In Vitro Investigation of the Hematological Effects of Non-Pulsatile and Pulsatile Blood Flow, Sarns Pump vs. Cobe-Stöckert Pump

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Abstract

The in vitro effects of pulsatile and non-pulsatile blood pumps on human red blood cells were investigated. Group I (Sarns, Inc., non-pulsatile pump) showed significantly less hemolysis ($p < 0.05$) than Group II (Cobe-Stöckert, non-pulsatile roller pump). Group III (Sarns, pulsatile roller pump) and Group IV (Stöckert, pulsatile roller pump showed significantly less hemolysis ($p < 0.05$). Both Sarns and Stöckert non-pulsatile roller pumps showed significantly ($p < .05$) less hemolysis at three and four hours of circulation than the Sarns and Stöckert pulsatile pumps.

Introduction

The literature is not replete regarding the effects of pulsatile perfusion on increased hemolysis during extracorporeal circulation (ECC). The literature currently available does not address the parameters which affect hemolysis during ECC, i.e., osmolality, electrolyte concentration, red blood cell deformability, adenine triphosphate (ATP) concentration, temperature, and pH.

Much of the uncertainty to the possible danger of non-pulsatile flow and the theoretical physiological advantage of pulsatile flow may be attributed to two principal factors: 1) the lack of clear, objective data on organ metabolism during non-pulsatile ECC, and 2) the fear of increased hemolysis from pulsatile flow. Investigators have shown the index of hemolysis is lower in the pulsatile pump.[1-3] However, a large number of these studies have variables that affect the parameters that are being measured.

An in vitro method has been designed to study the effects of non-pulsatile and pulsatile blood pumps on human red blood cell damage, as measured by plasma free hemoglobin levels, where all variables were eliminated other than the roller pump.

Materials and Methods

Figure 1 illustrates the extracorporeal circuit used. All extracorporeal circuits were primed with the following fluid: Normosol[R] with a pH of 7.40, 195 cc; fresh human blood, 450 cc; fresh human red cells, 275 cc; with a total circulating volume of 920 cc. An

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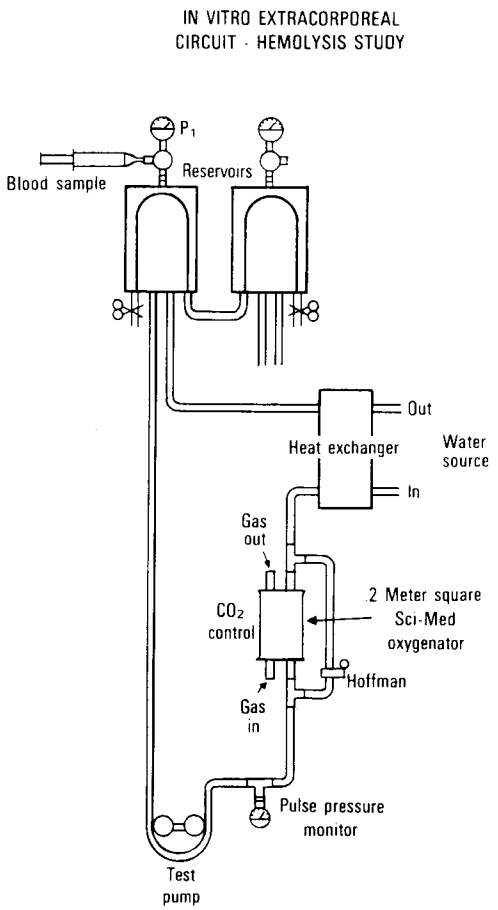

Figure: 1 This is a schematic drawing of the experimental circuit. Each reservoir represents a separate circuit. The total prime of each circuit is 920 cc. Experiments were accomplished in pairs and simultaneously with the entire prime pooled.

average of 15 milliequivalents of sodium bicarbonate was used to buffer the prime in each circuit. No further additions were necessary during the entire four hour experiment. Blood flow was maintained at 6 liters per minute. Group I and II experiments were conducted simultaneously in pairs. Prior to initiating the experiment, a cross clamp was placed on the circulating line between the two reservoirs[a], the experiments became separate, maintaining the same prime composition including hematocrit, temperature, pH, and osmolality. Experiments for Groups III and IV were conducted in the same way as Groups I and II. A one-half inch size Tygon tubing[b] was utilized throughout the entire circuit. The inflow was connected to its respective reservoir. After its insertion into the pump raceway, the outlet tube was connected to a one-half by one-half inch polycarbonate connector with a luer lock port where a pressure monitoring line was placed and connected to a pressure gauge manometer to monitor the pressure generated on the positive side of the roller pump. The tubing was then connected through a bypass system to a 0.2 M^2 silicone membrane oxygenator[c] and then to an adult heat exchanger[a]. The heat exchanger was connected to the reservoir to complete the circuit. Occlusion was set with one roller occluding the tubing while the other roller was totally disengaged from the tubing. Occlusion adjustment was made to allow the prime in the tubing to drop at a rate of one inch per minute in a thirty inch fluid column above the pumphead. The reservoirs were open to air and held below the pumphead during occlusion setting. In order to maintain the pH of the circulating fluid at normal ranges from plasma, it was necessary to insert a 0.2 M^2 silicone membrane oxygenator in the circuit. The pH was adjusted by removing excess carbon dioxide. This was accomplished during the priming of the circuit. In addition, it was used whenever necessary to adjust for pH. The average use was three times during the entire length of the experiment, and an average of 100 cc was shunted to the oxygenator for five minutes, then discontinued.

After priming the circuit, a control sample was drawn for pH, osmolality, and hemoglobin measurements. The experiments then started in the non-pulsatile pumps by increasing the blood flow to six liters per minute and samples were taken on the hour for four hours. The following steps were taken for initiation of the experiments with pulsatile circulation.

- Blood Flow Control was set to maximum.
- The EKG Internal Simulator was set at 60 beats per minute.
- The pulsatile blood flow/non-pulsatile blood flow ratio was set on 100% pulsatile.
- The pulse width was adjusted to deliver a blood flow of six liters per minute.
- The blood flow read-cut was checked with a graduated cylinder.

This method assured the investigators of not only having the same flow rates, but also the timing of the pulses was the same on both pumps.

Test

Plasma Free Hemoglobin: Modified Crosby and Furth.

After the samples were drawn from the circuit, they

a Travenol, Deerfield, IL 60015
b Norton, Akron, OH 44309
c SciMed Life Systems, Minneapolis, MN 55441

were centrifuged for ten minutes at 2,000 RPM. The plasma was decanted in a clean test tube and processed for plasma free hemoglobin. The sample was read against a standard and controls of known absorbency units. The measure of the absorbency or concentration of the processed sample was read on a spectrophotometer with 515 nm wavelength adjusting the spectrophotometer to zero absorbency with the reagent blank. The concentration of plasma free hemoglobin in mg/dl was calculated by the following formula:

Sample or unknown Concentration of mg/dl free
Standard x Standard = hemoglobin

Serum Potassium: This was directly measured by IL Flame Photometer Model 443 with an automatic diluter, model 444.[d]

Blood Gases (pH): These were measured by an IL Blood Gas Analyzer.[d]

Hemoglobin: This was directly measured by an IL cooximeter, model 282.[d]

Osmalality: This was measured by a Fisher Freeze point depression in milliosmols.

Index of Hemolysis: This was calculated by the following formal:

$$\frac{\textit{Plasma Free Hemoglobin—Control}}{\text{Flow Rate x Time}}$$

Index of hemolysis is represented in milligrams per 100 liters of blood pumped.

d Instrumentation Laboratories, Lexington, MA 02173

Table 1

Sarns Non-Pulsatile Roller Pump

	PLASMA FREE HEMOGLOBIN (mg/dl)	SERUM POTASSIUM (mEq/liter)	pH	IH*	HEMOGLOBIN (mg/dl)	HEMATOCRIT (PERCENT)	OSMOLALITY (millisomols)
Controls	8.563	4.72	7.36		11.6	35	300
1st hr.	61.61	4.78	7.37	0.041	10	30	300
2nd hr.	67.86	5.34	7.44	0.011	9	27	300
3rd hr.	73.99	5.12	7.44	0.006	9	27	299.2
4th hr.	87.57	5.5	7.44	0.004	9	27	299.2
Mean	59.91						
St. Error	13.54						
95% Confidence	± 91.2						
	32.35						

This table represents the averages of five experiments, the mean, standard error and 95% Confidence of the Mean present for plasma free hemoglobin. #IH—Index of Hemolysis.

Table 2

Stöckert Non-Pulsatile Pump

	PLASMA FREE HEMOGLOBIN (mg/dl)	SERUM POTASSIUM (mEq/liter)	pH	IH*	HEMOGLOBIN (mg/dl)	HEMATOCRIT (PERCENT)	OSMOLALITY (millisomols)
Control	6.68	4.43	7.38		9	27	300.4
1st hr.	82.24	4.8	7.35	0.058	9	27	299.6
2nd hr.	126.35	4.6	7.39	0.023	9	27	299.6
3rd hr.	137.3	4.3	7.43	0.013	9	27	298.8
4th hr.	160.8	4.3	7.44	0.007	9	27	298.8
Mean	102.673						
St. Error	27.17						
95% Confidence	± 165.32						
	40.01						

This table represents the averages of five experiments, the mean, standard error and 95% Confidence of the Mean present for plasma free hemoglobin. #IH—Index of Hemolysis.

Results

Sarns Non-Pulsatile vs.
Stöckert Non-Pulsatile

Tables 1 and 2 represent the mean, standard error (S.E.) and the 95% confidence interval (CI) of the mean for plasma free hemoglobin. Potassium concentration did not change in either group (Tables 1 and 2) throughout the entire experiment; neither did the osmolality, pH, hemoglobin or hematocrit. After three hours of circulation, there was a significant elevation of plasma free hemoglobin (Figure 2) with the Stockert non-pulsatile pump. This elevation was more significantly pronounced at four hours of circulation in Group II. There was no significant elevation (Figure 2) of plasma free hemoglobin throughout the entire four hour experiment in Group I (Sarns non-pulsatile roller pump).

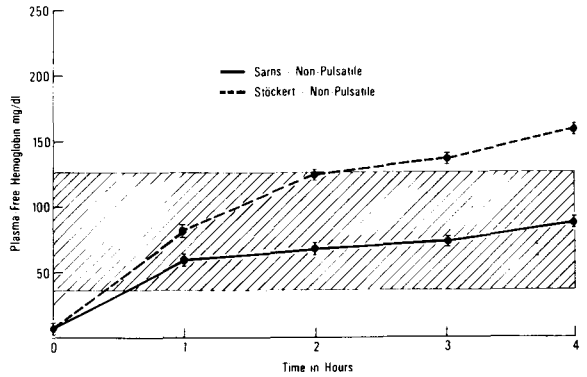

Figure 2: The ordinate is plasma free hemoglobin concentration in mg/dl. The abscissa is time in hrs. The shaded area between the two heavy lines represent the 95% confidence of the mean levels of Group I and Group II. Any point that is located outside of the 95% confidence of the mean is significant. Group I (Sarns non-pulsatile pump) is represented by solid line _____ . Group II (Stöckert non-pulsatile pump) is represented by the broken line _____. The bars on either side of the mean experimental point represent one standard error.

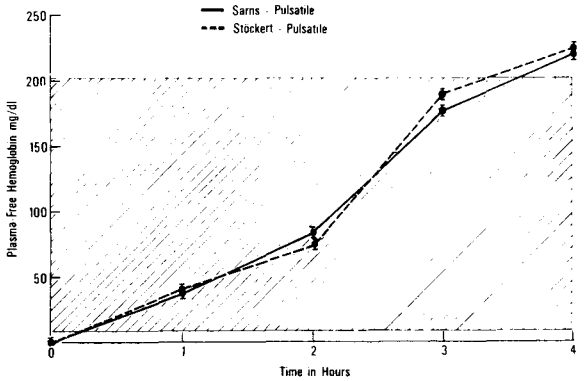

Figure 3: The ordinate is plasma free hemoglobin concentration in mg/dl. The abscissa is time in hrs. The shaded area between the two heavy lines represent the 95% confidence of the mean levels of Group III and Group IV. Any point that is located outside of the 95% confidence of the mean is significant. Group III (Sarns pulsatile roller pump) is represented by solid line _____ . Group IV (Stöckert pulsatile roller pump) is represented by the broken line _____. The bars on either side of the experimental point represent one standard error.

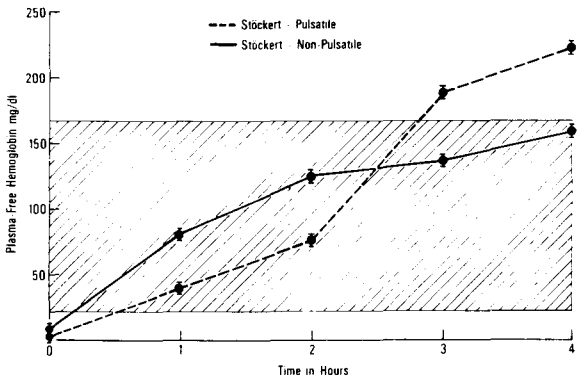

Figure 4: The ordinate is plasma free hemoglobin concentration in mg/dl. The abscissa is time in hrs. The shaded area between the two heavy lines represent the 95% confidence of the mean levels of Group II and Group IV. Any point that is located outside of the 95% confidence of the mean is significant. Group II (Stöckert non-pulsatile roller pump) is represented by solid line _____ . Group IV (Stöckert non-pulsatile roller pump) is represented by the broken line _____.

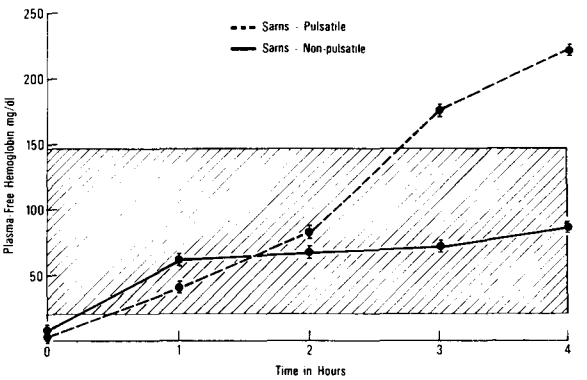

Figure 5: The ordinate is plasma free hemoglobin concentration in mg/dl. The abscissa is time in hours. The shaded areas between the two heavy lines represent the 95% confidence of the mean levels of Group I and Group III. Any point that is located outside of the 95% confidence of the mean is significant. Group I (Sarns non-pulsatile roller pump) is represented by a solid line _____ . Group III (Sarns non-pulsatile roller pump) is represented by a broken line _____. The bars on either side of the mean experimental point represent one standard error.

Table 3

Sarns Pulsatile Roller Pump

	PLASMA FREE HEMOGLOBIN (mg/dl)	SERUM POTASSIUM (mEq/liter)	pH	IH*	HEMOGLOBIN (mg/dl)	HEMATOCRIT (PERCENT)	OSMOLALITY (millisomols)
Control	2.87	4.9	7.38		10.94	32	300.6
1st hr.	39.27	4.9	7.39	0.03	10.28	30.84	300.0
2nd hr.	84.15	5.7	7.39	0.02	10	30	300.6
3rd hr.	176.6	6.8	7.35	0.02	10.9	30	299.8
4th hr.	223.9	7.2	7.35	0.01	10.1	30	300.6
Mean	105.4						
St. Error	41.51						
95% Confidence	± 200.7						
	9.7						

This table represents the averages of five experiments, the mean, standard error and 95% Confidence of the Mean present for plasma free hemoglobin. #IH—Index of Hemolysis.

Table 4

Stöckert Pulsatile Roller Pump

	PLASMA FREE HEMOGLOBIN (mg/dl)	SERUM POTASSIUM (mEq/liter)	pH	IH*	HEMOGLOBIN (mg/dl)	HEMATOCRIT (PERCENT)	OSMOLALITY (millisomols)
Control	2.4	5.44	7.38		10.3	30.8	300.8
1st hr.	40.92	5.46	7.40	0.03	10	30	300.6
2nd hr.	76.6	5.68	7.41	0.014	10	30	301.0
3rd hr.	191.0	6.64	7.38	0.02	10.1	30	300.4
4th hr.	226.0	7.85	7.37	0.01	10	30	300
Mean	107.2						
St. Error	43.21						
95% Confidence	± 206						
	7.7						

This table represents the averages of five experiments, the mean, standard error and 95% Confidence of the Mean present for plasma free hemoglobin. #IH—Index of Hemolysis.

Sarns Pulsatile vs.
Stöckert Pulsatile

Potassium concentrations were elevated at three and four hours (Tables 3 and 4) of circulation with both pulsatile pumps. Temperature, pH, osmolality and hemoglobin remained relatively constant. Plasma free hemoglobin was significantly elevated in both pulsatile pumps at four hours of circulation (Figure 3). The mean plasma levels of free hemoglobin during the entire length of the experiment were less in the Sarns pulsatile pump (Group III, Figure 3).

Plasma free hemoglobin levels were significantly elevated at three and four hours circulation (Figure 4).

Again, there was a significant elevation of plasma free hemoglobin in the pulsatile group (Figure 5) at three and four hours of circulation.

Discussion

Short-term utilization of roller pumps in the pulsatile mode is not damaging to red blood cells. However, the currently available pulsatile pumps have significant red blood cell hemolysis beyond three hours of perfusion under controlled in vitro conditions. However, the Sarns pulsatile pump showed consistently lower free plasma hemoglobin than the Stöckert pulsatile pump.

There was no change of index of hemolysis in either group, but they were significantly higher than the non-pulsatile roller pump. The Sarns non-pulsatile roller pump showed significantly less hemolysis than the Stöckert non-pulsatile pump.

We have shown that an in vitro model to study the hematological effects of pulsatile and non-pulsatile pumps is a reliable method to investigate red blood cell hemolysis. Variables such as temperature, osmolality, pH (acid-base imbalance), and electrolyte imbalance are eliminated. The lack of standardization of hemolysis protocols makes it difficult to compare products which are being currently utilized in the clinical situation. The investigators feel this model should be presented as a standard model for hemolysis investigations for artificial devices.

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