Osmotic Fragility of Human Blood Platelets

By J. Gurevitch and D. Nelken

During previous studies on the ABO antigens of blood platelets, it was observed that storage of platelet suspensions in normal saline resulted in a gradual decrease of their agglutinability by antisera, similar to the decrease of agglutinability of red blood cells stored in saline.

This similarity in the behavior of erythrocytes and thrombocytes suggested the study of other aspects of the physiology of thrombocytes in comparison with erythrocytes. The present paper reports the behavior of thrombocytes in hypotonic saline.

Materials and Methods

All glassware was siliconized before use. A solution composed of 1 per cent disodium sequestrene and 1 per cent Triton W.R. 1339 was prepared in 0.7 per cent sodium chloride. One ml. of this solution, added to 9 ml. of blood prevented both coagulation and irreversible clumping of platelets during centrifugation.

Preparation of the platelet suspension. Blood was taken into a siliconized syringe containing the disodium sequestrene-Triton solution. The blood was rapidly transferred into a large centrifuge tube with a flat bottom (a conical bottom made the resuspension of platelets difficult), and centrifuged in a horizontal centrifuge for five to seven minutes at 800 r.p.m. The supernatant plasma, containing a pure suspension of thrombocyte, was then centrifuged for ten minutes at 2000 r.p.m. to obtain a sediment of blood platelets. To a platelet sediment prepared from 15–20 ml. of blood, 1 ml. of normal saline was added and the tube, after standing for 30 minutes at 6°C, was gently shaken every 10 minutes to obtain a homogeneous concentrate of platelets.

Preparation of NaCl dilutions. Serial dilutions of saline from 0.60 per cent to 0.22 per cent in steps of 0.02 per cent were prepared in 20 clean dry serologic test tubes, 100 x 13 mm. One milliliter of each solution was transferred to a siliconized serologic tube of the same size for the examination of each platelet sample.

Estimation of 5-Hydroxytryptamine (serotonin). Serotonin was estimated by using an isolated segment of a rat's atropinized colon as described by Delgleich, Toh and Work.

Experimental

Fifty platelet samples obtained from normal healthy individuals were examined as to their osmotic fragility. To each saline dilution 0.05 ml. of the platelet suspension was added with a siliconed pipet. The tubes were then gently shaken by hand to insure homogeneous distribution of the platelets, and left at room temperature, about 25°C, with gentle shaking every 30 minutes. Results were read after 2 hours. Before reading, the tubes were again gently shaken and one drop transferred with a siliconed pipet to a siliconed slide and covered with a siliconed cover glass. This preparation was examined immediately with the oil immersion of a phase contrast microscope (750 X–1000 X).

We are indebted to Miss L. Boiato for technical assistance and for the drawings, and to Dr. L. Wislicki and to Dr. G. Izak for help with the serotonin estimations.

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This investigation was supported by a grant from the Meir Levinsohn Memorial Fund.

Submitted February 7, 1956; accepted for publication February 17, 1956.
At a concentration of 0.85 per cent NaCl the platelets were fairly uniform in size. They were polygonal, many of them possessing spicules; no internal structure could be recognized.

At 0.60 per cent NaCl (fig. 1) no changes in size occurred, the platelets being somewhat more rounded than at 0.85 per cent NaCl. Most cells had spicules and resembled small spiders, no internal structure was visible. In further salt concentrations from 0.60-0.44 per cent the platelets were enlarged, their spicules longer and fine granules appeared within the cells. This granulation usually started at one side of the cell, seldom at the center.

At a concentration of 0.44 per cent (fig. 2), certain new features appeared: most platelets were still small and spiderlike; a very few cells possessed one long swordlike spicule. Platelets having this process, which was 5 to 7 times longer than the cell, were still small and had no internal structure.

At a concentration of 0.38 per cent, about a third of the platelets showed distinct central granulation. They were enlarged, rounded, and had a sharp process which was about five times longer than the whole platelet. Some platelets had two such processes which were somewhat smaller and thinner. These swordlike processes were always clear and without any granulation.
As the salt concentration decreased, the platelets tended to form aggregates of 5 to 10 cells. Each cell possessed a swordlike process and was very large and round without normal spicules. Many cells were empty "ghost cells," others showed eccentric coarse granules. At a concentration of 0.34 per cent NaCl about 80 per cent of the cells had this appearance (fig. 3).

In the last five tubes (0.30–0.22 per cent) almost all platelets were disintegrated, forming large clumps of cells with characteristic swordlike processes. Many cells were empty "ghost cells."

Fig. 3.—Normal human platelets in 0.34 per cent NaCl.

Fig. 4.—Typical curve of serotonin released from platelets in hypotonic NaCl solutions.
With the decrease in the salt concentration a fall in turbidity occurred. Tubes with concentrations of 0.60–0.44 per cent had a milky appearance, the last 5 tubes, however (0.30–0.22 per cent), showed very little turbidity. The assessment of fragility by a turbidimetric method was found to be impracticable because of the interference of the siliconized tubes, the small quantity of fluid and the very small differences in turbidity between the tubes.

It was of interest to examine the serotomine released from the platelets disintegrated at the different saline concentrations. To this end, the tubes left for two hours at room temperature were centrifuged at 3000 r.p.m. for 20 minutes and the supernatants frozen at −20 °C. The serotomine contents of the supernatant solutions of six different samples of platelets were estimated using serotomine creatinine sulfate* as a standard. As may be seen in the graph there was a steady increase in the amount of serotomine released from the platelets with decrease in the NaCl concentration.

**Comment**

The problem of platelet fragility was studied by Muhrer, Bogart and Hogan in 1944. These authors examined swine platelets and measured their fragility by the release of thromboplastin in different hypotonic solutions. The thromboplastin was then added to recalcified plasma and the rate of coagulation was an indirect measure of the extent of platelet disintegration. Day in 1947 studied the fragility of human blood platelets in various hypotonic saline solutions. He used platelet-rich plasma which was added to hypotonic saline solutions, recalcified, and the coagulation time measured. He assumed that the coagulation time is quantitatively related to the speed of thrombin formation, which is in turn directly dependent upon the amount of thromboplastin released by platelets. If platelet-rich plasma exposed to hypotonic solutions clotted faster than normal plasma this was due to more complete lysis of platelets. Olef in 1936 described a method of examining the rate of disintegration of platelets by observing microscopically, at regular intervals, the quantitative changes of platelets kept in a solution of sodium metaphosphate. Platelets from normal persons and from patients suffering from various diseases were examined by this method. In 1939, Gram and Louis studied the osmotic fragility of human platelets by examining the platelets microscopically after incubation in hypotonic saline solutions. They found that at a NaCl concentration of 0.3 per cent the platelets were clumped and liberated many granules.

In our studies, fragilities of 50 samples of normal platelets were examined in hypotonic NaCl solutions. All these platelet samples showed identical behavior in the different NaCl concentrations. Dissolution started at a concentration of about 0.44 per cent NaCl and was complete at a concentration of about 0.34 NaCl. This range of fragility shows a striking similarity to the results obtained with red blood cells.

In connection with the gradual disintegration of blood platelets in hypotonic saline solutions, the release of serotomine from the platelets into these solutions.

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* We are grateful to the Upjohn Company, Kalamazoo, Michigan, for the supply of this material.
was examined. Results showed that the amounts of serotonin released from the platelets increased with the decrease of the NaCl concentration.

The application of the thrombocyte fragility test to platelets of patients with various bleeding tendencies is under examination.

**Summary**

The osmotic fragility of 50 samples of platelets from normal healthy donors has been studied. It has been found that fragility of platelets starts at a concentration of about 0.44 per cent NaCl and is complete at a concentration of 0.34 per cent. The serotonin released from the platelets increased with the gradual disintegration as the saline concentration fell.

**Summario in Interlingua**

Esseva studiate le fragilitate osmotic de 50 specimenes de plachettas ab normal donatores human in bon stato de sanitate. Esseva constatatque le fragilitate del plachettas comencia a un concentration de circa 0,44 pro cento de NaCl e es complete a un concentration de 0,34 pro cento. Le secroronina relaxata per le plachettas se augmentava in le curso del gradual disintegration correspondente al reduction del concentration salin.

**References**

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