CONTRIBUTION OF RED BLOOD CELLS AND PLATELETS TO SHEAR-INDUCED THROMBOXANE A2 GENERATION IN LAMINAR FLOW

Taha M. Alkhamis

Mu'tah University, Faculty of Engineering, Department of Chemical Engineering, Mu'tah University, P.O.Box 7, Mu'tah - Karak -Jordan

ABSTRACT

This experimental study was designed to investigate the contribution of red blood cells to the low shear induced thromboxane A2 generation as a result of arachidonic acid release, and to quantify such contribution if any. Red blood cells (RBCs) contribution to shear induced thromboxane A2 generation is quantified by this study. Such contribution is due to RBCs self action and through either augmented diffusion of platelets to the artificial surface or via collaboration with platelets utilizing their enzymatic system. Results may indicate that design of artificial organs has to take into consideration the existence of RBCs. These conclusions are consistent with the significant size difference between RBCs and platelets and the concentration of platelets agonists or their initial compounds, such as arachidonic acid, within RBCs and their membranes. Results indicate that RBCs significantly contribute to shear induced thromboxane A2 generation. About 60% of thromboxane A2 originates from RBCs, while platelet contribution is about 15%. The remaining 25% was suggested to be a possible collaboration between RBCs and platelets.

Key words: Shear-induced generation, Thromboxane A2, Red blood cells, Platelets aggregation, Arachidonic acid release.

INTRODUCTION

Adhesion of platelets either to surfaces or to each other (aggregation) does not occur in normal circumstances. However, in abnormal modification of circulatory system (atherosclerosis) or in contact with foreign surfaces, platelets seem to get activated and start adhesion and/or bulk aggregation which can be catalyzed by shear stress application [1-5]. In the United States about two million cardiovascular procedures, which involve cardiac catheterization, peripheral vascular procedures, and open heart surgery, are performed annually [6]. All such procedures require, at least, short-term blood cell contact with different biomaterials and a large portion of them involves implantation of permanent prosthetic devices.

Microaggregates and microembolic formation has been observed in cardiopulmonary bypass and hemodialyses. Also thrombocytopenia and thromboembolism have been found to occur as a result of contact of blood with cardiac protheses and flow of blood in extracorporeal circulatory devices [2-5]. Jen and McIntire [7] have suggested that embolic phenomena associated with thrombogenesis induced by mechanical trauma in extracorporeal flow systems, possibly caused by altered blood flow through plaque-laden bifurcations and stenotic vessels, may play a role in coronary thrombosis and stroke. Many past studies suggest that these problems stem primarily from platelet injury (aggregation and adhesion) following shear. Adenosine diphosphate (ADP) has been shown to be a primary aggregation agent of platelets [1-5] and may act through binding with ADP-platelet membrane receptors on the platelet membrane [8] to activate platelets and induce the release reaction.

However, substantial evidence has been found to suggest that red blood cells (RBCs) exposed to shear potentiate platelet aggregation, and adhesion to artificial surfaces, due to shear induced release of ADP from RBCs [1-

5]. A large number of past studies on low stress, shear induced blood damage, particularly hemolysis have dealt with various aspects of hemoglobin (Hb) and ADP release from RBCs, especially those related to artificial surface and/or bulk flow [4].

Thromboxane A2 is another chemical that was reported to be an important stimulator of platelet aggregation [9-14]. Evidence has been reported to suggest that RBCs exposed to low shear stress (below 200 dynes/cm^2) contribute to thromboxane A2 generation [15]. Thromboxane A2, a possible platelet agonist, is formed as a result of arachidonic acid (AA) release from platelet membrane via enzymatic path within the membrane itself [16,17]. AA is one of the major fatty acids making up red blood cell (RBC) and platelet membrane. However, previous study [1] could not detect any RBC-derived phospholipid. Moreover, the results presented did not exclude the possibility of some shear-induced rearrangement (flip-flop) of phospholipid in the RBC membrane. Furthermore, these results did not exclude the possibility of shear-induced loss of phospholipid-derived material from the RBC membrane such as AA [1].

This experimental study was planned to investigate the RBCs contribution to shear induced thromboxane A2 generation, and to evaluate such contribution through a systematic experimental approach that involves partial elimination of RBCs and platelet contribution, in addition to radiolabeling of RBCs.

APPARATUS, MATERIALS, AND METHODS

APPARATUS

A Weissenberg rheogoniometer model R16/R18 (Sangamo Weston Controls Ltd., Sussex, England) was used in this study with a 0.5° cone-and-plate 10 cm diameter arrangement to generate viscometric flow [1,18]. Figure 1 shows a schematic diagram for the blood test compartment. Generation of thromboxane B2 was correlated with shear rate levels up to 5424 s^{-1}. The maximum shear rate value gives an equivalent shear stress of less than 200 dynes/cm^2 for the highest viscosity of samples tested. This is considered to be low shear stress range [4]. Mathematical expressions of these two parameters as well as that for sample volume that is contained within the experimental compartment are shown in Table 1. The cone-and-plate geometry is useful at small cone angles, where a good approximation to uniform shear is achieved, if rotational speed is slow enough [18]. In this geometry, the steady-flow hydrodynamics for Newtonian fluids are known exactly and for non-Newtonian fluids approximately.

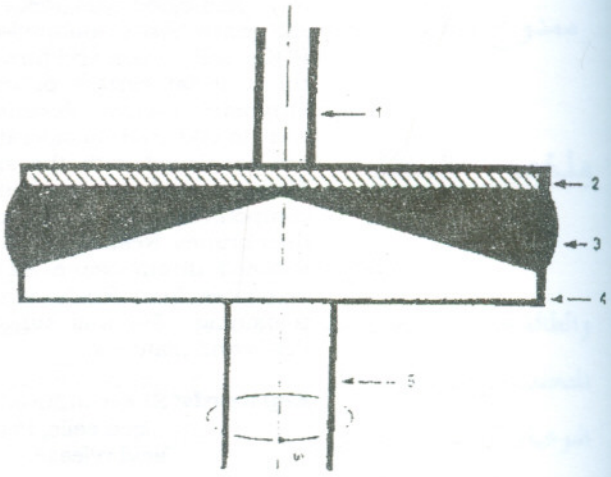

Figure 1 Schematic of shear compartment:
1. stationary upper shaft
2. stationary upper plate
3. sample compartment
4. rotating lower cone
5. rotating lower shaft

Materials

Blood:

Blood was obtained from healthy donors who fasted overnight. Donors were asked if they had taken any medications, such as aspirin (ASA), at least 14 days before donation. Only those who had not any medication, as pointed above, were considered for donation purposes. Samples of 100 to 150 ml were withdrawn from every subject in tubes containing acid citrate dextrose (ACD)/formula B (Fisher Scientific, Itasca, IL, U.S.A.)

Table 1 Physical parameters that control hydrodynamic flow within test compartment

Physical parameter	Mathematical Expression
Share rate (γ)	$\gamma = \frac{\partial u}{\partial y} = \frac{\varpi}{v}$
Share rate (τ)	$\tau = \mu \frac{\partial u}{\partial y}$
Sample volume (v)	$v = \frac{2}{3}\pi R^3 \tan v$

ω: angular velocity of the one is the cone; v: cone angle; $\frac{\partial u}{\partial y}$ velocity gradient; R: radius of platen

Phosphate buffered saline (PBS):

This buffer was used for suspending and washing purposes and was prepared following methods that were previously described [16,19].

Methods

Blood component mixtures: Platelet rich plasma (PRP)

Whole blood was centrifuged at 150 x g and room temperature for 10 minutes, then the supernatant was separated and collected with a plastic pipette and stored in a plastic container until use (within a three-hour period). The number of platelets in PRP was adjusted with platelet poor plasma (PPP) of the same individual to obtain a concentration of 210,000 ± 20,000 platelets/μl (standard deviation) .

Platelet poor plasma

Centrifugation of PRP at 1000 x g at room temperature for an additional ten minutes yielded a supernatant collected as PPP [1].

Red blood cell suspension (RBC-PBS)

Packed RBCs, the lower portion resulting from centrifugation of whole blood to obtain the PRP above, was obtained and washed twice by mixing and resuspending in an equal volume of PBS, followed by centrifugation at 150 x g for 10 minutes at room temperature. The resultant RBCs, after the second centrifugation, were resuspended and adjusted to the required hematocrit level (45%) which was equivalent to the average hematocrit of whole blood samples obtained.

PRP-PBS

Platelet rich plasma was diluted with PBS in a ratio of 11 to 9 (by volume) to have the same basis as whole blood with respect to platelet count.

Thromboxane B2 determination:

A radioimmunoassay in a kit form (Amersham Corporation, Arlington Heights, IL, U.S.A.) was used to determine the concentration levels of thromboxane B2 in the different samples. Thromboxane B2 was used as an index for thromboxane A2 which was considered to be less stable since it is readily converted to its B2 form.

Radio-labeled Red Blood Cells:

Tritiated arachidonic acid [5,6,8,11,12, 14,15- ^{3}H(N)]- was obtained (NEN research product - Division of DUPONT, Boston, MA, U.S.A.) with specific activity of 100 C/mmol. Portions of packed RBCs were resuspended in equal volumes with isotonic buffer containing tritiated AA. These samples were mixed gently for 1 hr following methods described elsewhere [20]. Then, the RBCs were washed several times with the same buffer. The efficiency of labeling was more than 85%.

Experimental approach

The first set of experiments involved shearing whole blood samples for 2 minutes period followed by centrifugal separation for 10 minutes. The supernatant was checked by the above radioimmunoassay for thromboxane B2 concentration. The second set of experiments involved shearing of PRP-PBS samples. RBCs samples in PBS were tested in the third set of experiments. The final set of experiments consisted of shearing of radio-labeled RBCs samples. The above experimental approach was designed to differentiate between different

blood components contribution to shear induced thromboxane A2 generation and to investigate the hypothesis of AA release from RBCs. Thromboxane A2 generation was shown to be shear rate rather than shear stress-dependent, therefore, comparison between samples under different imposed shear stresses but under similar shear rate conditions was justified [15]. All experiments were carried out at room temperature (22 ± 2) °C.

RESULTS AND DISCUSSION

Results obtained in this study indicate that RBCs contribute to thromboxane A2 gene-ration. Such contribution is significant and obvious from the figures representing the data obtained from the different sets of experiments comprising the experimental approach.

Figure 2 shows thromboxane B2 (index of thromboxane A2) generation in whole blood samples (WHB) as a function of shear rate. At the lowest shear rate (542 s^{-1}) its concentration was about 4 ng/100 ml, while at moderate shear rate (2712 s^{-1}) the concentration was about 9 ng/100 ml, and at the highest shear rate (5424 s^{-1}) it was about 26 ng/100 ml. Therefore, the concentration was doubled between low to moderate shear rate, while at the highest shear rate it was more than six fold the value at the lowest shear rate. This may indicate that within the range 2712 - 5424 s^{-1} there exists a threshold where significant damage to blood cells occurs. Moreover, since the same trend was seen earlier for ADP [4], therefore, thromboxane A2 generation, as a result of shear induced AA release, may be suggested to correlate with ADP shear induced release, or at least similar mechanisms, if not the same one, are responsible for the two phenomena. Since the shear rate levels for all experiments were the same, the average value of thromboxane B2 was calculated and compared to each other to indicate the relevant contribution [1, 4, 18]. Shear average concen-tration of thromboxane B2 generated was about 13 ng/100 ml.

Figure 3. shows data on thromboxane B2 generation from PRP-PBS samples. Lowest concentration was about 1.5 ng/100 ml at 542 s^{-1}, followed by 2 ng/100 ml at 2712 s^{-1}, and 2.5 ng/100 ml at 5424 s^{-1}. The differences between concentrations of the three shear levels were not significant compared to WHB results above. This may indicate that the threshold pointed above is related to RBCs rather than to platelets. An average value of about 2 ng/100 ml is obtained over the whole shear rate range. This result suggests a 15% contribution of platelets to shear induced thromboxane A2 generation when PRP-PBS samples are compared to WHB samples. However, it does not exactly reflect platelet contribution to shear induced thromboxane A2 generated as a result of AA release from RBCs, which may utilize platelet enzymatic system.

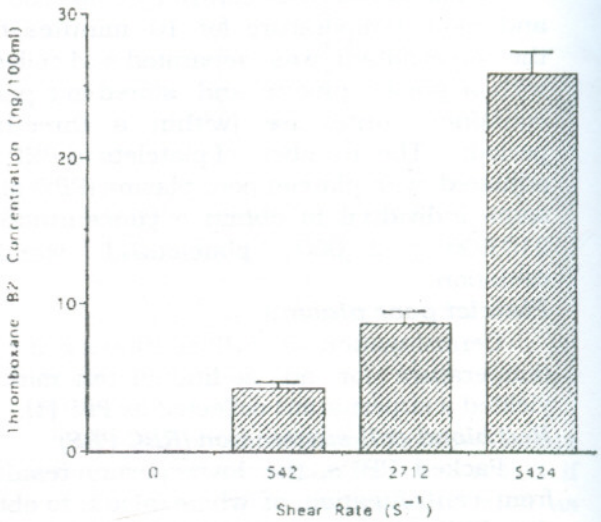

Figure 2 Thromboxane B2 generation from WHB samples

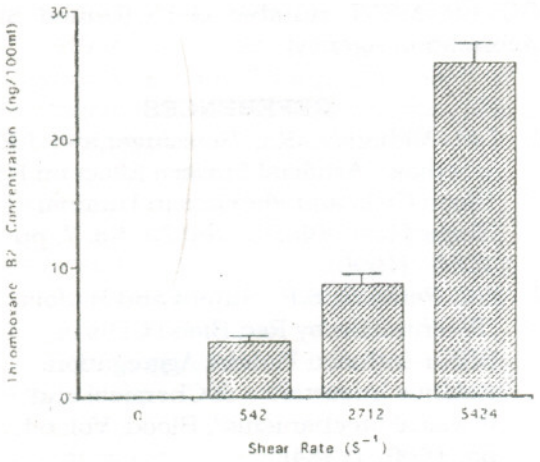

Figure 3 Thromboxane B2 generation from PRP-PBS Samples

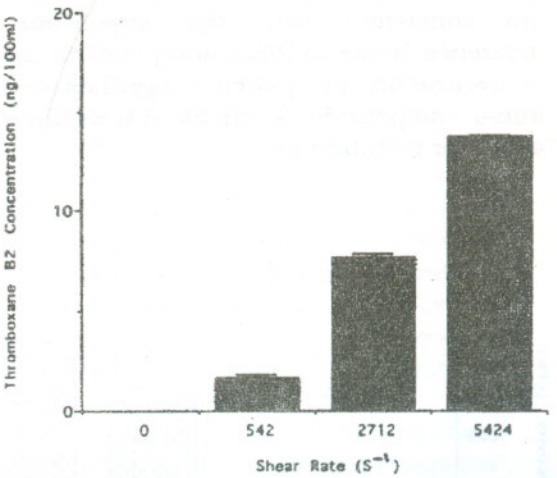

Figure 4 Thromboxane B2 generation from RBC-PBS Samples

Figure 4 shows results on thromboxane B2 generation from RBC-PBS samples. Lowest throm-boxane B2 concentration was about 2 ng/100 ml at 542 s^{-1}, followed by about 8 ng/100 ml at 2712 s^{-1}, and about 14 ng/100 ml at 5424 s^{-1}. Average concentration was about 8 ng/100 ml, which was about 60 % of that for whole blood samples. If this contribution is added to platelets' contribution of 15% it will lead to 75%, which results in 25% that are not accounted for. This 25% contribution may be due to the utilization of platelets enzymatic system, mentioned above, and/or due to a physical mechanism caused by RBCs which involves augmentation of platelet diffusion to the surface that will lead to the creation of platelet rich area near the surface, which in turn increases their interaction with the surface, and hence causes more thromboxane A2 generation. Therefore, the pure contribution of RBCs to shear induced thromboxane A2 generation is 60%, platelets pure contribution was 15%, and the combined RBCs-platelet contribu-tion was 25%. Paysant *et al.* [16] reported weak phospholipase activity in RBC membrane, which may play a role in AA metabolism. It was indicated by Rajagopalan *et al.* [20], that physical forces may indicate different pathways for eicosanoid meta-bolism than most commonly used stimuli. Therefore, shear application may activate a different mechanism of AA metabolism, which will be a topic for future research.

Figures 5 and 6 show results on RBCs radio-labeled with AA-^{3}H. These experiments were done to verify whether AA is released from RBCs as a result of imposing shear rate. Counts per minutes (CPM) were plotted versus shear rate. It is clear that CPM increase in the supernatant of RBCs radio-labeled samples, while they decrease in packed RBCs samples. This is consistent with the fact that, initially, radiolabeled molecules are all associated with the RBCs membranes. As samples were exposed to shear fields radiolabeled molecules started to leak to supernatant to form thromboxane A2, which reduces radioactivity associated with the membranes. This indicates that part of AA present in the membranes of RBCs is released due to shear rate application on an artificial surface. Moreover, this result supports the hypothesis of RBCs contribution to AA shear-induced release which may be followed by its conversion to thromboxane A2 as pointed above.

In conclusion, RBCs contribution to shear induced thromboxane A2 generation is quantified by this study. Such contribution is due to RBCs self action and through either augmented diffusion of platelets to the artificial surface or via collaboration with platelets utilizing their enzymatic system. Results may indicate that design of artificial organs has to take into consideration the existence of RBCs, since also previous studies pointed out their contribution to ADP shear-

induced release [1,3,19]. These conclusions are consistent with the significant size difference between RBCs and platelets and the concentration of platelets agonists or their initial compounds, such as AA, within RBCs and their membranes.

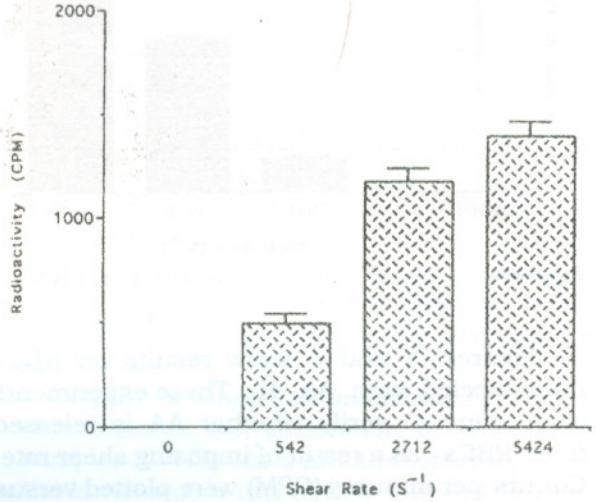

Figure 5 Radioctivity in sample of RBC-PBS supernatant

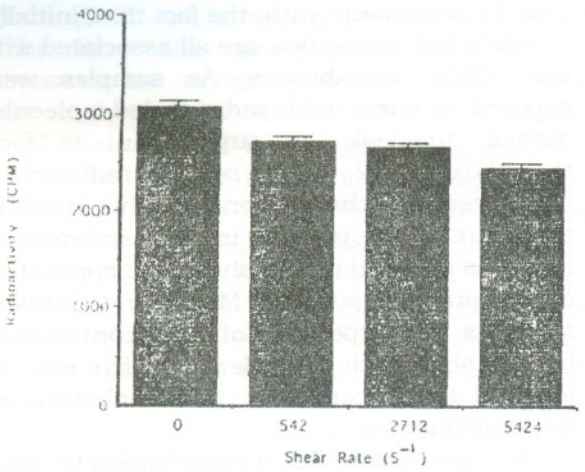

Figure 6 Radioactivity from packed RBCs samples

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REFERENCES

1. T.M. Alkhamis, R.L. Beissinger,and J.R. Chediak, "Artificial Surface Effect on Red Blood Cells and Platelets in Laminar Shear Flow", Blood, Vol. 75, No. 7, pp. 1568, (1990).
2. R.C. Reimers, S.P. Sutera and H. Joist "Potentiation by Red Blood Cells of Shear-Induced Platelet Aggregation: Relative Importance of Chemical and Physical Mechanisms", Blood, Vol. 64, pp. 1200, (1984).
3. T.M. Alkhamis, R.L. Beissinger and J.R. Chediak, "Red Blood Cell Effect on Platelet Adhesion and Aggregation in Low-Stress Shear Flow: Myth or Fact", TASAIO, Vol. 34, pp. 868, (1988).
4. T.M. Alkhamis, "Effect of Red Blood Cells on Platelet Adhesion and Aggregation in Low-Stress Shear Fields", Ph.D. Thesis, Illinois Inst. of Tech., Chicago, Il, (1988).
5. V.T. Turitto and H.J. Weiss, "Red Blood Cells: Their Dual Role in Thrombus Formation", Science, Vol. 207, pp. 541-543, (1980).
6. Guide Lines for Blood Material Interactions -Report of the National Heart, Lung, and Blood Institute Working Group, U.S. Department of Health and Human Services, (1983).
7. C.J Jen and L.V. McIntire, "Characteristics of Shear-Induced Aggregation in Whole Blood", J. Lab. Clin. Med., Vol. 103, pp. 115, (1984).
8. A.J. Marcus, "The Role of Prostaglandins in Platelet Function", Prog Hematol, Vol. 11, pp. 147, (1979).
9. G.W. Dorn, and A. DEJesus, "Human Platelet Aggregation and Shape Change are Coupled to Separate Thromboxane A2-Prostaglandin H2 Receptors", Am. J. of Physiology, Vol. 260 No. 2, pp. 327, (1991).
10. M.J. Hamberg, J. Svensson, and B. Samuelsson, "Thromboxanes: A New Group of Biologically Active Compounds Derived from Prostaglandin

Endoperoxides", Proc. Natl. Acad. Sci, USA, Vol. 72, pp. 2994, (1975).
11. S. Moncada, and J.R. Vane, "Pharmacology and Endogenous Roles of Prostaglandin Endoperoxide, Thromboxane A2 and Prostaglandin", Pharmacol. Rev., Vol. 30, pp. 293, (1979).
12. P.J. Piper and J.R. Vane, "Release of Additional Factors in Anaphylaxis and its Antagonism by Anti-Inflammatory Drug, Nature, Vol. 223, pp. 29, (1969).
13. R. Verheggen and K. Schror, "The Modification of Platelet -Induced Coronary Vasoconstriction by Thromboxane Receptor Antagonist", J. of Cardiovascular Pharma-cology, Vol. 8, pp. 483, (1986).
14. H. Kawai, and Y. Tamao, "The Combination of Thrombin Inhibitor and Thromboxane Synthase Inhibitor on Experimental Thrombosis and Bleeding", Thrombosis Research, Vol. 80, No. 5, pp. 429, (1995).
15. T.M. Alkhamis and R.L. Beissinger, "Evidence of RBC Contribution to TXA2 Generation in Laminar Shear Flow", Mu'tah Journal for Research and Studies - Natural and Applied Sciences Series, Vol. 7, No. 2, pp. 177, (1992).
16. S. Moncada, and J.R. Vane, "Arachidonic Acid Metabolites and Interaction between Platelets and Blood Vessel Walls", Physiology in Medicine, Vol. 300, No. 20, pp. 1142, (1979).
17. R.L. Beissinger and M.C. Williams, "Effects of Blood Storage on Rheology and Damage in Low-Stress Shear Flow", Biorheology, Vol. 22, pp. 477, (1985).
18. T.M. Alkhamis, R.L. Beissinger and J.R. Chediak, "Effect of Red Blood Cells on Platelet Adhesion and Aggregation in Low-Stress Shear Flow", TASAIO, Vol. 33, No. 10, pp. 636, (1987).
19. S.C. Srivastava, "Radiolabeled Red Blood Cells: Status, Problems, and Prospects", in "Radiolabeled Cellular Blood Elements", Editor: M.L. Thakur, Plenum Press, pp. 363, (1983).
20. S. Rajagopalan, L.V. McIntire, E.R. Hall and K.K. Wu, "The Stimulation of Arachidonic Acid in Human Platelets by Hydrodynamic Stresses", Biochemica et Biophysica Acta, Vol. 958, pp. 108, (1988).

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مساهمة كريات الدم الحمراء والصفائح الدموية لانتاج ثرمبوكسين أ٢ والمحفز باجهاد القص فى السريان

طه موسى الخميس

قسم الهندسة الكيماوية -جامعة مؤته - الأردن

ملخص البحث

صممت هذه الدارسة العملية لبحث مساهمة كريات الدم الحمراء في انتاج ثرمبوكسين أ٢ تحت تأثير اجهاد القص ونتيجة تحرير حامض الأراكيدونيك وكذلك لتحديد قيمة المساهمة ان وجدت .

وقد تم استخدام اسلوب عملي لتحديد مساهمة كريات الدم الحمراء والصفائح الدموية تحت تأثير اجهادات قص منخفضة لا تصل في حدها الاعلى الى ٢٠٠ داين/ سم^{٢} ولمدة دقيقتين في جهاز وايزنبرغ ريوميتر ، حيث تم بداية اجراء اختبارات على عينات دم تحتوي على جميع المكونات، بعدها تم فحص عينات في مجموعة ثانية من الاختبارات مكونة من البلازما الغنية بالصفائح الدموية والمعلقة في محلول ملحي متعادل كما تم فحص عينات في مجموعة ثالثة مكونة من كريات دم حمراء معلقة في محلول ملحي متعادل وفي مجموعة رابعة من الاختبارات تم تمييز كريات الدم الحمراء بمادة تريتيوم المشعة ومن ثم اجراء الفحص عليها.

تشير النتائج الى ان كريات الدم الحمراء تساهم بشكل واضح الى انتاج مادة ثرمبوكسين أ٢ نتيجة لتأثير اجهاد القص المنخفض وقدرت مساهمة كريات الدم الحمراء لانتاج مثل هذه المادة بحوالي

٦٠ % ومساهمة الصفائح الدموية بحوالي ١٥ % بينما يرجع الباقي الى مساهمة مشتركة محتملة بين كريات الدم الحمراء والصفائح الدموية وبواقع ٢٥ % .