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STUDY OF RED CELL AGGREGATION IN PULSATILE FLOW FROM ULTRASONIC DOPPLER POWER MEASUREMENTS

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ABSTRACT **Human red cell aggregability and disaggregability represent important hemorheological parameters of blood. Several techniques have been proposed to evaluate the tendency of red cells to form aggregates and to disrupt in the presence of shear stress. One of the most recent approaches is based on the characterization of the intensity of ultrasonic scattered signals. A pulsatile flow loop model is used in the present study to demonstrate the potential applicability of Doppler ultrasound to detect and characterize the hemodynamic behavior of red cell aggregates. Porcine whole blood specimens collected from 20 different pigs were circulated in the flow model (tube diameter of 0.476 cm) at different mean velocities and pulsation rates. At a pulsation of 70 beats/min for mean velocities of 13 cm/sec and 63 cm/sec, no cyclic variation of the Doppler power was observed, suggesting the absence of rouleaux build-up and rouleaux disruption. At a pulsation of 20 beats/min and mean velocities of 11 cm/sec and 38 cm/sec, statistically significant cyclic variations ($p < 0.01$) were measured. It is suggested that aggregate size enlargement, rouleaux orientation with the flow field and the effect of shear stress on rouleaux disruption are possible causes for the observed cyclic variation of the Doppler power within the flow cycle at a pulsation of 20 beats/min. A discussion of the potential application of this technique for *in vivo* study in large vessels is given.**

Introduction

Clinical Significance

The aggregation of red blood cells is a dynamic and reversible process that occurs when macroproteins in the plasma such as fibrinogen, haptoglobin, ceruloplasmin, α_1 acid-glycoprotein, α_1 antitrypsin, c-reactive protein, and immunoglobulins (Dacie and Lewis, 1991) link two or more red cells to form cylindrical rouleaux, branched rouleaux, loop and very complex three-dimensional network (Shiga *et al.*, 1983; Skalak, 1984; Samsel and Perelson,

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1984; Fabry, 1987). It is now generally accepted that several factors may influence the growth of the aggregates in *in vitro* laboratory preparation including the concentration of plasma macromolecules, the balance between attractive and repulsive forces, the shape and deformability of red cells, the hematocrit, the temperature, and the pH which affects the volume of red cells (Shiga *et al*, 1983; Miale, 1982; Tomita *et al.*, 1986; Maeda *et al.*, 1988; Donner *et al.*, 1988). Under flow conditions, the formation and break-up of aggregates are governed by another factor, specifically the shear forces (Murata and Secomb, 1988, 1989; Chabanel and Samama, 1989; Chien *et al.*, 1990). At low shear rates, the aggregation of red cells tends to increase because the flow dynamics help in bringing the cells closer together and increase the probability of cell bridging (Chien, 1976). However, as the shear rate further increases, it was found that for human blood, the size of the aggregates decreases. For shear rates higher than approximately 46 s^{-1}, no more aggregates are observed for human blood (Schmid-Schönbein *et al.*, 1968). A value of disaggregation shear rate of 59 s^{-1} has recently been reported for normal human blood adjusted to 40% hematocrit (Chabanel and Samama, 1989).

One of the rheological properties of human blood is an increase in its viscosity at low shear rates associated with the presence of red cell aggregation. The evaluation of the erythrocyte aggregation tendency, disaggregation shear rate and the measurement of the sedimentation rate can be of valuable interest in clinical medicine. The vast majority of acute or chronic infections, tissue damage and most degenerative diseases are associated with changes in the concentration of plasma proteins which can lead to an increase in red cell aggregation and sedimentation rate (Dacie and Lewis, 1991; Miale, 1982; Bicher, 1972). Moreover, the slowdown in blood circulation due to an increase in blood viscosity and presence of aggregates may reduce capillary perfusion and cause ischemia, necrosis or tissue infarction. It may also cause anemia due to red cell trapping, decreased cardiac output, increased peripheral resistance, and decreased oxygen consumption and carbon dioxide elimination (Bicher, 1972). Over the last few years, studies have been reported on the increase in red cell aggregation in patients with coronary artery disease (Neumann *et al.*, 1989, 1991; Hahn *et al.*, 1989), vascular occlusion (Tanahashi *et al.*, 1989; Ohta, 1989; Glacet-Bernard *et al.*, 1990), arterial hypertension (Razavian *et al.*, 1991; Zannad *et al.*, 1988), myocardial infarction and cerebrovascular accidents (Hayakawa and Kuzuya, 1991), diabetes (Hayakawa and Kuzuya, 1991; Le Devehat *et al.*, 1990), pregnancy (Huisman *et al.*, 1988), vulvar, cervix, endometrial, and ovarian cancers (Miller and Heilmann, 1989), and sickle cell anemia (Obiefuna and Photiades, 1990). The detection and quantification of red cell hyper-aggregation has even recently been proposed as a prognostic method to identify a subgroup of patients with unstable angina at a high risk of acute myocardial infarction (Neumann *et al.*, 1991).

Laboratory Evaluation of Erythrocyte Aggregation

Several *in vitro* laboratory methods based on the transmission and reflection of light have been proposed to quantify the aggregation of erythrocytes under varying shear rate conditions induced generally by circulating blood in concentric tubes or in a commercially available viscometer (Shiga *et al.*, 1983; Tomita *et al.*, 1986; Donner *et al.*, 1988; Chabanel and Samama, 1989; Brinkman *et al.*, 1963; Volger *et al.*, 1975). Some methods involve individual counting of the aggregates (Shiga *et al.*, 1983; Obiefuna and Photiades, 1990), while others are based on the quantification of the variation in light intensity. In methods involving cell counting and sizing, a dilute preparation of the blood sample (= 0.25% hematocrit) is needed to allow visualization of cells.

Only limited shear rate conditions can be tested because at high shear rates the erythrocytes move too rapidly to be visualized and at low shear rates, sedimentation of the red cell particles dominates over rouleaux formation (Shiga *et al.*, 1983). Another difficulty with the technique based on the characterization of light intensity is that it can only be applied with a thin blood layer at normal hematocrit because of the high absorption of light in blood by the hemoglobin. A compromise has to be made between the light source intensity and wavelength, the hematocrit and the blood layer thickness used in the concentric tube or in the viscometer. An approach using infrared light produced by gallium arsenide diodes has been proposed in the design of a red blood cell aggregameter to reduce the light absorption limitation (Tomita *et al.*, 1986).

The use of ultrasound has recently been proposed as an alternative to estimate the size of red blood cell aggregates, rouleaux dynamics and sedimentation rate (Boynard *et al.*, 1987; Kim *et al.*, 1989; Boynard and Lelièvre, 1990). The feasibility of the ultrasonic approach to the presence of aggregates induced by dextran and fibrinogen was first demonstrated by Shung and Reid (1979). Later, the shear rate dependence of rouleaux formation and the associated changes in ultrasound echogenicity was evaluated by others (Sigel *et al.*, 1982; Beitler *et al.*, 1983; Kallio and Alanen, 1988; Yuan and Shung, 1988; Shung *et al.*, 1992). In a recent study (Shung and Lam, 1991), a linear correlation was found between the ultrasonic backscattering coefficient and blood fibrinogen concentration. Using the commercially available SEFAM aggregameter (Chabanel and Samama, 1989), a linear correlation had also been established between the aggregation index and fibrinogen concentration; a non-linear correlation was measured between the disaggregation shear rate and fibrinogen concentration.

Backscattering of Ultrasound from Blood

While experimental results showed that ultrasound backscattering may allow measurement of the size of red blood cell aggregates (Boynard and Lelièvre, 1990), efforts were also underway over the last few years to develop theoretical models that can be used to interpret the experimental findings concerning factors affecting the echogenicity of blood (Twersky, 1988; Mo and Cobbold, 1992). From these models, it was understood that the ultrasonic backscattered signal from blood is a function of the frequency of the transmitted signal, the volume of the scatterers, the compressibility and density of red cells and plasma, the hematocrit, and the packing factor which is a measure of the interaction among scatterers. In Twersky's model (Twersky, 1988; Berger *et al.*, 1991), the packing factor was shown to be related to the hematocrit, the shape and correlation among scatterers and the variance in particle size. In the Mo and Cobbold model (1992), the packing factor was replaced by a variable describing the average variance of local red cell concentrations within elemental voxels defined in relation to the wavelength of the transmitted wave. Based on this last model (1992), it was postulated that ultrasound backscattering should be sensitive even to a small degree of aggregation (2 or 3 cells). However, the influence of the other parameters on the scattered signal has to be considered as well.

In the present study, of all factors that can theoretically affect the backscattering from blood, only the volume of the scatterers and the packing factor can change within the flow cycle. The change in the volume of the aggregates within the flow cycle represents the basis of the hypothesis supporting the present work. Possible modification of the packing factor

within the flow cycle has, however, to be kept in mind when interpreting our results.

Hypothesis and Objective

It is hypothesized that the changes in Doppler scattering power from whole blood during the flow cycle may be primarily associated with the formation and break-up of red cell aggregates caused by the changes in shear stress and flow dynamics within the flow cycle. In other words, it is assumed that contributions from other factors to changes in Doppler power, such as flow disturbance, red cell alignment, and changes in the correlation among scatterers (variation of the packing factor) are significantly smaller or not present. Based on this hypothesis, the objective of the present study is to demonstrate that Doppler ultrasound can be a valuable tool for studying the dynamics of red cell aggregation in pulsatile flow. The approach proposed relies on the measurement of the variation in Doppler backscattering power within the flow cycle. Observations of changes in Doppler power and ultrasound echogenicity within the flow cycle were made recently in *in vitro* and *in vivo* experiments with whole blood (Cloutier and Shung, 1991; De Kroon *et al.*, 1991). In these studies, the variation of the backscattered signals was presumed to be associated with changes in the state of erythrocyte aggregation. A second objective of the present study is, then, to confirm the influence of the state of erythrocyte aggregation and disaggregation on the variation of the backscattered power within the flow cycle.

Methods

A complete description, along with a diagram of the flow loop model used in the present study, can be found in Cloutier and Shung (1993). The description of the experimental arrangement, blood preparation, technical specification of the Doppler ultrasonic system, and digital processing of the data is summarized here. The main flow conduit, immersed horizontally in a water tank to allow acoustic coupling, was a cylindrical high-pressure polyethylene tube with an inner diameter of 0.476 cm and a length of 90 cm. A polyethylene tube was selected because of its good acoustic properties. The polyethylene tube was connected to larger size Tygon tubes on both sides to close the flow loop. On one side, the Tygon tube was joined to a blood reservoir of 1 liter and on the other side to the output of a Harvard pulsatile pump (model 1423). Another tube was located between the reservoir and the input of the pump. Depending on the flow velocity needed in the main polyethylene flow conduit, a shunt tube fitted to a valve was connected between the output of the pump and the reservoir to obtain a wider range of flow adjustment. A volume of approximately 1.5 liters of blood was needed to fill the flow loop model.

Porcine whole blood collected from a local slaughterhouse was circulated in the model. A solution of 1 g of EDTA in 10 ml of saline was added to the blood at a concentration of 30 ml per liter of blood to prevent coagulation. The blood was stored at 4° C for an averaged period of 1.5 ± 0.9 day (mean ± standard deviation, n = 20) before the experiments. The day before the experiment, the whole blood was centrifuged and the top white cell layer was removed. The concentrated erythrocytes were recombined with the plasma to 40% hematocrit and circulated in the flow loop for 1 to 2 hours, at high flow and pulsation rates, to eliminate bubbles before starting measurements. The monitoring of the Doppler signal allowed visual assessment of air bubbles (Spencer *et al.*, 1969). Another advantage to circulating blood before

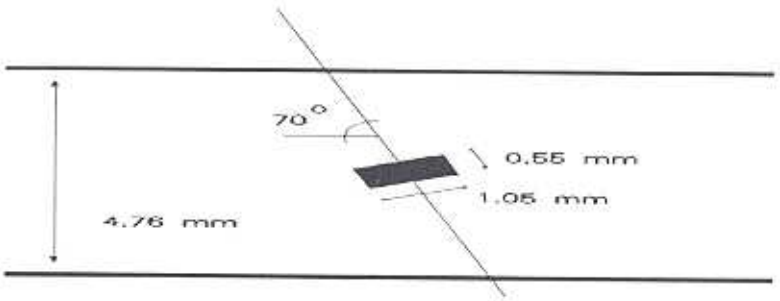

Fig. 1. Relative position and size of the Doppler sample volume.

measurements was to ensure that large aggregates formed during blood storage were disrupted before digitizing Doppler signals. Continuous mixing of the blood solution in the reservoir was performed by a magnetic stirrer. A Clay-Adams microcentrifuge (Readacrit, 8000 rpm/6300g) was used to measure the hematocrit by centrifuging blood over 5 minutes. All experiments were performed at room temperature (25.3 ± 1.8° C, n = 20). For each experiment, the Reynolds number was evaluated to confirm the presence of laminar flow.

The Doppler ultrasonic probe used to transmit and receive the pulsed-wave acoustic signals was located approximately 50 cm from the entrance of the polyethylene tube in the water tank. The angle between the tube and the Doppler probe was maintained at 70°. The distance between the face of the transducer and the location of the Doppler sample volume was 1.5 cm. The size of the sample volume determined experimentally was 0.5 mm^3. The ultrasonic beam at the site of measurement was axially symmetrical and cylindrical. The beam divergence was minimal over the length of the sample volume. All Doppler measurements were performed by positioning the sample volume at the center of the tube. The Doppler flow-meter developed by Hartley (1980) was operated with a 10 MHz non-focused transducer fired at a pulse repetition frequency of 40 kHz. The frequency response of the flow-meter has been compensated numerically to obtain a flat band-width between frequencies of 0 Hz to 20 kHz. The position and dimension of the Doppler sample volume are shown in Fig. 1.

A square-wave electromagnetic flow-meter (Carolina Medical Electronics, model 501) was used along with a blood flow probe (*In Vivo* Metric, model K) to monitor the instantaneous mean flow rate within the tube. The flow-meter was calibrated by timed volume measurements. The off-set of the flow-meter was adjusted before each experiment and its output signal was digitized to monitor the flow wave-form. The output signal was also used to synchronize the digitization of the audio Doppler signals and to monitor and adjust, if necessary, the pulsation rate of the Harvard pulsatile pump.

The forward audio output of the pulsed-wave Doppler system and the flow rate signal from the electromagnetic flow-meter were digitized by a Hewlett-Packard (HP) digitizing scope model 54501A to 8 bits at a rate of 51.2 kHz. Acquisition of the Doppler signals at different moments within systole and early

diastole was realized by triggering the flow-rate signal and adjusting the delay of the HP oscilloscope. An IEEE-488 interface board (Metrabyte, model MBC-488) was used to transfer the digitized data to a 386 personal computer. The digitized signal was windowed with a 10 ms Hanning function (512 samples), zero padded to 1024 samples and fast Fourier transformed (FFT) to obtain a spectral representation of the Doppler signal. Mean Doppler power spectra were then calculated at different moments of the flow cycle by averaging power spectra over 100 cycles. The mean power of the Doppler signals in decibels (dB) was determined in the frequency domain by computing the following equation:

$$\text{mean power} = 10 \times \log \left(\frac{1}{N} \sum_{k=1}^{N} P_k(f) \right),$$

where P represents the power, f the frequency, k the sample number, and N the number of samples between 0 Hz and 20 kHz. The mean power in dB provides a relative measurement of the backscattered Doppler signal. Fig. 2 shows an example of a mean Doppler power spectrum of porcine whole blood at 40.3% hematocrit for a mean velocity within the sample volume of 228 cm/sec.

The mean velocity within the sample volume was obtained by computing the mathematical mean value of the Doppler spectrum. The transformation of the frequency axis to a velocity axis can be obtained by using the Doppler equation ($V = c\, f_d / 2\, f_o \cos \theta$), where V is the blood velocity, c the velocity of sound in blood (1570 m/sec), f_d the Doppler frequency shift, f_o the transmitted frequency of the Doppler sine bursts (10 MHz), and θ the angle between the ultrasonic beam and the flowing blood (70°) assuming that blood flow is parallel to the tube axis.

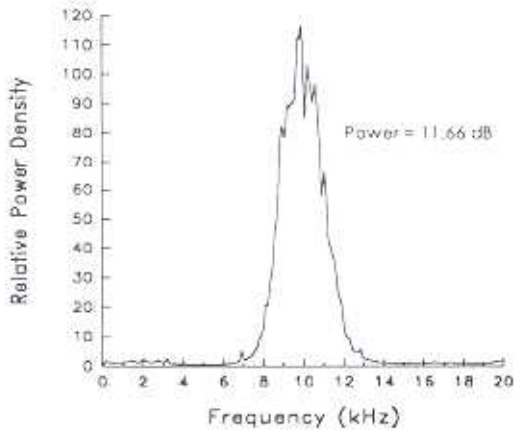

Fig. 2. Example of a mean Doppler power spectrum averaged over 100 cycles for porcine whole blood at 40.3% hematocrit flowing at a mean velocity within the sample volume of 228 cm/sec (mean Doppler frequency shift = 9,947 Hz). The power of the mean spectrum in dB is also shown.

Results

The behavior of red cell aggregates was studied by circulating blood in the model under four different experimental pulsatile flow wave-forms. The instantaneous mean velocity *vs.* time traces shown in the present study were obtained from an electromagnetic flow-meter and a Doppler flow-meter. The electromagnetic flow-meter allows measurements of the mean velocity within the tube, while the Doppler flow-meter gives measurements of the range of velocity present within the sample volume located at the center of the tube. Consequently, the Doppler mean velocities presented in Figs. 4.1 and 4.2 are greater than the velocities obtained with the electromagnetic flow-meter. These wave-forms obtained from the output of the electromagnetic flow-meter are presented in Fig. 3. A first series of experiments was performed at a pulsation rate of 70 beats/min and another series at a pulsation of 20 beats/min. Two ranges of velocity were used for both pulsation rates. Experiments were undertaken at velocities at peak systole of approximately 205 cm/sec and approximately 75 cm/sec. The instantaneous mean flow velocity *vs.* time trace (Fig. 3C) at a pulsation of 70 beats/min and at a velocity at peak systole of 75 cm/sec is similar to that found in normal human abdominal aorta (Milnor, 1989). Estimates of the mean velocity and mean Reynolds number (Re) calculated over a flow cycle, and Re at peak systole for each instantaneous mean flow velocity *vs.* time trace are presented in Table 1. In the computation of the Reynolds number (Milnor, 1989), the dynamic viscosity of whole blood and density were assumed at 4 cP and 1.093 g/cm^3, respectively. The Reynolds number was computed even if a Poiseuille flow was not present throughout the flow cycle (Milnor, 1989).

Results obtained at a velocity at peak systole of approximately 205 cm/sec, as measured with the electromagnetic flow-meter, are presented in Fig. 4.1. Each data point shown in this figure corresponds to the average of 5 experiments performed with different blood samples. No significant variation of the mean power within the flow cycle (one-way analysis of variance with repeated measurements, $p = 0.967$) was obtained at a pulsation of 70 beats/min (panel A), suggesting perhaps the absence of rouleaux formation and disruption. The average mean power within the flow cycle for this series of experiments was 6.6 ± 0.6 dB (mean ± standard deviation). By keeping the range of velocities the same but decreasing the pulsation rate to 20 beats/min (panel B), the red cells were probably brought into contact during the stoppage of flow (approximately 2 secs) and eventually formed rouleaux (Kim *et al.*, 1989). In this case, a significant cyclic variation of the Doppler power was measured ($p < 0.0001$). In early systole, the mean power first increased by 4.7 dB and then abruptly dropped by 6.8 dB before peak systole. In early diastole, the mean power slowly increased by 0.8 dB. The minimal and maximal mean power values within the flow cycle were 6.4 ± 0.4 dB and 13.4 ± 1.4 dB, respectively. This important power difference within the flow cycle reveals significant modifications in the dynamics of the aggregates. The abrupt reduction of the mean power before peak systole is probably associated with the disruption of rouleaux. The increase in mean power during the acceleration of flow suggests an increase in the size of the aggregates and possible rouleaux orientation with the flow field which will be discussed later.

Table 1

Mean Velocity (V_{mean}), Mean Reynolds Number (Re_{mean}) and Reynolds Number at Peak Systole (Re_{peak}) of the Instantaneous Mean Velocity *vs.* Time Traces Presented in Fig. 3

Flow Traces	V_{mean} (cm/sec)	Re_{mean}	Re_{peak}
A	63	822	2692
B	38	497	2655
C	13	174	980
D	11	144	952

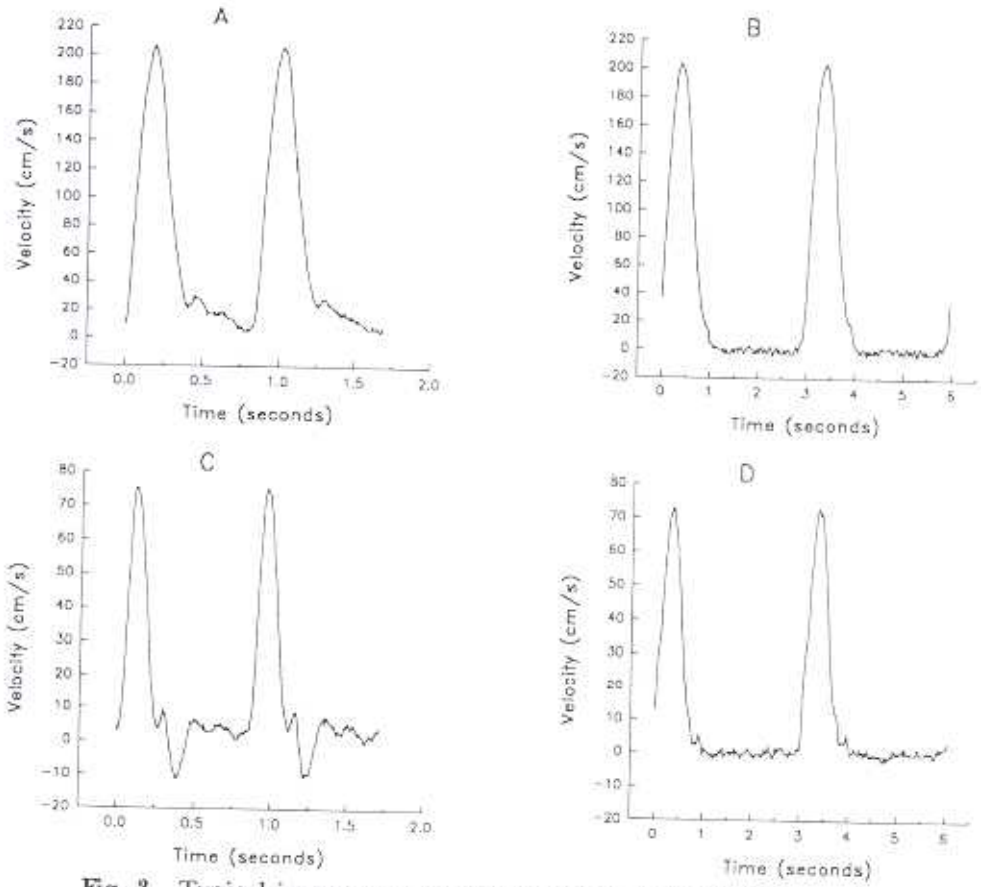

Fig. 3. Typical instantaneous mean velocity *vs.* time traces used. The velocities at peak systole for panels A and B were adjusted to approximately 205 cm/sec and those for panels C and D to approximately 75 cm/sec. Panels A and C correspond to a pulsation of 70 beats/min and panels B and D to a pulsation of 20 beats/min.

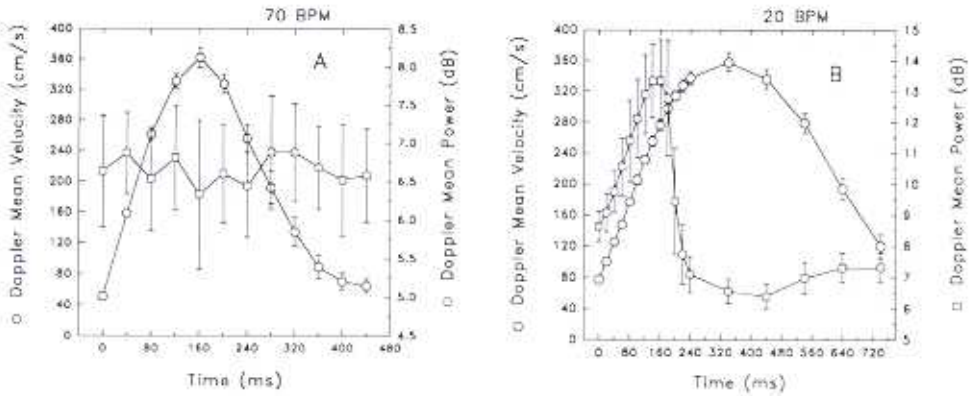

Fig. 4.1. Mean velocity within the Doppler sample volume and mean power as a function of the timing within systolic and early diastolic periods. Panel A corresponds to a pulsation of 70 beats/min (mean hematocrit H = 40.3 ± 0.1%) whereas panel B corresponds to a pulsation of 20 beats/min (H = 40.2 ± 0.3%). Both series of experiments were performed at a velocity at peak systole of approximately 205 cm/sec, as measured with the electromagnetic flow-meter. Each data point represents the mean ± standard deviation computed over 5 experiments.

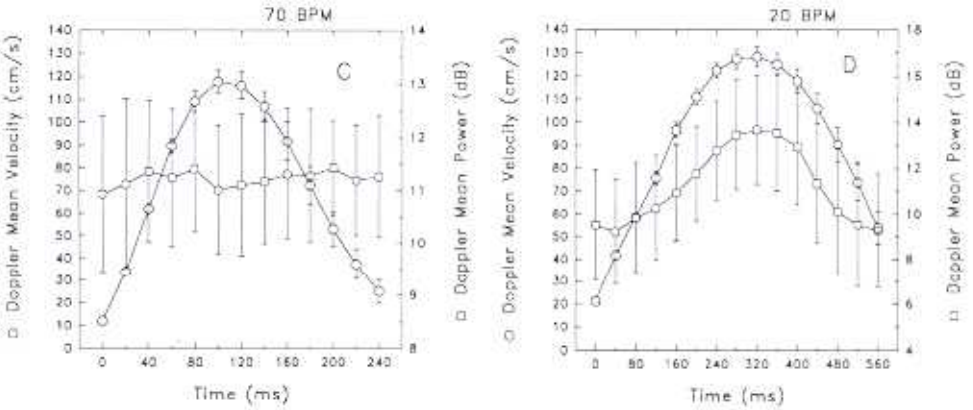

Fig. 4.2. Mean velocity within the Doppler sample volume and mean power as a function of the timing within systolic and early diastolic periods. Panel C corresponds to a pulsation of 70 beats/min (H = 39.9 ± 0.7%), whereas panel D corresponds to a pulsation of 20 beats/min (H = 39.9 ± 0.6%). Both series of experiments were performed at a velocity at peak systole of approximately 75 cm/sec as measured with the electromagnetic flow-meter. Each data point represents the mean ± standard deviation computed over 5 experiments.

Table 2

Summary of Minimal, Mean, and Maximal Power Values of Doppler Spectra Corresponding to the Instantaneous Mean Flow Velocity *vs.* Time Traces Shown in Fig. 3

Flow Traces	Minimal Power (dB)	Mean Power (dB)	Maximal Power (dB)
A	6.3 ± 1.0	6.6 ± 0.6	6.9 ± 0.6
B	6.4 ± 0.4	9.6 ± 2.6	13.4 ± 1.4
C	10.9 ± 1.5	11.2 ± 1.2	11.4 ± 0.9
D	9.2 ± 2.3	11.2 ± 2.7	13.7 ± 2.4

Experiments were also performed at a lower mean velocity at peak systole to simulate physiological conditions. Panel C of Fig. 4.2 shows results obtained at a velocity at peak systole (measured with the electromagnetic flow-meter) of 75 cm/sec, mean velocity of 13 cm/sec and pulsation of 70 beats/min. Under this experimental condition simulating the flow in normal human abdominal aorta, no variation of the Doppler mean power was obtained ($p = 1.0$). The average mean power within the flow cycle was 11.2 ± 1.2 dB. In panel A of Fig. 4.1, we had also observed the absence of Doppler power variation within the acceleration and deceleration phases of the flow cycle for a pulsation of 70 beats/min. However, the average mean power was lower, at 6.6 dB. The reduction of the maximal velocity from 205 cm/sec to 75 cm/sec, and consequently, the reduction of the mean shear rate within the tube, seems to suggest that aggregates are present for the experimental conditions presented in panel C. However, the short duration of the diastole, on one hand, and the reduced shear stress, on the other, may have provided a steady state of rouleaux formation and disruption with a greater average size of aggregates.

In panel D of Fig. 4.2, the dynamics of the aggregation also varied within the flow cycle. A statistically significant variation of the mean power was obtained ($p = 0.009$). During the acceleration of blood, the mean power increased by 4.5 dB to reach a maximum at peak systole. During the deceleration of flow, the mean power dropped by 4.4 dB. This cyclic variation of the Doppler power suggests a dynamic increase in the size of the aggregates in systole and a decrease during early diastole. Rouleaux orientation might also be involved. The maximal mean power at peak systole was 13.7 dB, which is similar to the maximal value of 13.4 dB (panel B), measured also at 20 beats/min, but at a velocity at peak systole of 205 cm/sec instead of 75 cm/sec. It is also interesting to observe that the average mean power values within the flow cycle for panels C and D are similar. In both situations, the mean power was 11.2 dB, suggesting that the size of the aggregates was similar on average for both experiments. Table 2 summarizes values of minimal, mean and maximal power of Doppler spectra within the flow cycle.

Discussion

Justification of the Working Hypothesis

As reported previously, our working hypothesis was that the changes in Doppler scattering power from whole blood during the flow cycle were primarily associated with the presence of red cell aggregates. It was also postulated that contributions from the presence of flow disturbance, red cell

alignment and changes in the correlation among scatterers were significantly smaller or not present. The most significant factor affecting the interaction among scatterers is the presence of blood flow turbulence. Turbulence is known to reduce correlation among scatterers (Twersky, 1988) and consequently increase fluctuation in cell concentration (Mo and Cobbold, 1992). As demonstrated experimentally in previous studies (Shung *et al.*, 1992; Cloutier and Shung, 1992), turbulence increases the Doppler backscattering power. In turbulent pulsatile flow, it was shown that the mean Doppler power increases in systole to reach a maximum early after peak systole and then decreases in diastole. In pulsatile flow, laminar flow often persists even when Re computed from mean or peak velocities rises well above the steady flow critical value (Milnor, 1989). As reported in Milnor (1989), laminar flow in models persists up to a mean Reynolds number of 2900. Disturbed but not fully turbulent flow exists up to at least 7900. In the present study, Re_{mean} did not go beyond 1000, which insured the absence of blood flow disturbance in our experiments.

From Twersky's model (Berger *et al.*, 1991) on the backscattering of ultrasound from blood, small aggregates are modeled by elongated particles. However, for large rouleaux, the packing factor derived by considering ultrasonic scatterers as a mixture of cylinders of different sizes may be a more appropriate model (Twersky, 1988; Berger *et al.*, 1991). The presence of cylinders leads to a larger backscattering power. Another parameter not considered in Twersky's model that is important to discuss is the influence of the orientation of the rouleaux within the flow field. It is known that shear stress induces rouleaux rotation and that the angular velocity of rotation is shear stress dependent (Goldsmith, 1986). Since rouleaux rotate most slowly when aligned with the flow, at any given instant, the largest fraction of rouleaux might have their major axes aligned with the flow field. In a study of Mottley and Miller (1988), it was shown that the backscattered ultrasonic power from muscular fibers is angular dependent. They showed a maximal backscattered power when the fibers were perpendicularly oriented with the ultrasonic beam. By using the analogy between fiber orientation and the orientation of aligned networks of cylindrical rouleaux, it would be plausible to expect an increase in mean power for aligned rouleaux, since the angle between the ultrasonic beam and the flow field was 70° in our experiments, *i.e.*, close to 90°." Rouleaux alignment may be an additional factor contributing to the mean power increase in systole for a pulsation of 20 beats/min. However, the exact contribution of this phenomenon is unknown and cannot be appreciated using the experimental protocol proposed in the present study. Rouleaux formation and disruption are still believed to be the main determinants of the observed changes in mean power values.

In addition to the effect of turbulence on the packing factor, other experimental conditions affecting the correlation among red cells may influence the packing factor and consequently the mean power within the flow cycle (Cloutier and Shung, 1993). In a recent study performed by our group (Cloutier and Shung, 1993), changes in the value of the packing factor were hypothesized to explain a cyclic variation of the Doppler power, observed when the flow velocity variation within the Doppler sample volume was of the order of 330 cm/sec, in measurements made using washed red cells as ultrasonic scatterers. In that study, Doppler measurements were performed using instantaneous mean velocity *vs.* time traces similar to those presented in Figs. 3A and 3C (70 beats/min). At high flow velocities (Fig. 3A) and a hematocrit of 40%, the mean power dropped during flow acceleration and increased during flow deceleration. An overall mean power change of approximately

2.5 dB was observed. In the present study, no cyclic variation of the Doppler power was measured at high flow velocities (Fig. 4.1A) for whole blood at 40% hematocrit and a pulsation of 70 beats/min. The absence of a variation is believed to be associated with the effect of the plasma on the rheological properties of red cells. The attracting forces induced by the macroproteins present in the plasma for porcine whole blood may be responsible for the absence of the cyclic variation. In the present study, reducing the pulsation to 20 beats/min had a significant effect on the Doppler power (Fig. 4.1B). An overall cyclic variation of 7 dB was measured with whole blood at 20 beats/min, which is significantly higher than the variation of 2.5 dB observed with washed red cells (Cloutier and Shung, 1993). Moreover, the cyclic variation obtained at 20 beats/min followed a totally different pattern of variation to that found with red cell suspensions (Cloutier and Shung, 1993), which supports the hypothesis that the presence of aggregates is the main determinant involved in the cyclic variation seen in Fig. 4.1B. At lower flow velocities (Fig. 3C), no cyclic variation of the Doppler power was observed within the flow cycle for both porcine red cell suspensions (Cloutier and Shung, 1993) and porcine whole blood (Fig. 4.2C). Therefore, it is justified to associate the cyclic variation of Fig. 4.2D (20 beats/min) to the presence of aggregates.

Experimental Limitations and Comparison with Results Found in the Literature

It is known that temperature influences the aggregability of red cells and consequently ultrasound echogenicity (Sigel *et al.*, 1982; Beitler *et al.*, 1983). In the present study, experiments were performed at room temperature. The cyclic variation of Doppler power might have changed if blood at 37° C had been used, since temperature increases red cell aggregation. Another limitation of the present study is the delay needed between blood collection and Doppler measurements. A recent study (Caswell and Stuart, 1992) showed that the storage of blood up to 7 days does not change blood viscosity but does affect erythrocyte sedimentation rate. The effect of blood storage on the aggregation index and disaggregation shear rate has also been documented (Chabanel and Samama, 1989). It was recommended to perform all red blood cell aggregation tests within 3 hours after blood withdrawal (Chabanel and Samama, 1989). The exact influence of this factor on our results is not known. However, this limitation would not exist in *in vivo* measurements. The last drawback of the present study is associated with the ultrasonic technique selected. Doppler ultrasound has the advantage of making measurements on blood velocities within the sample volume. On the other hand, this technique does not allow measurement of the backscattered power when blood does not move, because no Doppler frequency shift occurs. Consequently, as seen in Figs. 4.1 and 4.2, no measurement was performed in middle and late diastole. The use of A-mode or B-mode systems or intravascular ultrasonic imaging would solve this problem if velocity information is not necessary.

Very few studies can be found in the literature on factors affecting the cyclic variation of the ultrasonic backscattered signal within the flow cycle. However, a very interesting *in vivo* study reporting observations of changes in ultrasound echogenicity within the flow cycle was made recently by De Kroon *et al.* (1991). De Kroon *et al.* found a cyclic variation at 70 beats/min, while no such variation was measured in our study at that pulsation rate. Several factors may explain this difference: the temperature difference between both studies (room and body temperatures); the influence of blood storage; the influence of the frequency of the ultrasonic signals, and the selection of patients possibly with hyper-aggregability in the study of De Kroon *et al.* In the present study,

measurements were performed at 10 MHz, while De Kroon *et al.* used an intravascular transducer operating at 30 MHz. The use of a carrier frequency at 30 MHz may have amplified the influence of aggregates on the variation of the ultrasonic backscattered signal within the flow cycle because at that frequency, Rayleigh scattering may no longer exist. Another explanation suggested to explain the discrepancy between both studies is the possibility that an abnormal concentration of macroproteins, responsible for the aggregation mechanism, was present in the blood of the 3 patients studied by De Kroon *et al.* Such abnormal concentration may have induced a cyclic variation of the ultrasonic power.

In the present study, it has been demonstrated that Doppler ultrasound may be a valuable tool for studying the dynamics of red cell aggregation. In addition to the classical use of Doppler ultrasound (quantification of flow velocity), other important applications of the technique have been proposed in the literature. The detection of air emboli, gas emboli and formed element emboli of platelet orthrombus origin is certainly a very promising approach. Arterial microemboli produce short transient backscattered signals of high amplitude that can be detected visually on the Doppler spectrum. Since the work by Spencer *et al.* (1969), *in vivo* studies have recently been reported (Spencer *et al.*, 1990; Spencer and Klepper, 1992; Russell *et al.*, 1991; Stump *et al.*, 1991). The quantification of the relative changes in backscattered power may certainly broaden the possible clinical applications of Doppler ultrasound.

Dynamics of Red Cell Aggregation and Disaggregation

The results shown in Figs. 4.1 and 4.2 confirm the capability of Doppler ultrasound to characterize the dynamics of red cell aggregation. Postulating that the cyclic variation of the Doppler power is mainly attributable to changes in scatterer size and shape, our results showed that aggregate formation is influenced by the mean velocity (consequently, the mean shear rate within the Doppler sample volume) and the pulsation rate. The disaggregation of rouleaux was shown to be affected by the mean shear rate. The influence of the pulsation rate on red cell disaggregation is not clear from our results. An intriguing observation is that the mean power increased during the acceleration of flow, before rouleaux disruption, for a pulsation of 20 beats/min. A review of several observations on pulsatile flow behavior may help in understanding the following discussion.

In pulsatile flow, the velocity profile within a rigid tube or a vessel changes significantly as a function of the timing within the cardiac cycle (Milnor, 1989). The profile is blunt at the beginning of systole, becomes almost parabolic at peak systole, depending on the hematocrit, and then flattens again in diastole. During the deceleration of blood, the velocity profile is almost parabolic and reverse flow is observed near the wall. Based on our results, it seems that a prolonged diastole is necessary to observe an aggregate size enlargement in systole because no cyclic variation was found at a pulsation of 70 beats/min. During the 2 seconds of flow stoppage, the red cells are more likely to be brought into contact and tend to align themselves with axial symmetry as suggested by Skalak (1984). However, this 2-second period is certainly insufficient to form aggregates because, as observed in Figs. 4.1B and 4.2D, the difference in mean power between the end of flow deceleration and the beginning of systole (which corresponds approximately to the period with no flow) is minimal (less than 1.3 dB). The most important changes in mean power for a pulsation of 20 beats/min occurred during acceleration and deceleration of flow. As the flow accelerates with a blunt and more parabolic profile later in systole, the aligned cells then probably form rouleaux until the

shear rate within the Doppler sample volume becomes sufficient to break them totally or partly. Rouleaux formation in systole was not observed at a pulsation of 70 beats/min, probably because red cells do not have enough time in diastole to be brought into contact and to align themselves.

The disaggregation process differed between experiments performed at a high flow velocity (Fig. 4.1B) from those at a more physiological velocity range (Fig. 4.2D). At a high flow velocity, the rouleaux abruptly disrupted, while at a low flow velocity, no such rapid break-up of aggregates was observed, probably because of the reduced shear stresses involved. Other differences are the remaining aggregate sizes after rouleaux disruption and the timing in systole when break-up occurred. At high flow, the break-up of aggregates occurred 160 ms before peak systole; at low flow, it occurred at peak systole. The mean power after rouleaux disruption reached a value of 6.4 dB, as shown in Fig. 4.1B, and 9.3 dB, as shown in Fig. 4.2D. This last result suggests that the shear rate present in the low flow experiments is not sufficient to disrupt the aggregates located at the middle of the tube completely. Within the flow cycle, the rouleaux size may increase by a few cells, on the average, during systole and decrease also by a few cells during early diastole.

Rheological Properties of Porcine Whole Blood

Porcine whole blood was selected as an ultrasonic scattering medium for several reasons. The use of porcine blood was necessitated by its availability and by the need for large volumes of blood. Another important criterion in the selection of porcine blood was its similar viscous and elastic components of viscosity to those of human blood over a large range of shear rates (Brookshier and Tarbell, 1991). It is known that erythrocyte aggregability differs depending on the animal species selected (Ohta *et al.*, 1992). The aggregation tendency and sedimentation rate of porcine blood were shown to be higher than human blood (Schneck, 1988). Porcine blood thus represents a reasonably adequate model for studying the mechanisms of human erythrocyte aggregation.

Conclusion

It has been demonstrated in the present study that Doppler ultrasound may be a promising tool for studying the rheological properties of red cell aggregates. All the techniques proposed in the literature to study the rheological properties of red cell aggregates involve laboratory manipulations. Significant changes are known to take place (*e.g.* clotting, oxidation, *etc.*) when blood is removed from its natural habitat. The use of ultrasound to study the rheological properties of red cell aggregates may offer one major advantage over light reflection techniques: Ultrasound, which propagates through soft tissues and is only slightly attenuated in blood, may allow measurement of the hemorheological and hemodynamic properties of red cell aggregates *in vivo* in large vessels. The quantification and characterization of Doppler power variations within the flow cycle may help detect pathological hyper-aggregating processes. In the study by De Kroon *et al.* (1991) using intravascular ultrasonic imaging and in a preliminary study of our group (Cloutier and Shung, 1991) using Doppler ultrasound, changes in blood echogenicity and mean power within the cardiac cycle were thought to be associated with red cell aggregation. The present study reinforces this postulation.

Some difficulties need to be solved before using ultrasound as a diagnostic tool. The instrument would have to be calibrated. A moving reflector would need to be used as a standard to normalize the mean power scattered by blood. This technique would allow establishment of a 0 dB reference with which

Doppler backscattered power from blood could be compared. Each system, including the transducer, has its own gain factor and sensitivity, and it would be difficult to compare results without normalization. The results obtained in the present study can be compared with the studies by Cloutier and Shung (1991; 1992; 1993) because the same system and the same polyethylene tube were used. If no normalization is performed, at least information concerning the cyclic variation of the mean power in dB can be compared because this measure is relative. Other important parameters that will have to be considered for *in vivo* studies are the hematocrit and the flow characteristics (*e.g.*, the presence of turbulence).

In the present study, aggregation and disaggregation within the flow cycle were observed only for a pulsation of 20 beats/min. For the instantaneous mean flow velocity *vs.* time trace simulating conditions found in normal human abdominal aorta, no variation of the Doppler mean power was found at room temperature. These results do not necessarily signify that the dynamics of aggregation is not a function of the cardiac cycle for *in vivo* human studies of blood flow in arteries. This remains to be investigated. The presence of a high concentration of bridging macromolecules and a temperature of 37° C may result in cyclic changes similar to those shown in Figs. 4.1B and 4.2D, even if a pulsation rate of approximately 70 beats/min is present.

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