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Experimental Determination of the Normalized Index of Hemolysis for the Sputnik Implantable Pediatric Rotary Blood Pump

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The paper describes the procedure for evaluation of hemolysis induced by the Sputnik implantable pediatric rotary blood pump. In a series of five experiments the normalized index of hemolysis (N.I.H) for the Sputnik pump was found to be 0.0099 ± 0.0015 g/100 L.

Introduction

Evaluation of hemolysis is a necessary component of clinical monitoring of the performance of circulatory assist devices. It also provides comparison between different devices and evaluation of their conformance with international standards specifying requirements on blood-contacting gas-exchange medical devices.

The index of hemolysis is an estimate of the amount of plasma free hemoglobin per unit volume of blood released in the process of blood circulation through an extracorporeal circuit. Previously, this index was considered inappropriate for evaluating the hemolysis induced by small pumps with a low priming volume because in this case the number of passages of a unit volume of blood through each component of the circuit is higher than in the case of large pumps. To solve this problem, the trauma index – the ratio of the free hemoglobin concentration to the number of passages of each blood cell through the extracorporeal circuit – was introduced. Later, the preference has been given to the normalized index of free hemoglobin production in plasma.

Use of a left ventricular-assist pump involves partial breakdown of red blood cells (hemolysis). Evaluation of hemolysis is necessary to assess the effect of the ventricular-assist pump on blood and to estimate the degree of blood trauma.

The relative (δ) and normalized indices of hemolysis are the characteristics of the degree of hemolysis [1]. The relative hemolysis index characterizes the degree of red blood cell breakdown leading to release of hemoglobin into blood plasma.

The normalized index of hemolysis characterizes the concentration of free hemoglobin in plasma (per 100 L of pumped blood) taking into account the hematocrit, the blood flow rate, and the circulation time.

The paper describes the testing procedure for evaluation of hemolysis from the relative and normalized hemolysis indices.

Materials and Methods

The Sputnik implantable pediatric rotary blood pump (IPRBP) is described in [2-5]. The design of the Sputnik IPRBP consists of a fixed flow tube housing the main units of the pump, a straightener at the inflow, an impeller with an embedded magnet rotating at a rate of several thousand rpm, and a fixed diffuser on the output. The straightener has three blades at angles of 120° to each other. The main purpose of the straightener is to direct flow to the impeller blades with the aim of minimizing eddies before entry to the impeller. The three-bladed

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impeller directs the flow to the three stationary blades of the diffuser, which are curved in the opposite direction. The straightener and diffuser are also the bearings between which the single rotating part of the pump – the impeller – is suspended [5].

A test bench for evaluation of hemolysis was constructed [1]. It consisted of a laboratory bench with a 50-L tank (AVAT TS-100, OAO Chuvashorgtekhnika, Cheboksary, Russia), a TW 2.02 thermostat (ELMI Ltd., Riga, Latvia), a 400-mL closed venous reservoir (Terumo Corporation, Tokyo, Japan), flexible polyvinylchloride tubes (OD 3/4" ID 1/2", OD 11/16" ID 1/2", OD 1/2" ID 3/8"; TYGON E-3603, Ile-de-France, France), four 3/8"-1/2"-LL (luer lock) adapters (Terumo Corporation, Tokyo, Japan), four three-way cocks (Terumo Corporation, Tokyo, Japan), two adjustable screw resistors, Sputnik IPRBP, an Angioton-4K pressure meter with power supply (BIOSOFT-M, Moscow, Russia), two TruWave Edwards Lifesciences pressure sensors with connecting cables, a ME11PXL349 ultrasonic clamp-on tubing flow sensor (Transonic Systems Inc., Ithaca, New York, USA), and a T402 multi-channel research console (Transonic Systems Inc., Ithaca, New York, USA). A diagram of the test bench is shown in Fig. 1.

According to the standards [6], donor blood with 30% hematocrit level (measured immediately after blood was drawn) was used in the experiment. If the hematocrit level did not meet the standard requirements, 0.9% saline solution was added to reach the required level of 30%. Evaluation of hemolysis was performed within 1 h after blood was drawn.

During the experiment, 7 sessions of blood drawing were performed at 1-h intervals. The first session was performed 5 min after the work bench started to operate. Fourteen samples were taken (7 samples from the circuit and 7 samples from the control reservoir). An additional blood sample from the control reservoir taken during the first session was refrigerated at 0°C.

During each blood drawing session a small amount (0.5 mL) was preliminarily bled off to avoid sampling stagnant blood from the three-way cock. Then, a 2-mL sample was taken using a syringe and transferred to a test tube. Each test tube was plugged and labeled. The label contained information about the time when the sample was taken and the place from which it was drawn (circuit or control reservoir). The test tubes with blood samples were placed in a rack and stored at +4°C for at least 6 h to obtain a stable layer of blood plasma.

The free hemoglobin level was measured using a Thermo Scientific GENESYS 10S UV-Vis spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). 15 blood samples were studied using the spectrophotometer: 7 samples from the circuit, 7 samples from the control reservoir, and the additional sample that had been frozen and thawed up.

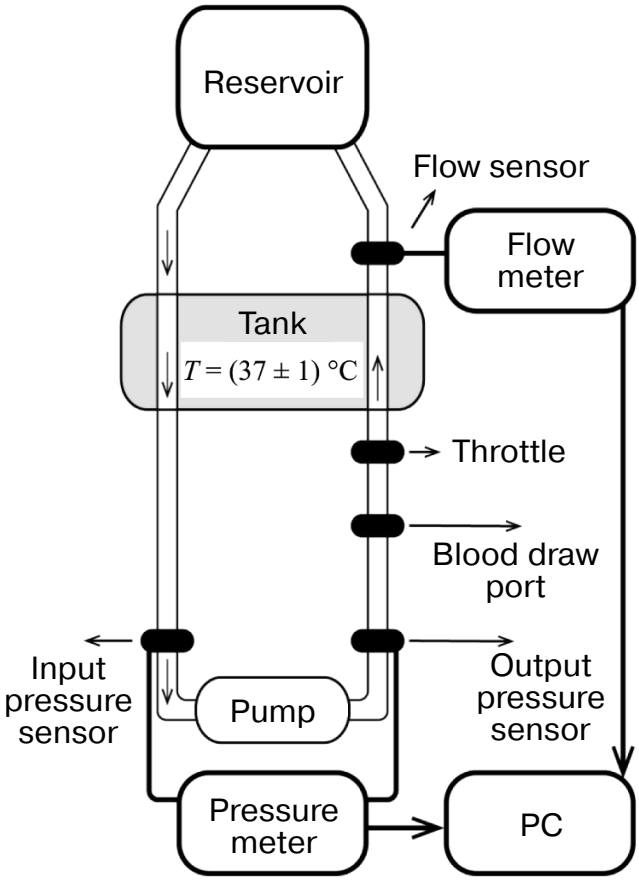

Fig. 1. Diagram of the test bench.

Results and Discussion

Data on the dynamics of the optical density of plasma samples were obtained (Fig. 2). Spectra were measured within the range of 300-700 nm with a step of 0.2 nm.

The level of hemolysis was determined from the optical density of plasma at wavelength $\lambda = 540$ nm for each of the 7 sessions in which blood was drawn from the circuit. Calculations took into account continuously occurring spontaneous autohemolysis using the data obtained by testing the samples from the control reservoir.

The relative hemolysis δ (g·%) was calculated by the following formula:

$$\delta = [(A_e - A_c)/(A_{ch} - A_c)]\cdot 100\%, \quad (1)$$

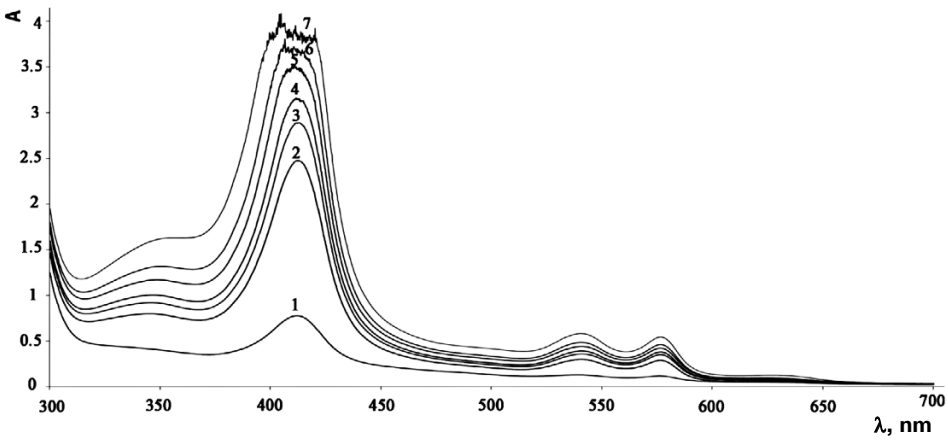

Fig. 2. Optical densities of plasma samples taken 5 min (curve 1) to 360 min (curve 7) after the beginning of the test.

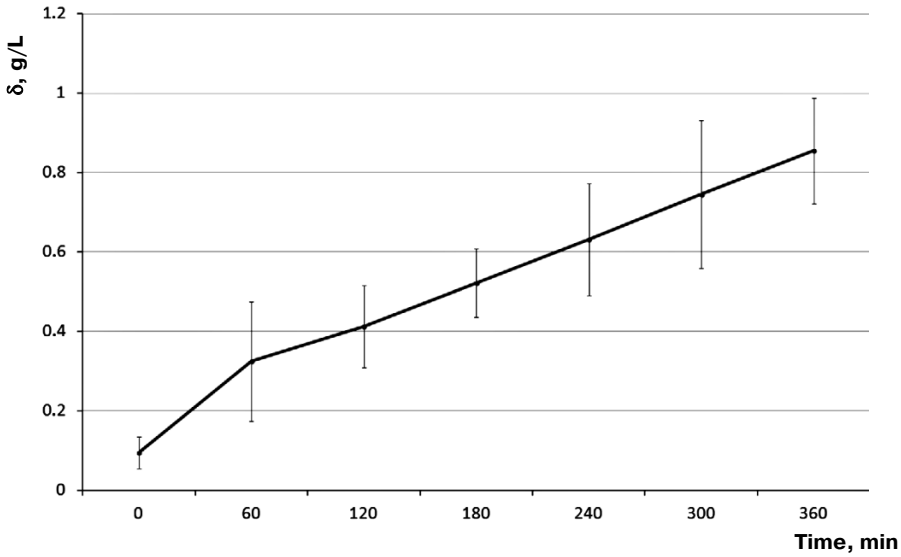

Fig. 3. Relative hemolysis determined in five experimental sessions (error bars are shown).

where A_e is the optical density of given plasma sample taken from the circuit at wavelength $\lambda = 540$ nm; A_c is the optical density of the control sample taken from the control reservoir at $\lambda = 540$ nm; A_{ch} is the optical density of plasma in the case of complete hemolysis (thawed-up sample) at $\lambda = 540$ nm.

Further calculations were performed using the arithmetic mean values of optical density within the range of 539-541 nm with a step of 0.2 nm. The mean optical density of plasma in the case of complete hemolysis was $A_{ch} = 7.033993$.

The relative hemolysis δ (g·%) was calculated using Eq. (1) on the basis of the obtained data on the optical density of blood plasma. Figure 3 shows the dynamics of relative hemolysis during the experiment.

The obtained values of the relative hemolysis allowed the normalized index of hemolysis *N.I.H.* (g/100 L) to be calculated for each of the 7 sessions in which blood was drawn from the circuit.

The normalized index of hemolysis was calculated by the following formula:

$$N.I.H. = \delta \cdot V \cdot \frac{100 - Ht}{Q \cdot T}, \quad (2)$$

where δ is the relative hemolysis, g/L; V is the total volume of blood in the circuit, L; Ht is the hematocrit level, %; Q is the blood flow rate in the circuit, L/min; T is the time interval between blood drawing sessions, min.

In the tests, the blood volume was 0.5 L; blood flow rate, 5 L/min. $N.I.H$ was found to be 0.0099 ± 0.0015 g/100 L. Although this value is below the maximum acceptable level of free hemoglobin in blood [7], it is sufficiently high to make further tests of the Sputnik IPRBP necessary. It should also be noted that the evaluation of hemolysis was performed at the blood flow rate of 5 L/min. This rate is considerably higher than the mean blood flow rate for pediatric patients. A decrease in the flow rate through the circuit can reduce the level of breakdown of blood corpuscles during the tests.

Conclusion

The results of the tests show that the level of hemolysis induced by the Sputnik IPRBP does not exceed the maximum acceptable level. The obtained data will be used in further research for development, manufacture, and *in vitro* and *in vivo* testing of the Sputnik IPRBP intended to provide pediatric cardiac surgery with a useful tool for treatment of acute cardiac failure.

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