/kg bw ASA/day for one week, one month and two months, respectively). At the end of the experiment, the apparent viscosity of the plasma and of three different hematocrits was measured with a Wells-Brookfield cone-plate viscometer at ten different shear rates. In addition, protein concentration was determined for the plasma of each rat. The data suggest that as the hematocrit increased from 30% to 45%, the viscosity of the group which received 40 mg/kg bw ASA/day for one week was significantly higher than all other groups, particularly as shear rate increased. No significant differences were observed in any of the other experimental groups when compared to the control group. This study suggests that the effects of acetylsalicylic acid on blood viscosity

are dose and time dependent. It is possible that doses higher than 40 mg/kg bw or ASA treatment longer than one week trigger an adaptive response which is initiated upon an initial increase in blood viscosity. As a result, blood viscosity is unaltered.

EFFECTS OF ACETYLSALICYLIC ACID ON BLOOD VISCOSITY IN HEALTHY RATS, *RATTUS NORVEGICUS.*

A Thesis

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the Division of Biological Sciences

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by

Jessica Andrea Filosa

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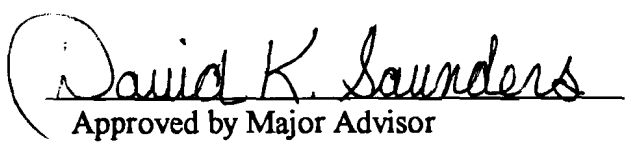

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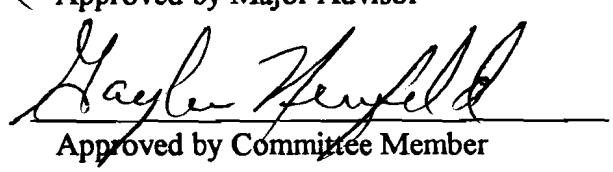

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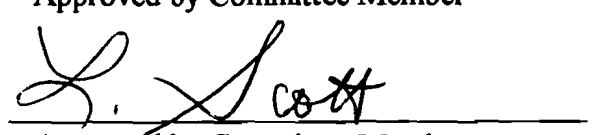

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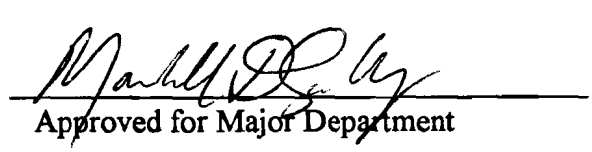

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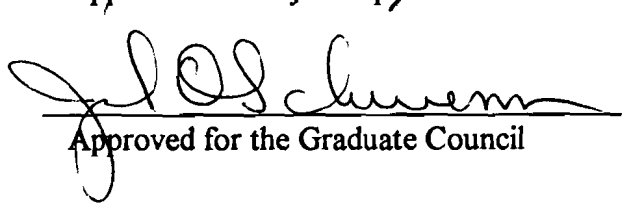

Approved for the Graduate Council

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A special thanks for Dr. David Saunders who was always present when I needed, knowledge, guidance and encouragement. Dr. Saunders has helped in the development of my critical thinking as well as in my desire to continue in this field of study. I thank the members of my committee, Dr. Neufeld, Dr. Robbins, and Dr. Scott for their comments and helped in the writing of this thesis.

I would like to dedicate this thesis to my parents, Jorge and Maria Ester Filosa, and family who had given me the opportunity to come to this country in order to attain a quality education.

PREFACE

This thesis was written in the style required by the journal of *Biorheology*.

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INTRODUCTION

One early pioneer to study the physical properties of blood flow was J. L. M. Poiseuille (1799-1869). In 1846 Poiseuille described the relationship between pressure, flow, fluid properties, and vascular dimensions. This relationship is known as Poiseuille's Law (Milnor, 1989). Poiseuille's ideas opened a new field of study called rheology, the study of fluid dynamics. Rheological studies soon branched into the study of blood flow or hemorheology.

Poiseuille studied steady flow in cylindrical tubes and empirically derived the following formula:

$$Q=\Delta P\pi r^4/8\eta L \qquad \text{(equation 1)}$$

where Q=fluid flow; ΔP=change in pressure; r=radius; L=length and η=viscosity. However, blood flow is not constant but pulsatile, blood vessels are neither straight nor rigid, and blood is not a simple fluid but a suspension of cells (Lowe, 1994). The anomalous properties of the blood can be better studied if Ohm's Law is used in addition to Poiseuille's Law, resulting in an equation that expresses vascular resistance to flow:

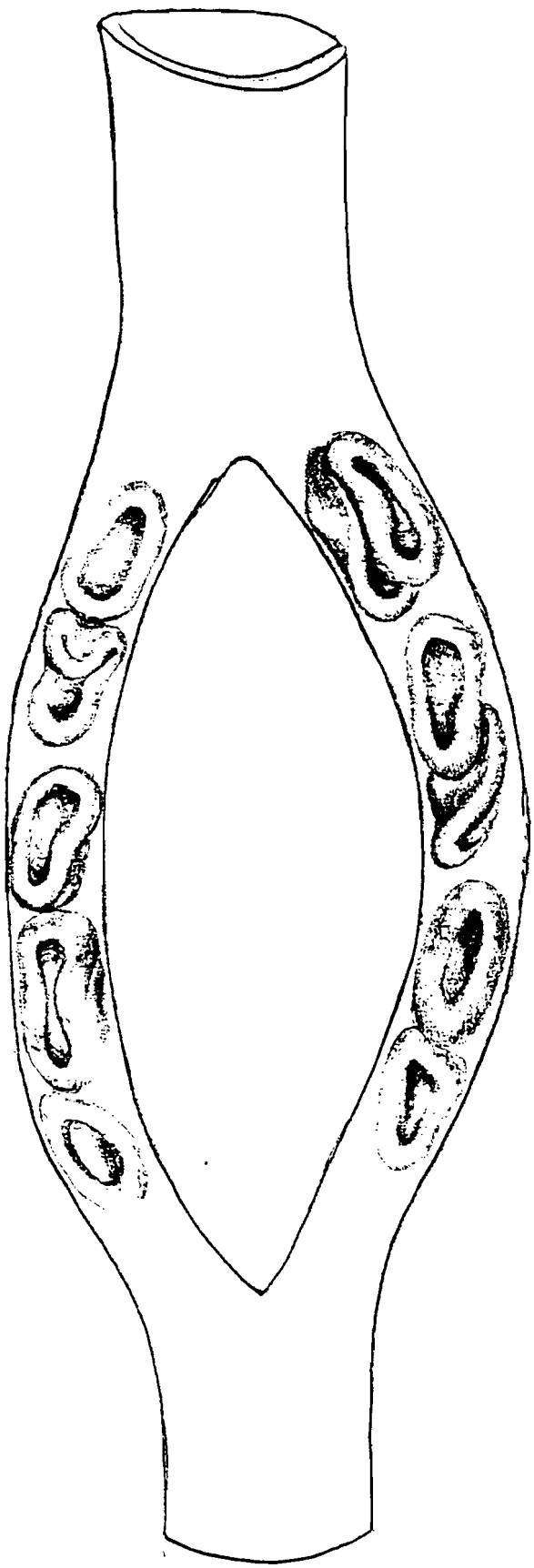
$$R_{vas}=8\eta L/\pi r^4 \qquad \text{(equation 2)}$$

The importance of this relationship is that it defines the resistance blood has to overcome in order to flow to organs and tissues. That is, blood flows against a vascular resistance (created as a result of the frictional interaction between cell layers or laminae and the vessel

wall) and a viscous resistance (frictional interaction between cell laminae or cell components) (Lowe, 1994). From equation 2 one can interpret that a decrease in blood viscosity will decrease the resistance to flow, thus decreasing the force required to overcome the frictional interaction between cells and the vessel wall (Milnor, 1989).

In general, in the larger vessels, blood takes on characteristics similar to a Newtonian fluid in that the coefficient of viscosity is constant, such that viscosity does not vary at different blood velocity gradients (Fung, 1981). However, in narrow tubes or at the branching points of the circulatory tree where some turbulence is present, blood exhibits non-Newtonian behavior (Fung, 1981; Lowe, 1994). Non-Newtonian behavior is brought about by the unique physical characteristics of the red blood cells and their interaction with plasma proteins and other cellular components. In the capillaries, the red blood cells flow through narrow diameters of about 4 to 10 μm. Because a typical human red blood cell has an average diameter of 7-8 μm, they flow in single file, relying on their flexible membrane properties in order to flow through such small vessels (Figure 1) (Fung, 1981; Thruston, 1994). The behavior of the red blood cells is therefore governed by the

Figure 1. Diagrammatic representation of red blood cell deformability as seen flowing through capillaries. (Not drawn to scale.)



physical properties of these cells and the flow conditions to which they are exposed.

When blood flow is streamline or laminar, cylindrical layers of cells flow one past the other, with their rate of flow dependent upon the frictional (viscous) interaction between blood cell components (red blood cells, white blood cells, platelets and plasma proteins) (Chien, 1981). As blood exits the heart and enters the aorta, blood cells are exposed to a high degree of pressure while flowing at a high rate. The layer closest to the vessel wall has a velocity of almost zero, whereas the layer flowing in the center of the vessel exhibits the highest velocity (Milnor, 1989). The viscous interaction between laminae creates a velocity gradient between these layers of cells, causing blood to flow in the shape of a parabola (Figure 2a). The velocity gradient and pressure driving the cell layers are therefore two parameters that determine the viscous characteristics of the blood.

Blood viscosity is expressed as the blood's resistance to flow:

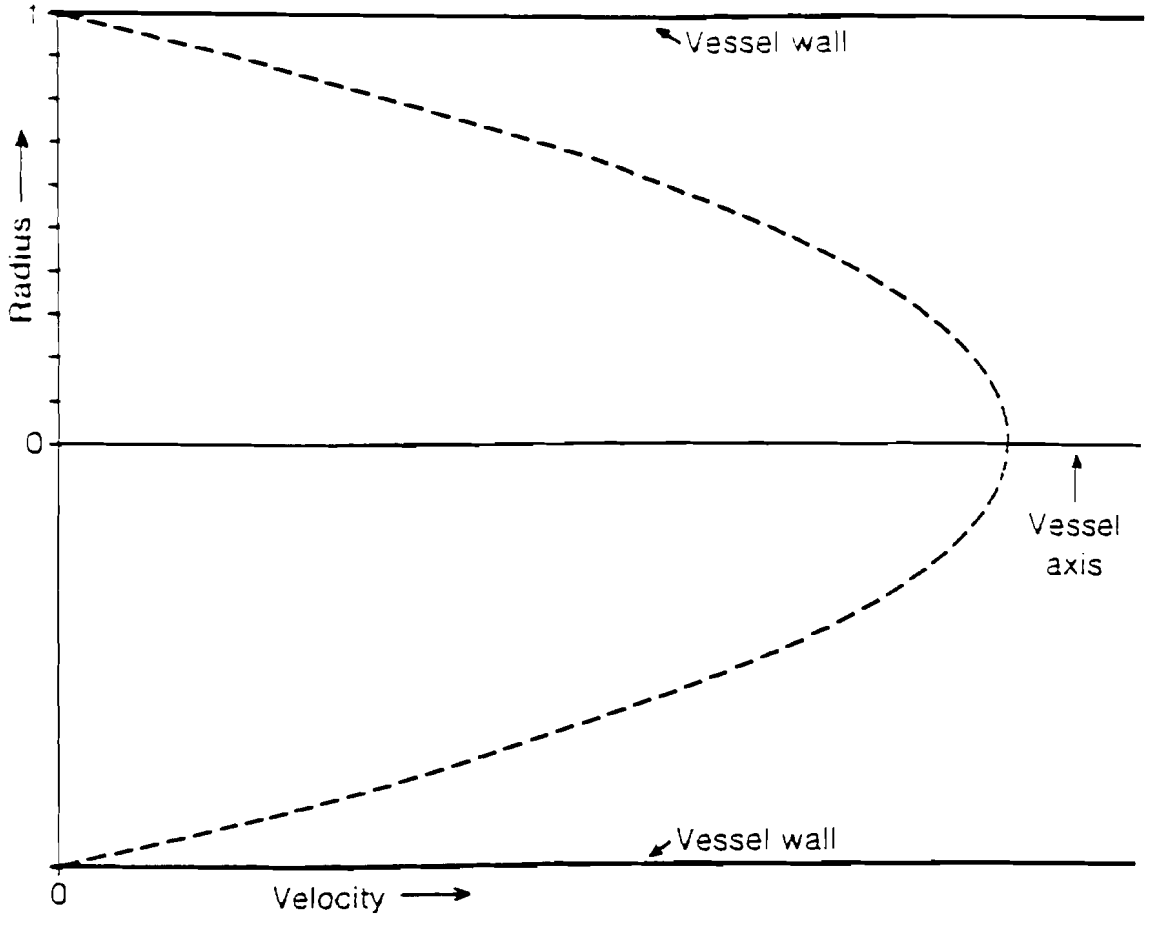
$$\eta = \text{shear stress}/\text{shear rate} \qquad \text{(equation 3)}$$

where η=viscosity(cP); shear stress(SS)=force per unit area ($dyne/cm^2$); and shear rate(SR)=velocity gradient over a given distance (sec^{-1}). In other words, viscosity relates to the force (SS) required to create motion (SR) between two

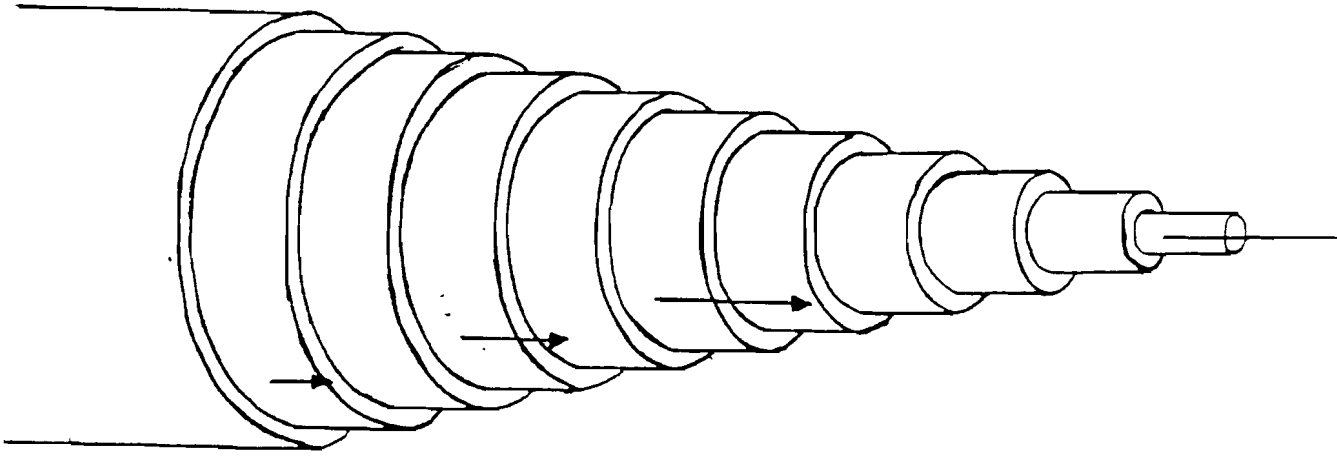
Figure 2a. Parabolic shape brought about by the different velocity gradients of cell layers (laminae) as the vessel radius decreases (Milnor, 1989).

Figure 2b. Physical model of flowing laminae across a given diameter. Arrows represent an increase in the magnitude of the velocity as the layers approach to the central axis (Milnor, 1989).

2a.



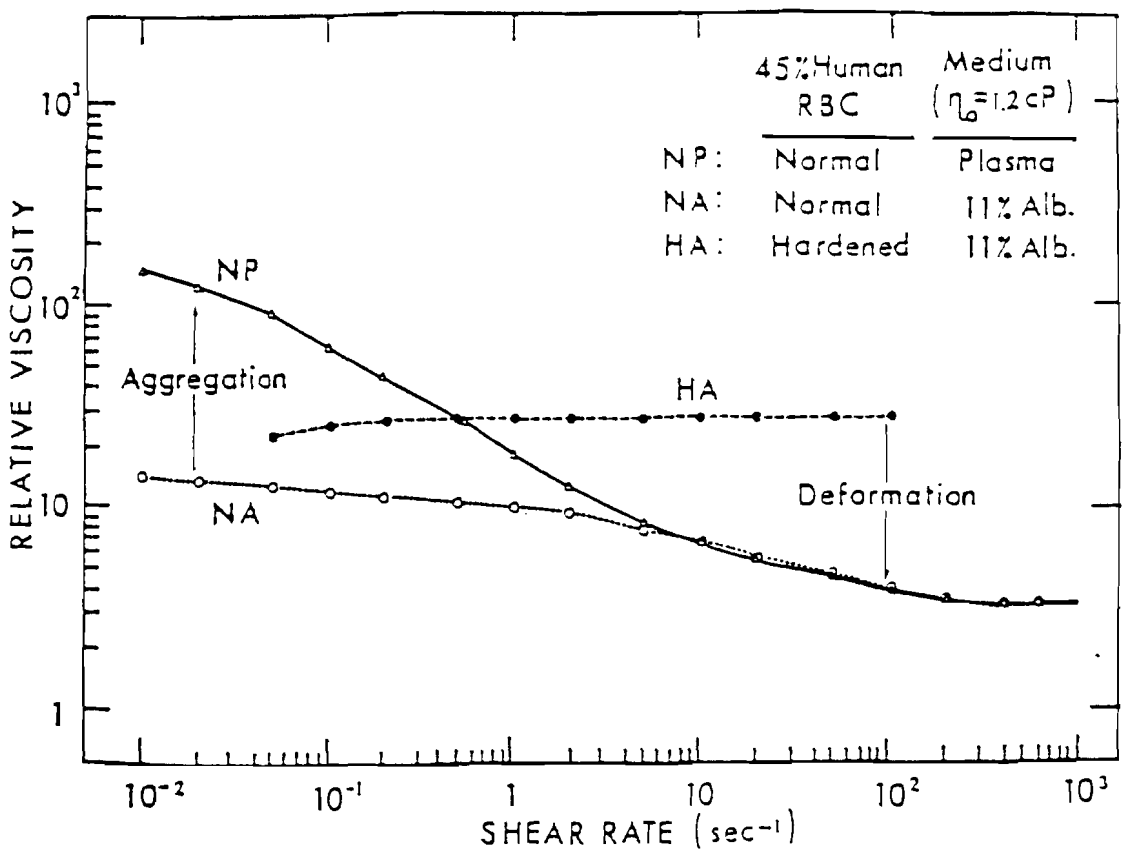
2b.



layers of fluid as they slide past one another (Figure 2b) (Milnor, 1989). Shear stress is created by the left and right ventricles during each contraction and the shear rate is the velocity gradient over a given area between laminae as they slide past one another (Chien, 1970).

Shear rate can be estimated as 4V/R, where V=velocity and R=vessel radius (Chien *et al.*, 1971). Changes in shear rate lead to changes in the behavior of the red blood cells as they flow, which in turn changes the overall viscous properties of the blood. Figure 3 shows viscosity values at different shear rates for normal red blood cells suspended in plasma, normal blood suspended in Ringers-albumin, and hardened red blood cells suspended in Ringers-albumin (Chien, 1970). The effects of red blood cell deformability and red blood cell aggregation are clearly shown in Figure 3. As shear rate increases, whole blood viscosity decreases, whereas the viscosity of hardened red blood cells suspended in Ringers-albumin solution remains constant. Therefore, increase red blood cell deformability leads to a decrease in blood viscosity at high shear rates. On the other hand, when shear rates are low, red blood cells suspended in their normal environment (plasma) exhibit a greater viscosity than the hardened cells suspended in Ringers-albumin solution. At low shear rates, cell deformability is no longer a factor in blood viscosity as red cell aggregation becomes the major determinant

Figure 3. Log apparent viscosity vs. shear rate of human red blood cells (RBC) in plasma (NP), normal RBC in 11% Ringers-albumin (NA) and hardened RBC in 11% Ringers-albumin (HA) Chien, 1970. By Permission).


45%Human RBC
Medium (η_0=1.2cP)
NP: Normal Plasma
NA: Normal 11% Alb.
HA: Hardened 11% Alb.
NP
HA
NA
Aggregation
Deformation
RELATIVE VISCOSITY
SHEAR RATE (sec^{-1})
10^3
10^2
10
1
10^{-2}
10^{-1}
1
10
10^2
10^3

(Chien *et al.*, 1967). Therefore, at high shear rates, the main determinant for blood viscosity is *red blood cell deformability* as high shear rates prevent the formation of intercellular bridges between the red blood cells (Chien, 1981). At lower shear rates, the main determinant for blood viscosity is *red blood cell aggregation* since there is ample time for plasma proteins to create cell-to-cell bridges between the red blood cells (Chien, 1981).

During high flow velocities (high SR) the red blood cells become elongated into an ellipsoid shape, orienting themselves in the direction of flow (Bull *et al.*, 1983). Such shape change is possible because these red blood cells possess a 40% excess membrane relative to their internal volume (Fung, 1981). Changing from the normal biconcave shape into an ellipsoidal shape leads to an overall decrease in blood viscosity (Figure 4) (Bull *et al.*, 1983). On the other hand, when flow rates are slow (low SR), the red blood cell behaves more like a visco-elastic fluid, allowing plasma proteins to form intercellular bridges between the cells, and therefore causes red blood cell aggregation (Wells *et al.*, 1962). Thus, increased red blood cell deformability and decreased red blood cell aggregation lead to an overall decrease in blood viscosity over a wide range of shear rates.

In addition to red blood cell aggregation and

Figure 4. Red blood cell shape changes relative to increases in shear rate (Re-drawn from, Bull *et al.*, 1983).

LOW SHEAR RATE (SEC^{-1})

HIGH SHEAR RATE (SEC^{-1})

deformability, blood viscosity is also affected by plasma protein concentration, hematocrit, and temperature. An increased concentration of the plasma proteins, fibrinogen, serum globulins, and albumin, leads to an increase in red blood cell aggregation, thereby increasing the viscosity of the blood (Agre and Parker, 1989; Chien, 1981; Koenig *et al.*, 1991). Increases in hematocrit (the ratio of red blood cells to total blood volume) increases the contribution from these cells to blood viscosity, thus increasing whole blood viscosity (Fung, 1981). Finally, an increase in temperature, in general, leads to a decrease in viscosity (Chien, 1970; Chien, 1981).

Some of the above factors might be influenced by the action of acetylsalicylic acid (ASA), the primary component of aspirin. Many research studies have shown the antithrombotic, antipyretic, and anti-inflammatory effects of aspirin in the blood. For example, aspirin is a common medication for individuals with cardiovascular related disorders such as myocardial infarction, unstable angina, hyperfibrinogemia, and atherosclerosis (Cairns *et al.*, 1985; Dormandy *et al.*, 1982; Ehrly, 1990). However, it is not clear whether ASA can elicit changes in the viscous properties of the blood and thus influence blood flow.

In vivo, ASA is hydrolyzed into acetic acid and salicylate. In the plasma, salicylate binds to albumin, but

as its concentration increases, more unbound salicylate is available to bind to other cells or tissues (Meyers *et al.*, 1980). Burgin and Schatzmann (1979) found that salicylate alters red blood cell membrane behavior. As salicylate serum concentration increased, these molecules adsorbed to the erythrocyte's membrane causing an increase in Ca^{++} permeability (Burgin and Schatzmann, 1979). Increasing calcium concentration within the red blood cell leads to a decrease in red blood cell deformability (Beutler *et al.*, 1995; Weed *et al.*, 1969). As the Ca^{++} concentration in the membrane and cytosol of the red blood cell increases, the cell uses its intracellular ATP to remove excess Ca^{++} from its interior in order to maintain cellular deformability as well as a normal internal environment. As a result, over time, the cell depletes its ATP levels, resulting in a decrease in membrane deformability (Fung, 1981; Weed *et al.*, 1969), which in turn, increases the overall viscosity of the blood, particularly at high shear rates.

In my study, I focused strictly on the effects of aspirin on blood viscosity, concentrating primarily on the red blood cells' properties and on ASA's effects on the viscous characteristics of the plasma. I treated healthy rats with the intention of finding other effects of ASA in addition to its common antithrombotic effects on the blood. As such, the purpose of this study was to investigate the effects of acetylsalicylic acid on blood viscosity in

healthy rats, *Rattus norvegicus*. The experiment was performed with different aspirin concentrations and within different time periods in order to determine if particular dosages of aspirin and/or duration of aspirin treatment improved the rheological properties of the blood in healthy rats.

MATERIALS AND METHODS

Aspirin administration: concentration dependence

Three groups of laboratory rats, A through C, were used throughout the experiment. Each group consisted of five male rats (*Rattus norvegicus*) with an average weight of 250 grams. Rats from group A were treated with a daily dose of aspirin (40 mg/kg body weight (bw)), for one week. Rats from group B were treated with 80 mg/kg bw/day for one week. Group C served as a control. High ASA concentrations were chosen with the intention of assuring high aspirin levels in the blood. Experimental and control rats were allowed free access to food and water.

Acetylsalicylic acid (99%) (Sigma, St. Louis, MO) was weighed with a Mettler 54H AR balance, and mixed with melted chocolate to form *ASA chocolate chips*. Treated rats received one *ASA chocolate chip* per day, and control rats received a chocolate chip without ASA. At the end of one week, viscosity measurements were performed on the blood of the treated and control groups.

Aspirin administration: time dependence

Three additional groups were tested, each containing five male rats with an average weight of 250 grams. Two groups (D & E) were given a daily dose of aspirin (40 mg/kg bw/day) for one month and two months respectively, using the method described above. Group C served as a control,

receiving a chocolate chip containing no aspirin.

Preparation of blood sample:

At the end of the experiment, rats were anesthetized by intraperitoneal injection of 20 mg ketamine hydrochloride (100 mg/ml). Surgical procedures were not performed until the rat's pupillary response was negative. Whole blood was removed from the inferior vena cava using a heparinized syringe and a 21 G needle. An average of 8 ml of blood was obtained from each rat and immediately placed in an ice bath.

Measurements of blood viscosity:

Viscosity measurements on 0.5 ml of whole blood were performed using a Wells Brookfield Cone-Plate Viscometer (Model DV-II+, Brookfield Engineering Lab. Stoughton, MA) with a CP-40 spindle. The viscometer was calibrated with distilled water at 38^0C and compared to a standard table in the Handbook of Chemistry and Physics prior to making viscosity readings from blood samples of individual rats. Blood viscosity readings were performed at a constant temperature of 38^0C with the aid of 38^0C water current flowing around the viscometer plate. Measurements were made at ten different shear rates (3.75, 7.5, 15, 18.8, 30, 37.5, 75, 150, 375, and 750 sec^{-1}) and on three different hematocrit readings (normal, below normal, and above normal). Different hematocrits were obtained upon centrifugation (1200 x g for 5 min) of three Eppendorf tubes

containing normal blood samples. Plasma was extracted from one Eppendorf tube and added to another, thus lowering the hematocrit in one tube and increasing it in the other. The third tube was unaltered. Hematocrit readings were determined using the microhematocrit method.

Plasma viscosity (0.5 ml) was determined from each rat at each of the ten different shear rates at a constant temperature of 38^{0}C. Additional plasma was collected and immediately frozen for later determination of protein concentration.

Viscosity measurements were also performed at the ten different shear rates and at a constant temperature of 38^{0}C on red blood cells suspended in Ringers-Albumin solution (0.86 g NaCl, 0.030 g KCl, and 0.033 g $CaCl_2$ in 100 ml of distilled water). Prior to this procedure, cells were washed three times in a 0.9% NaCl solution and then suspended in Ringers-albumin solution (Chien, 1971).

Protein assay measurement:

Protein concentration was determined for each plasma sample using the BioRad reagent method (Bradford, 1976).

Measurement of red blood cell deformability:

During high shear rate conditions (>100s^{-1}), the forces acting on the red blood cells as they flow through vessels are sufficiently large to prevent aggregation of these cells. As a result, under high shear rates the major determinants for blood viscosity are hematocrit and red

blood cell deformability (Aarts *et al.*, 1984). Dintenfass (1968) formulated an equation that described the deformable capacity of the red blood cells:

$$\mu s=\mu o(1-TH)^{-2.5} \qquad \text{(equation 4)}$$

where μs=whole blood viscosity, μo=plasma viscosity; H=hematocrit, and T="Taylor factor"(variable that describes the deformability characteristics of the RBCs). This can be rewritten as follows:

$$T=(1-(\mu s/\mu o)^{-0.4})/H \qquad \text{(equation 5)}$$

In order to calculate Taylor's factor from the obtained data, I first calculated the "relative viscosity" (μs/μo) of the blood by dividing the apparent viscosity of whole blood by the apparent viscosity of the plasma at each shear rate and at a particular hematocrit. I then applied the obtained ratio to equation 5. An increase in the T value indicates a decrease in red blood cell deformability (Aarts *et al.*, 1984).

Statistical analysis:

A SAS package version 5.16 (SAS Institute Inc., Cary, N.C.) was used for the statistical analysis of the viscosity data. The viscosity data were log transformed and grouped by experimental group, rat, and shear rate for a particular hematocrit. Viscosity values for each of the three different hematocrit samples per rat were used to create a regression equation for each rat at each shear rate (average

R^2=0.978). From the regression equation, I predicted viscosity values for three different hematocrit readings (30%, 38%, and 45%). Hematocrit values were chosen with the intention of predicting apparent viscosity values for a rat (average hematocrit of 38%) and a human (average hematocrit of 45%). A 30% hematocrit was chosen with in order to predict viscosity values below normal for a rat or a human. A one-way analysis of variance followed by Duncan's Multiple Range test was used in order to compare mean log viscosities for each of the three created hematocrits, plasma viscosity, protein concentration, and Taylor's factor between each group. Means were considered significantly different if $P<0.05$.

RESULTS

Viscosity values at a shear rate of 750 sec^{-1} were excluded from the data analysis. The viscosity values of the blood at this shear rate were beyond the limits of measurement of the viscometer.

Concentration dependence:

Whole blood viscosity: Figures 5 through 7 show mean values of log apparent viscosity for whole blood (±SE) vs. shear rate for group A (40 mg/kg bw ASA/day for one week), group B (80 mg/kg bw ASA/day for one week) and group C (control) at hematocrits of 30%, 38%, and 45% respectively. No statistically significant differences were found between the control and the experimental groups at any of the measured shear rates at a hematocrit of 30%. At a hematocrit of 38%, group A had a significantly higher log apparent viscosity than group B at shear rates greater than 3.75 sec^{-1}. In addition, at a hematocrit of 38%, significant differences were also seen at shear rates higher than 18.8 sec^{-1} between group A and the control group. Finally, at a hematocrit of 45%, group A had a significantly higher log apparent viscosity than group B at all shear rates and than the control group at shear rates above 3.75 sec^{-1}. As hematocrit and shear rate increased, the differences ($P<0.05$) between group A and the other two groups became more apparent with group A consistently showing the highest blood viscosity.

Plasma viscosity: No significant differences were found between groups for mean plasma viscosity (±SE) at any shear rate (Figure 8).

Time dependence:

Whole blood viscosity: Figures 9 through 11 show mean values (±SE) of log apparent viscosity vs shear rate for whole blood at three different hematocrits (30%, 38%, and 45%) for group A (40 mg/Kg bw ASA/day for one week), group D (40 mg/kg bw ASA/day for one month), group E (40 mg/kg bw ASA/day for two months) and control. No significant differences were seen at any of the measured shear rates between groups A, E, D, and C at a hematocrit of 30%. At a hematocrit of 38% group A had a significantly higher viscosity than groups D and E at a shear rate of 7.5 sec^{-1}. In addition, group A also showed significantly higher blood viscosities than did groups D, E and control, at shear rates greater than 18.8 sec^{-1}. Finally, at a hematocrit of 45%, group A had a significantly higher blood viscosity than did groups D, E, and control at shear rates greater than 3.75 sec^{-1}. At a shear rate of 3.75 sec^{-1}, significant differences were seen only between group A and group E, with group A having the highest blood viscosity. Despite an initial increase in blood viscosity after one week of treatment, prolonged treatments had little effect on blood viscosity as groups D & E showed no significant differences in blood viscosity after one and two months of treatment

respectively, as compared to control animals.

Plasma viscosity: Although no statistically significant differences were observed between the experimental groups and the control group, plasma viscosity was significantly different at a shear rate of 37.5 sec^{-1} between groups A, D, and E, with groups D and E having higher plasma viscosity than group A (Figure 12).

Plasma protein concentration:

Group D (40 mg/kg bw ASA/day for one month) had a significantly higher plasma protein concentration than all other groups (P=0.0003) (Table 1). These data contradict plasma viscosity data, because plasma viscosity was not increased as might be expected from an increase in plasma protein concentration.

Red Blood Cell Deformability:

No statistical differences were seen in the Taylor's factor value between the experimental groups and the control group (Table 2). However, group A (group with the highest viscosity values) did have the highest Taylor's factor value at a shear rate of 150 sec^{-1}, although this was not the case at a shear rate of 375 sec^{-1}. A high Taylor's factor indicates an increase in red blood cell rigidity and thus a decrease in red blood cell deformability.

Figure 5. Concentration dependence: Log apparent viscosity vs. shear rate at a constant hematocrit of 30% (Meand ± SE).

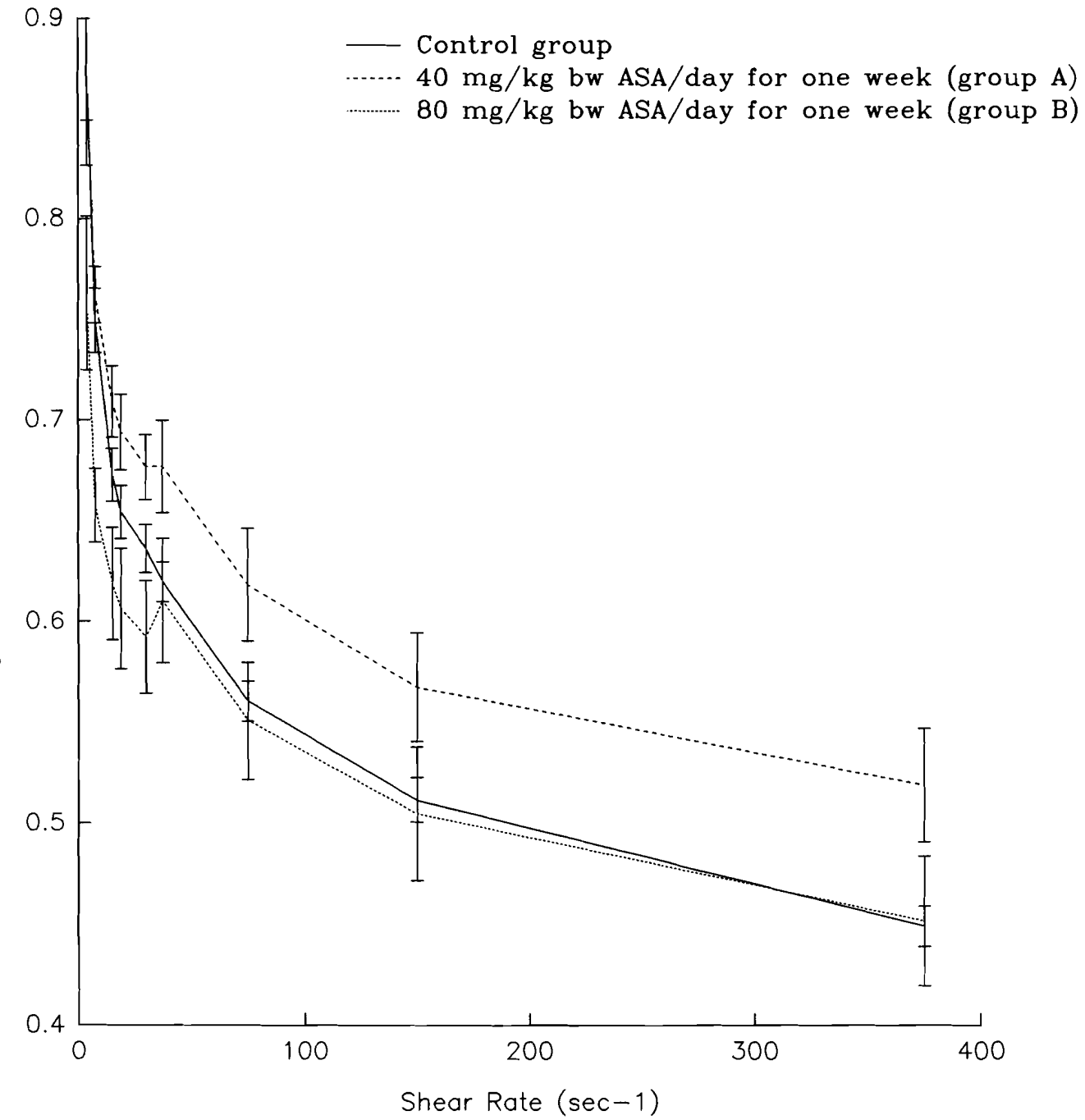

Control group
40 mg/kg bw ASA/day for one week (group A)
80 mg/kg bw ASA/day for one week (group B)
0.9
0.8
0.7
0.6
0.5
0.4
0
100
200
300
400
Shear Rate (sec−1)

Figure 6. Concentration dependence: Log apparent viscosity vs. shear rate at a constant hematocrit of 38% (Mean ± SE).

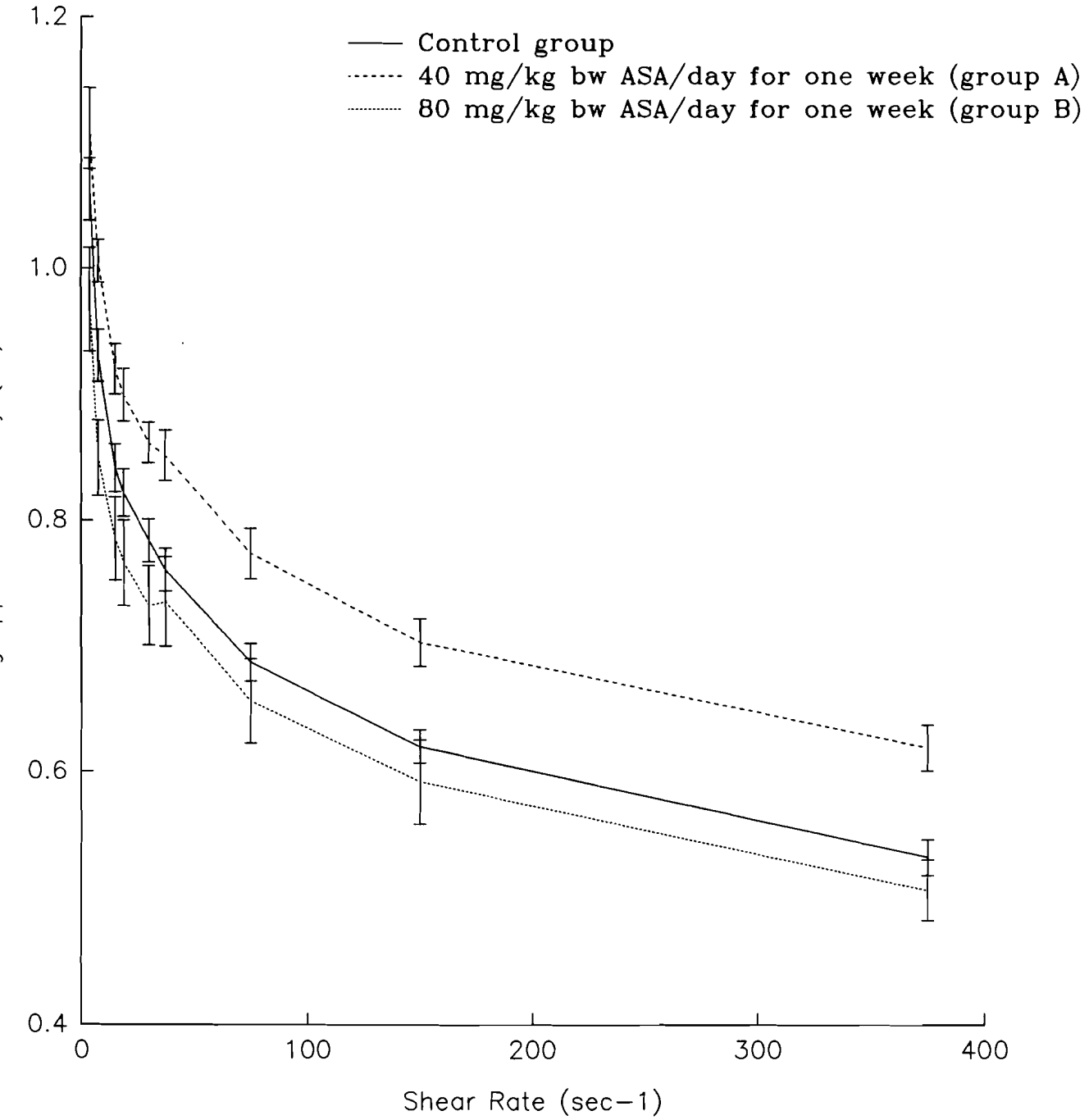

Control group
40 mg/kg bw ASA/day for one week (group A)
80 mg/kg bw ASA/day for one week (group B)
1.2
1.0
0.8
0.6
0.4
0
100
200
300
400
Shear Rate (sec−1)

Figure 7. Concentration dependence: Log apparent viscosity vs. shear rate at a constant hematocrit of 45% (Mean ± SE).

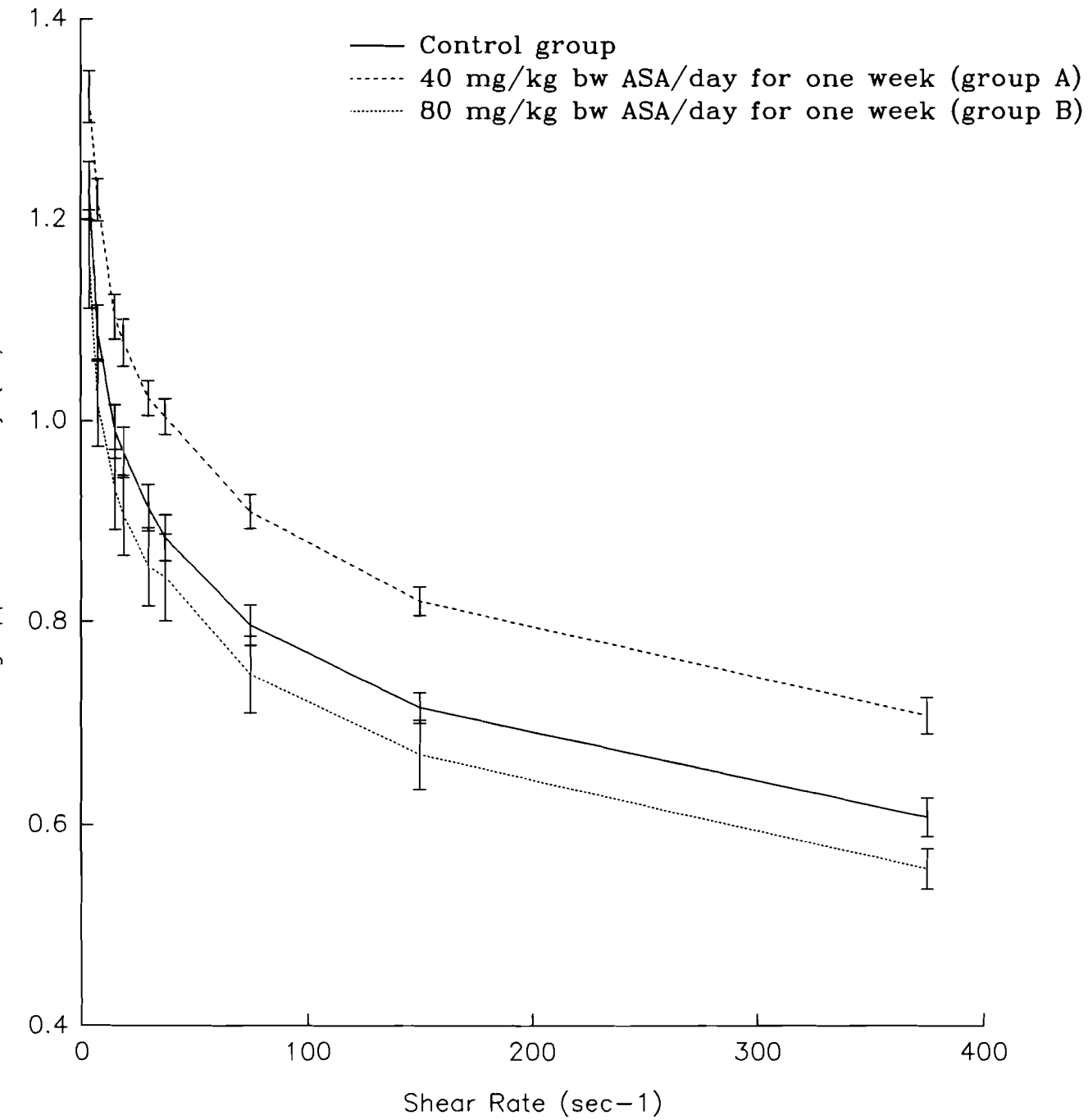

Control group
40 mg/kg bw ASA/day for one week (group A)
80 mg/kg bw ASA/day for one week (group B)
1.4
1.2
1.0
0.8
0.6
0.4
0
100
200
300
400
Shear Rate (sec−1)

Figure 8. Concentration dependence: Apparent viscosity vs. shear rate for plasma (Mean ± SE).

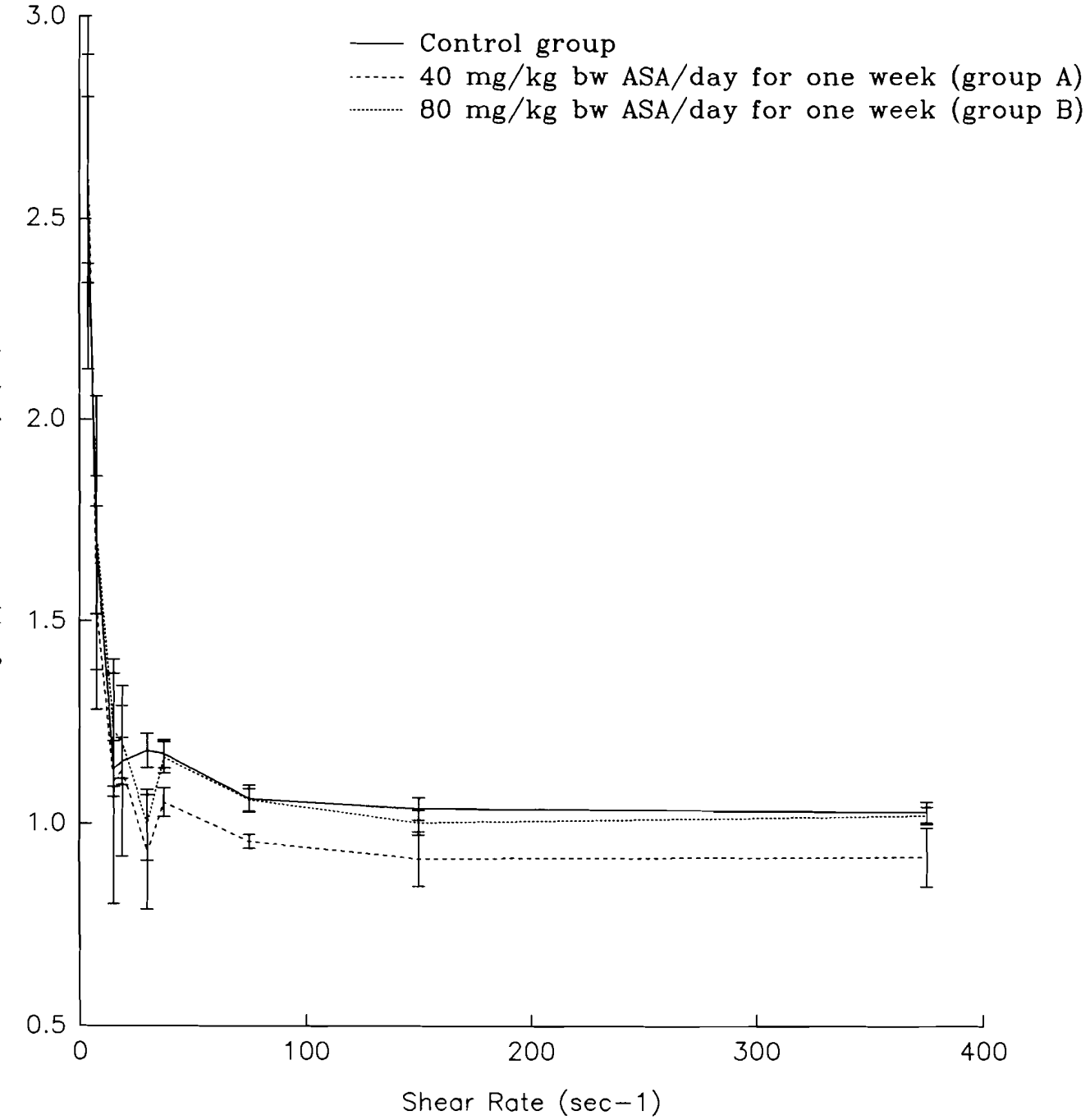

Control group
40 mg/kg bw ASA/day for one week (group A)
80 mg/kg bw ASA/day for one week (group B)
3.0
2.5
2.0
1.5
1.0
0.5
0
100
200
300
400
Shear Rate (sec−1)

Figure 9. Time dependence: Log apparent viscosity vs. shear rate at a constant hematocrit of 30% (Mean ± SE).

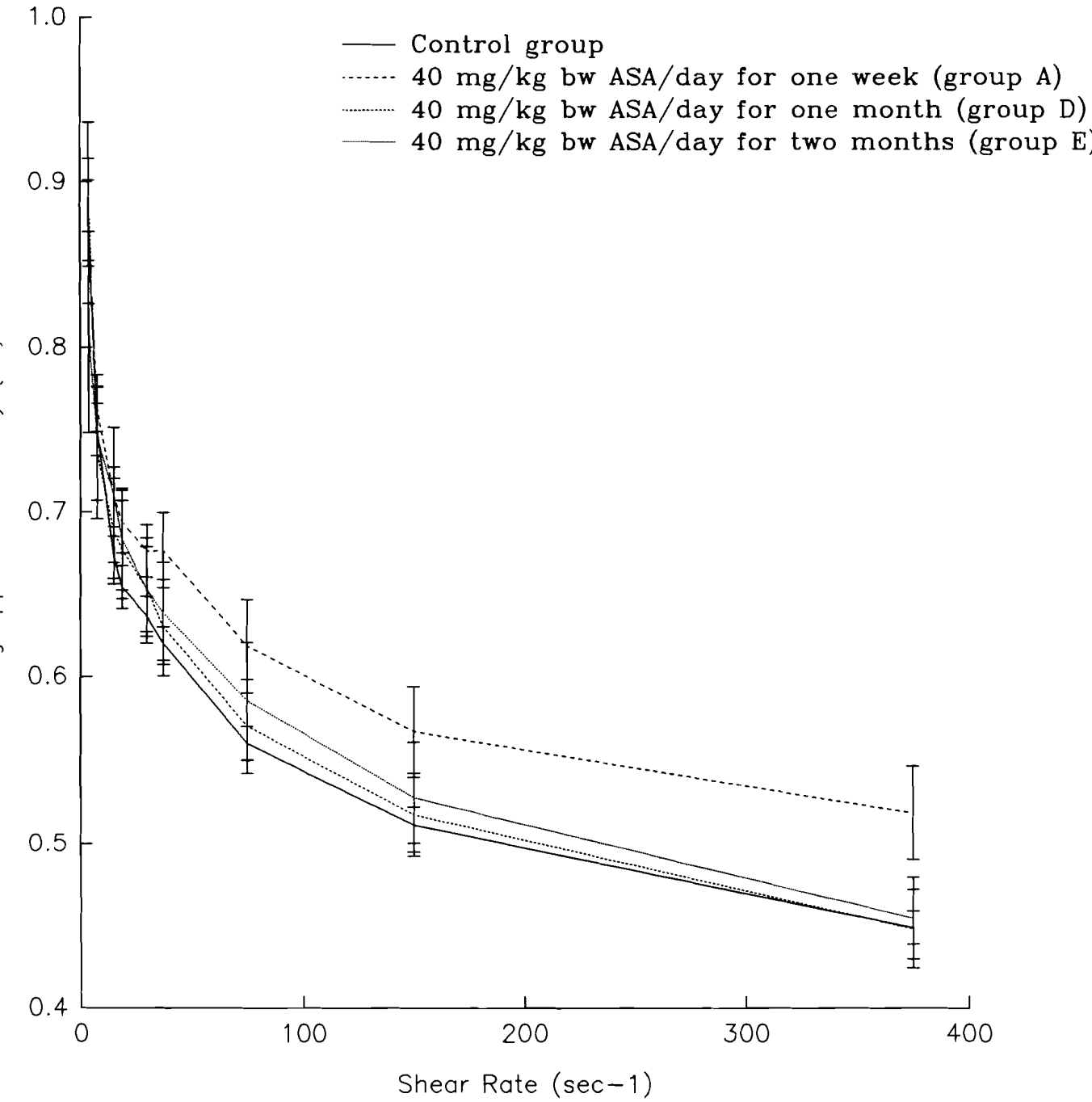

Control group
40 mg/kg bw ASA/day for one week (group A)
40 mg/kg bw ASA/day for one month (group D)
40 mg/kg bw ASA/day for two months (group E)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0
100
200
300
400
Shear Rate (sec−1)

Figure 10. Time dependence: Log apparent viscosity vs. shear rate at a constant hematocrit of 38% (Mean ± SE).

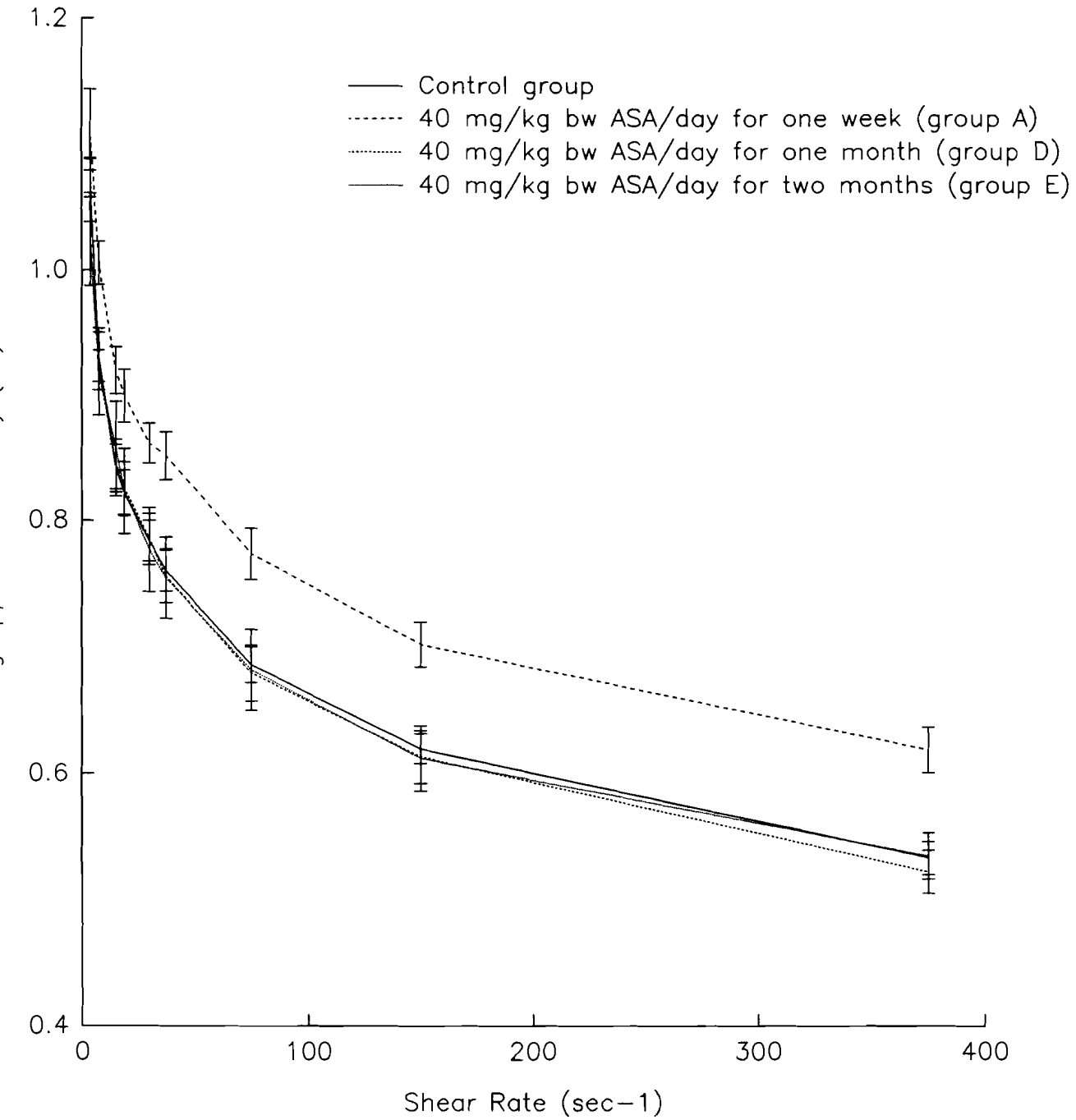

Control group
40 mg/kg bw ASA/day for one week (group A)
40 mg/kg bw ASA/day for one month (group D)
40 mg/kg bw ASA/day for two months (group E)
1.2
1.0
0.8
0.6
0.4
0
100
200
300
400
Shear Rate (sec−1)

Figure 11. Time dependence: Log apparent viscosity vs. shear rate at a constant hematocrit of 45% (Mean ± SE).

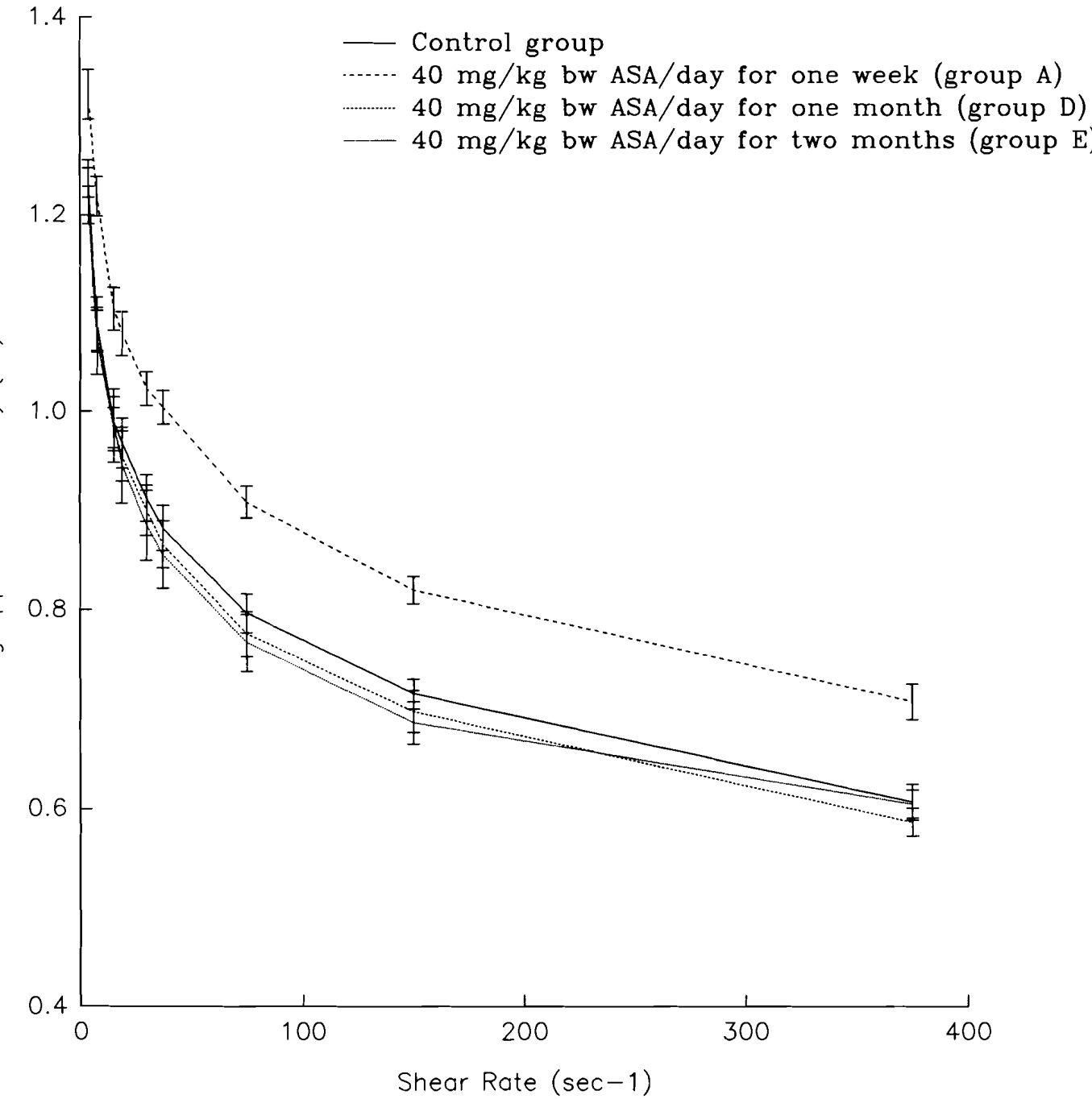

Control group
40 mg/kg bw ASA/day for one week (group A)
40 mg/kg bw ASA/day for one month (group D)
40 mg/kg bw ASA/day for two months (group E)
1.4
1.2
1.0
0.8
0.6
0.4
0
100
200
300
400
Shear Rate (sec−1)

Figure 12. Time dependence: Apparent viscosity vs. shear rate for plasma (Mean ± SE).

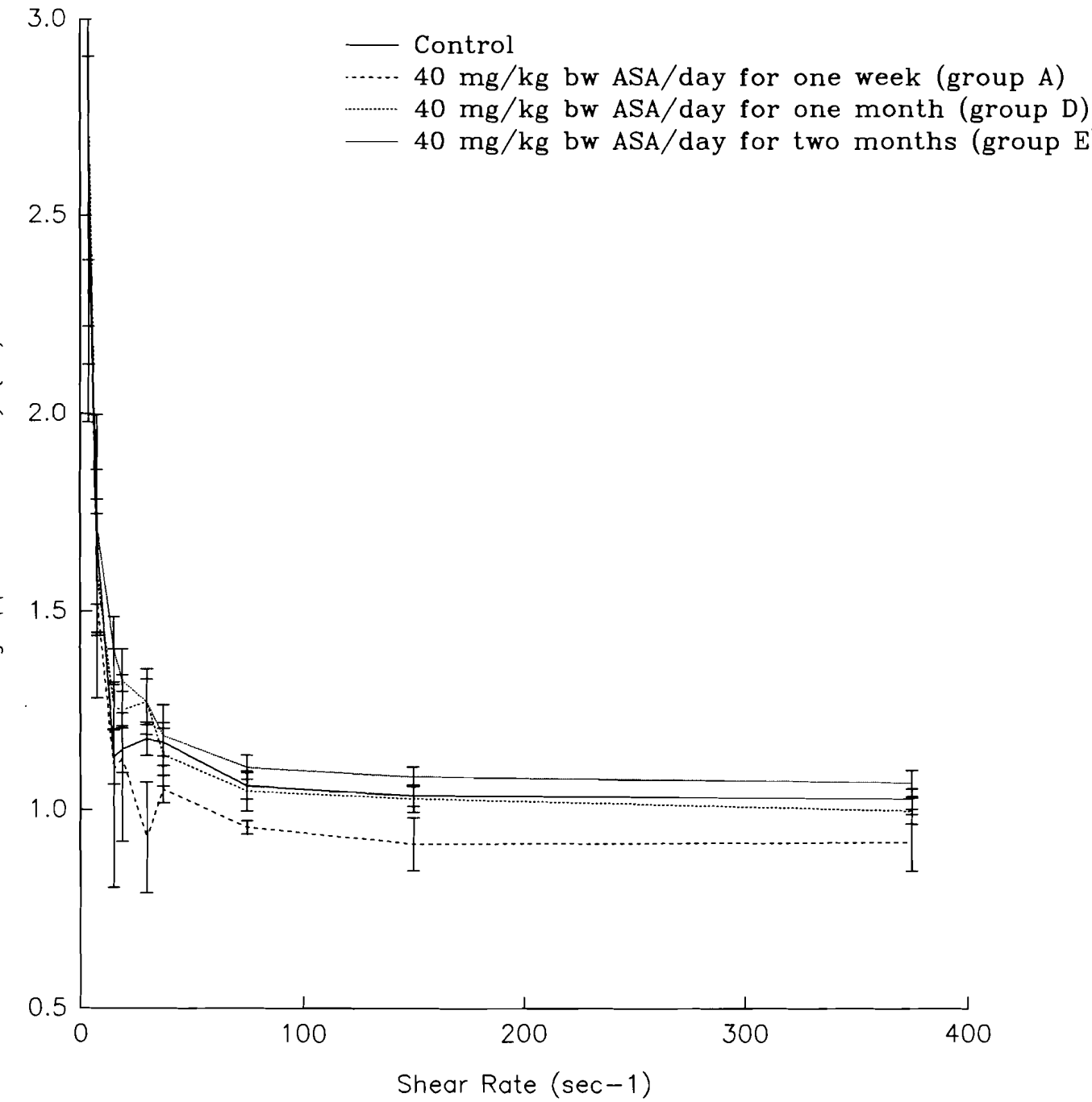

Control
40 mg/kg bw ASA/day for one week (group A)
40 mg/kg bw ASA/day for one month (group D)
40 mg/kg bw ASA/day for two months (group E
3.0
2.5
2.0
1.5
1.0
0.5
0
100
200
300
400
Shear Rate (sec−1)

Table 1. Mean protein concentration for control and experimental groups.

TREATMENT GROUP	PLASMA PROTEIN CONCENTRATION (g/dL)
Control	4.07
10 mg/kg bw ASA/day for one week	3.91
20 mg/kg bw ASA/day for one week	4.36
10 mg/kg bw ASA/day for one month	5.06[a]
10 mg/kg bw ASA/day for two months	3.90

[a] Treatment with subscript is significantly different from all other groups

Table 2. Mean Taylor's factor values for control and treated groups at a shear rate of 150 sec^{-1}.

TREATMENT GROUP	TAYLOR'S FACTOR
Control	0.01555
10 mg/kg bw ASA/day for one week	0.01851
20 mg/kg bw ASA/day for one week	0.01771
10 mg/kg bw ASA/day for one month	0.01693
10 mg/kg bw ASA/day for two months	0.01488

DISCUSSION

A normal circulatory system demands an efficient oxygen delivery, as well as undisturbed blood flow conditions. As Poiseuille postulated in 1846, blood flow is determined primarily by pressure differences, vessel radius, vessel length, and by the viscous properties of the blood (Milnor, 1989). The purpose of my study was to determine whether acetylsalicylic acid could alter blood viscosity and in turn, lead to changes in the hemorheological conditions of the circulatory system, thus benefiting blood flow.

Understanding blood flow dynamics allows scientists to prevent and help in the treatment of blood flow-related disorders. Aspirin does serve as a preventive medication for people who suffer from cardiovascular-related disorders (Dormandy *et al.*, 1982). However, are these effects the result of altered hemorheological conditions due to alterations in blood viscosity, and can ASA alter blood viscosity in *healthy* individuals?

In my study, I found that acetylsalicylic acid does significantly alter the viscosity of the blood in healthy rats. However, blood viscosity changes were found to be significant only in group A (40 mg/kg bw ASA/day for one week) with this group having a consistently higher blood viscosity at hematocrits greater than 30%, and primarily at the highest measured shear rates. Interestingly, I found that group A showed no significant differences in plasma

viscosity compared to all other groups, with the exception of having a significantly lower plasma viscosity compared to group D (40 mg/kg bw ASA/day for one month) at a shear rate of 37.5 sec^{-1}. It is possible that during the experiment, there might have been a shift in protein types, not detectable by the protein concentration determination procedure used in this study. This is important because, although no plasma viscosity differences were observed, changes in plasma composition might lead to changes in whole blood viscosity.

The plasma viscosity data suggest that for the blood of group A, the major effect of ASA was on the physical properties of the red blood cells (plasma viscosity was normal, and whole blood viscosity was above normal). In addition, the same conclusion can be drawn from the data obtained as the hematocrit was increased. Increasing the number of red blood cells increases the contribution from these cells to whole blood viscosity. Group A showed the highest contribution from these cells to whole blood viscosity when compared to all other groups at a constant hematocrit. Both results suggest that ASA's effects on blood viscosity were due to changes in the physical properties of the red blood cells.

Although no statistically significant differences were found in the Taylor's factor data, group A had a higher T value when compared to the other groups at a shear rate of

150 sec^{-1}, indicating that the red blood cells of group A were somewhat less deformable than the cells of the other groups. Lack of statistical differences in the Taylor's factor resulted from the equation being less sensitive at hematocrits less than 60% and at a shear rates less than 150 sec^{-1} (Aarts *et al.*, 1984). However, data is in accordance with the data of Saniabadi *et al.*, (1991) in which a *modest decrease* in red blood cell deformability was found when blood samples from healthy individuals were analyzed two hours after subjects were treated with 300 mg of aspirin.

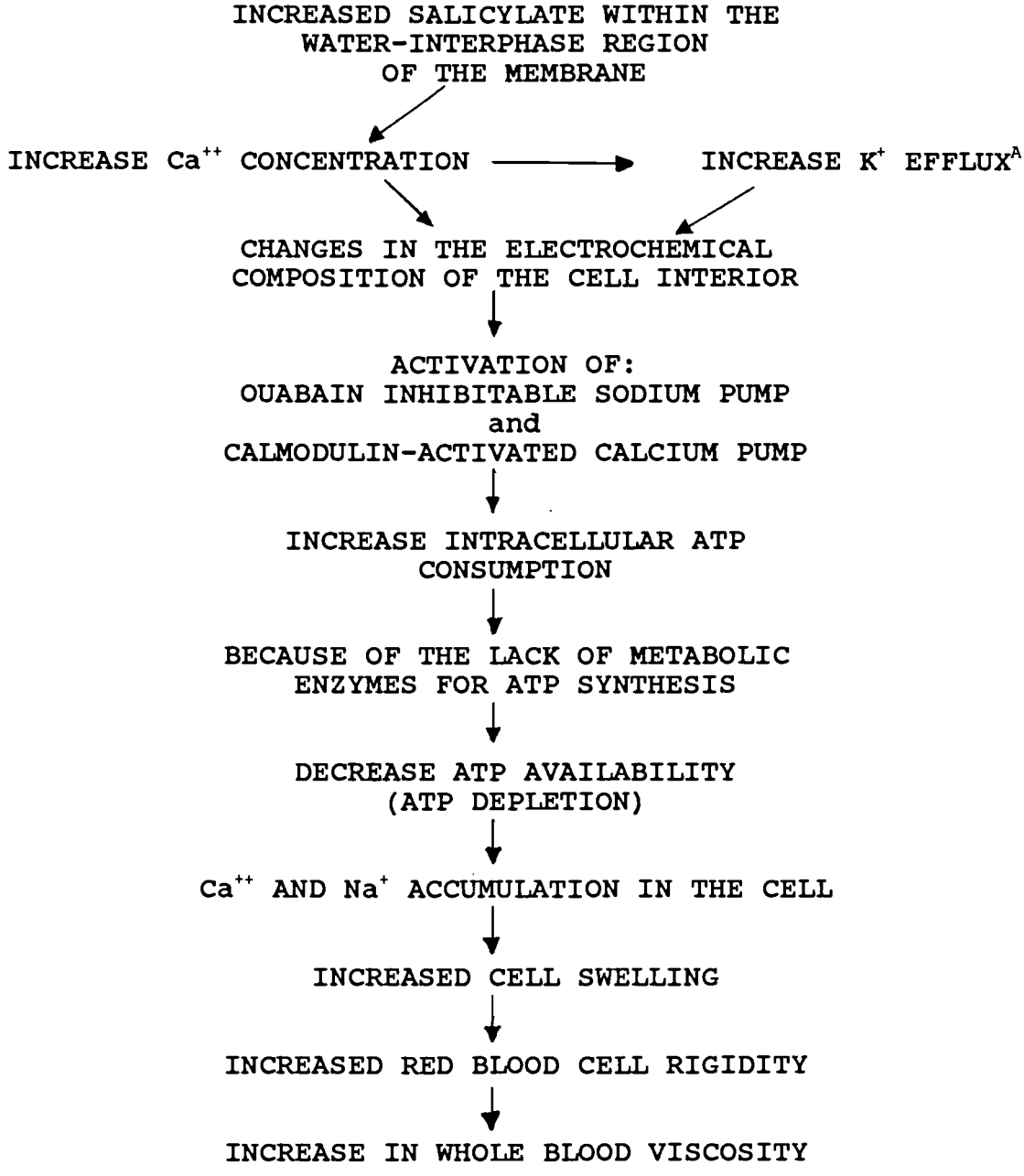
The effects of ASA on the red blood cells might be the result of ASA being hydrolyzed into acetic acid and salicylate, and in turn, salicylate acting on the red cell membrane. In 1979, Burgin and Schatzmann described the effects of salicylate and Ca^{++} on the red blood cell membrane. In their study, they found that salicylate penetrated the membrane-water interphase of the red blood cell, resulting in an increased Ca^{++} permeability, thereby leading to an increase in the intracellular [Ca^{++}]. As calcium penetrates the red cell membrane, it imparts negative charges on the membrane as well as changes to the biochemistry of the cell (Burgin and Schatzmann, 1979). Calcium penetration leads to phospholipid redistribution (Lin *et al.*, 1994; Williamson *et al.*, 1992), which in turn causes changes in red blood cell shape from a normal

biconcave discocyte to a crenated echinocyte (Lin *et al.*, 1994). Finally, increases in intracellular [Ca^{++}] lead to intracellular ATP depletion (Weed *et al.*, 1969) resulting in RBC membrane rigidity.

As intracellular [Ca^{++}] increases, there is an increase in Na^{+} concentration within the cell, which in turn results in water following Ca^{++} and Na^{+}, causing cell swelling (Beutler *et al.*, 1995). Cell swelling not only increases the volume of the red blood cell but also increases the internal viscosity of these cells (Mohandas *et al.*, 1980). As a result, the cell loses its flexible properties, and is unable to deform under high shear rates or as the cell flows through capillaries. Figure 13 is an attempt to explain the possible cascade of events brought about by salicylate penetration to the red blood cell membrane.

It was interesting to find that at the highest dosage used in my experiment (80 mg/kg bw ASA), the viscosity of the blood was neither increased nor decreased. It is possible that the circulatory system of animals exposed to high salicylate concentrations adapts to the effects of salicylate on the red cell membrane through the faster removal of rigid red blood cells. As a result, less rigid immature red blood cells (reticulocytes) are release into the blood in order to maintain a normal hematocrit, and thus normal oxygen supply to the tissues. This, in turn, results in viscosity being unaltered. At high concentrations,

Figure 13. Possible effects of salicylate on blood viscosity.



[A] (Lauf *et al., 1992)*

salicylate's excretion is time dependent (Akopov *et al.*, 1992; Pedersen and Fitzgerald, 1984). Therefore, in group B, there was likely a higher amount of unbound salicylate molecules available in the plasma, thus increasing the number of possible affected red blood cells. This would result in increased removal of these cells from the blood, coupled by an increase in the release of reticulocytes (Beutler *et al.*, 1995).

Reticulocytes have different metabolic characteristics than erythrocytes. Reticulocytes have a higher metabolic energy capacity as a result of a higher density of metabolic enzymes, and thus have greater ATP synthesis (Beutler *et al.*, 1995). This is an advantage as, although reticulocytes might be affected by salicylate, the rate at which red blood cell become rigid will be slower as compared to mature red blood cells because of the higher metabolic capacity of the reticulocytes.

I suggest that if the ratio of *rigid* to *non-rigid* red blood cells exceeds a certain limit, the circulatory system will respond to these changes by the removal of *rigid* cells and the addition of newer cells (highly deformable reticulocytes) into the blood. If the above hypothesis is true, the results observed in groups B, D, and E are the result of changes in the ratio of mature vs. immature red blood cells, which in turn, affects the overall viscosity of

the blood (Fedde *et al.*, 1996). As the ratio of rigid cells increases, these cells have greater resistance to flow because their decrease membrane deformability, larger volume, and greater internal viscosity, resulting in a greater contribution to the overall viscosity of the blood. On the other hand, younger red blood cells are highly deformable because of their high metabolic capacity, hence decreasing whole blood viscosity.

Blood viscosity is a coefficient of friction that integrates the resistance to flow of cell layers as well as the interaction between these cells layers with the vessel wall. As the number of rigid cells increase, there is an increase in the shear stress created by the interaction of these cells with the vessel wall and adjacent cell layer (Fung, 1981). Rigid cells flow in the layers closest to the vessel wall where the resistance to flow and the shear stresses are greater; therefore viscosity is greater. In other words, a greater ratio of rigid to deformable red blood cells, will lead to an overall increase in blood viscosity. In contrast, deformable cells flow at a faster rate along the central axis of the vessel where shear rates are high, and therefore viscosity is low (Fung, 1981; Milnor, 1989). If the ratio of rigid to non-rigid red blood cells is in balance, the overall viscosity of the blood remains unchanged.

Acetylsalicylic acid might initially cause an increase

in blood viscosity in healthy individuals, triggering an adaptive response that returns blood viscosity to normal values. From my experiment I found that 40 mg/kg bw of acetylsalicylic acid for one week affected the red blood cells of healthy rats. It is possible that 40 mg/kg bw ASA in one week was not a sufficiently large dosage nor long duration to stimulate the removal of rigid cells from the blood and the addition of newer red blood cells. On the other hand, increasing the ASA dose (80 mg/kg bw) might have affected a greater number of red cells in the blood, taking less time to reach the set point (initial increase in blood viscosity) that stimulates the adaptive response that resulted in an overall normal blood viscosity for group B. Finally, 40 mg/kg bw of ASA administrated to rats for one month and two months respectively, showed no change in blood viscosity. I suggest that during the early portion of the treatment periods the blood viscosity increased as a result of an increase in the ratio of rigid to non-rigid cells, again stimulating the removal of rigid cells from the blood and addition of reticulocytes. This removal likely must have occurred after one week but before one month of treatment.

Although neither 40 mg/kg bw nor 80 mg/kg bw of ASA are normal dosages for humans, it is possible to expect similar results observed in this experiment were humans to take an aspirin tablet (325 mg/day) for a relatively long period of

time. That is, there might be an initial increase in blood viscosity, but after a certain time period, the ratio of rigid (cells affected by salicylate) and non-rigid cells could reach the set point that would stimulate the removal of rigid cells and the addition of newer cells. As a result, blood viscosity would return to pre-aspirin treatment values.

With regards to future research, it would be interesting to test whether acetylsalicylic acid does change the ratio of erythrocytes to reticulocyte in the blood. In order to test such an adaptive mechanism to increased red blood cell rigidity, one could measure erythropoietin levels, reticulocyte numbers, osmotic fragility, or pass red blood cells through a sieve tube (Gregersen *et al.*, 1967) in order to determine the ratio of deformable to non-deformable cells.

In conclusion, at high dosages, ASA appears to alter the viscous properties of the blood by increasing the ratio of rigid to non-rigid cells. However, the circulatory system appears to respond to such changes by an unknown mechanism(s) that brings blood viscosity back to normal values. Although yet to be confirmed, such a mechanism might be the increased removal of rigid cells and the replacement of non-rigid cells into the blood.

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David K. Saunders
Signature of Major Advisor

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Effects of acetylsalicylic acid on blood viscosity in healthy rats (*Rattus norvegicus*)
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August 19, 1997
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