

PLATELET ADHESIVENESS

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IN 1941, Helen Wright¹ described a method for estimating the adhesive quality of platelets. Confirmation of the original findings by Spooner and Meyer² established the procedure as a means of distinguishing this property of platelets. It is the purpose of the present report to evaluate the usefulness of the method in clinical medicine.

METHOD

Five ml. of blood is obtained by "clean" venipuncture with a dry needle and syringe, and delivered into a small dry bottle containing 8 mg. potassium oxalate and 4 mg. ammonium oxalate.* The bottle is gently rotated to aid solution of the oxalate. Within 10 minutes a 2 ml. sample of this oxalated blood is pipetted into a glass tube with a bulbous dilatation at one end. The tube is then rotated at 7 r.p.m. Samples for serial platelet counts are withdrawn before rotation is begun, and every 20 minutes for 80 minutes. The platelet counts are made using the standard red cell pipet with Reese-Ecker diluting fluid to which 2 per cent formaldehyde has been added. The diluting fluid is autoclaved after it is prepared, and stored at refrigerator temperature. Each portion is filtered before it is used. The counting chambers are placed on wet filter paper and covered with a petri dish after being loaded. This permits the platelets to settle without drying on the chamber for about a half hour before the count is made. By this technic large clumps of platelets are rarely seen, and microorganisms which may be confused with platelets are kept at a minimum.

The serial platelet counts are calculated in terms of per cent of the initial count, and a curve is plotted with the number of remaining platelets as ordinate and the time in the rotating tube as abscissae. The curve then represents the proportion of platelets which have failed to adhere to the wall of the rotating tube. In normal individuals, platelet counts usually fall to about 30 per cent of the initial count in 80 minutes. Adhesiveness determinations carried out in 22 adults without evidence of disturbed coagulation or phenomena indicative of vascular alteration, yielded counts of from 25 per cent to 40 per cent of the initial count in 80 minutes. In almost every case, the estimations were made in duplicate. The initial counts ranged from 140,000 to 220,000 per cmm. The data obtained in the 22 subjects is summarized in the solid curves of figure 3.

Since platelet counts vary widely when made by different observers, the counts have been made by one person especially practiced in the procedure, who had attained thoroughly consistent manipulative characteristics. We have found the procedure time consuming and inconsistent when the technic was varied only slightly. Prothrombin estimations were made by a method previously described.³ Fibrinogen levels were established by determination of the protein content of plasma before and after the contained fibrinogen was coagulated and removed.

RESULTS

The present report embraces a study of the curves of platelet adhesiveness in 110 instances which include both normal and a large variety of disease states.

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* Since this paper has been submitted, we have been using 0.2 mg. heparin per ml. blood in place of the oxalate.

No abnormality in platelet adhesiveness was demonstrated in a large variety of diseases in which disturbed coagulation was not a factor. These include infectious mononucleosis, 2 cases; erythema multiforme, 1 case; pulmonary hemorrhage secondary to bronchiectasis, 1 case; duodenal ulcer, 2 cases, one actively bleeding; rheumatic heart disease, 3 cases; arteriosclerotic heart disease, 4 cases; hypertensive heart disease, 4 cases; diabetes mellitus, 2 cases; rheumatoid arthritis, 2 cases; amyloid disease, 1 case; subarachnoid hemorrhage, 1 case; and multiple sclerosis, 1 case. The detailed data which follow represent instances in which altered adhesiveness of the platelets and/or coagulability were demonstrated.

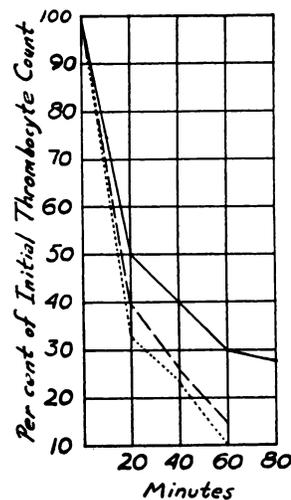


FIG. 1. PATIENT G: EFFECT OF HYPERPROTHROMBINEMIA INDUCED BY LARGE DOSES OF VITAMIN K
Solid line indicates Control (diluted, 12.5 per cent, plasma prothrombin. Time, 42 sec. normal).
Broken line indicates third day (12.5 per cent plasma prothrombin time, 35.2 sec.). *Dotted line* indicates fourth day (12.5 per cent plasma prothrombin time, 31.8 sec.).

1. Platelet Adhesiveness in Hypercoagulable Blood

The adhesiveness of the platelets was studied in conditions in which the blood was hypercoagulable. It is possible to induce this condition experimentally in some subjects with normal liver function.⁴ It occurs spontaneously in some cases of intravascular thrombosis. It is detectable by a reduction in prothrombin time to a level below the limits of normal (hyperprothrombinemia) and/or thrombocytosis.⁵

(a) Hyperprothrombinemia Induced by Vitamin K.⁴

Synthetic vitamin K (Synkayvite) (Tetrasodium 2-methyl-1,4-naphthohydroquinone diphosphoric acid ester) 76 mg. daily on four successive days was given to a subject with normal liver function, in order to induce hyperprothrombinemia (fig. 1).

Day of therapy	Prothrombin time of diluted (12.5%) plasma*	Fibrinogen	Initial platelets in 80 minutes
	<i>seconds</i>	<i>mgm./100 ml</i>	<i>%</i>
Control.....	42.2	410	29
Third.....	25.2	400	13
Fourth.....	31.8	450	9
Sixth.....	42.0		20

* Normal range 37-42 seconds.

(b) *Reactive Thrombocytosis and Hyperprothrombinemia in Thrombosis.*⁵

Diagnosis	Prothrombin time of diluted (12.5%) plasma*	Platelet count per cmm.	Initial platelets in 80 minutes
	<i>seconds</i>		<i>%</i>
Acute venous thrombosis.....	33	600,000	30
Acute coronary thrombosis.....	Not made	600,000	24

* Normal range 37-42 seconds.

2. *Platelet Adhesiveness in Hypocoagulable Blood*

(a) *Dicumarol-induced Hypoprothrombinemia*^{2, 6} (fig. 2).

Prothrombin time of whole plasma	Initial platelets in eighty minutes	Diagnosis and remarks
<i>seconds</i>	<i>%</i>	
24.0	53	Pulmonary embolus, on dicumarol therapy
30.0	53	
24.0	55	
15.0	30	

(b) *Thrombocytopenic Purpura.*

A 20 year old white male showed the clinical manifestations of thrombocytopenic purpura. The platelet counts varied between 22,000 and 220,000 and were usually below 80,000 per cmm. The intensity of the clinical symptoms could not be correlated with the degree of thrombocytopenia. The prothrombin time, fibrinogen concentration, clot retraction and clotting time were consistently normal. Four determinations of platelet adhesiveness were made. The initial counts varied between 60,000 and 220,000. In none of these did the count fall below 47 per cent of the initial value after eighty minutes. The average count after eighty minutes was 53 per cent. The average of the four determinations is compared to the normal in figure 3.

(c) *Spontaneous Purpura in Pregnancy.*

A 26 year old female para 1 gravida 1, commenced to show diffuse purpuric hemorrhages during the sixth month of pregnancy. Physical examination revealed no other positive findings. Prothrombin time, fibrinogen concentration and clotting

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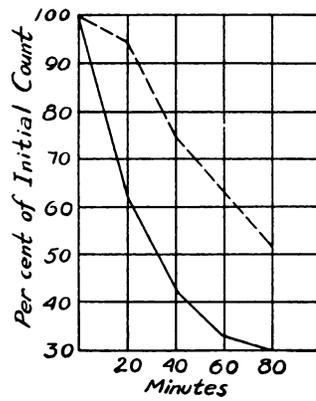


FIG. 2. PATIENT W.

Solid line indicates control. Broken line indicates after dicumarol (prothrombinopenia)

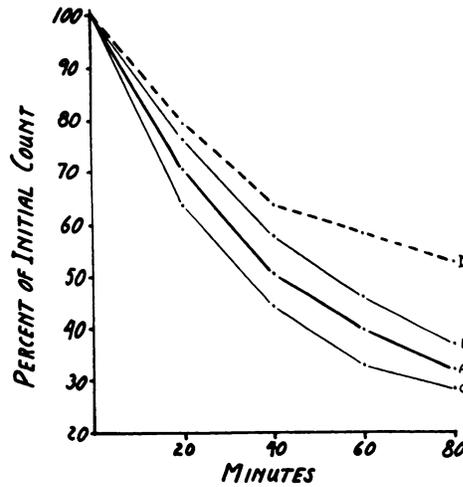


FIG. 3. PLATELET ADHESIVENESS IN THE NORMAL AND IN THROMBOCYTOPENIC PURPURA

Curve A represents the average value of 22 normal individuals. Curves B and C represent the average deviation of these normals. Curve D is the average of 4 estimations made in a patient with thrombocytopenic purpura. The initial platelet counts varied between 60,000 and 220,000.

time were normal. The platelet count was 158,000 and the adhesiveness curve yielded a fall to 67 per cent of the initial count in 80 minutes. The purpuric condition subsided spontaneously one month later, and the adhesiveness curve became normal.

minutes	During hemorrhagic phase	After recovery
	per cent of initial count	%
20	84	75
40	85	48
60	80	42
80	67	32

(d) *Prolonged Clotting Time and Hemorrhage*

A male adult with chronic rheumatic valvular disease, exhibited continuous oozing of blood from the gums for three days after a tooth was extracted. The bleeding and prothrombin times were normal, but the clotting time was slightly prolonged and prothrombin times were normal, but the clotting time was slightly prolonged to seven and one-half minutes by the rotating tube method.² (Upper limit of normal is six and one-half minutes.) At that time the platelet count was 140,000 and the adhesiveness curve fell to 30 per cent in 80 minutes. After the hemorrhage ceased, the clotting time became reduced to normal (four and one-half minutes) and the platelet count increased to 370,000 and the adhesiveness curve yielded a reduction to 18 per cent of the initial count in sixty minutes, which is indicative of an increase beyond normal.

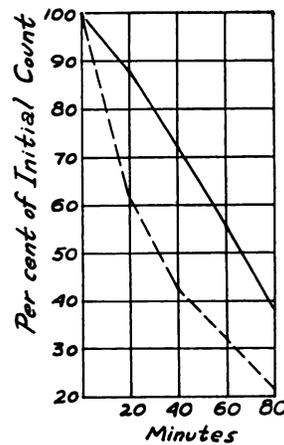


FIG. 4. PATIENT R: HEMOPHILIA

Solid line indicates control. Broken line indicates coagulation time reduced by Fraction 1

(e) *Chronic Myelogenous Leukemia*

A male adult with chronic myelogenous leukemia began to show diffuse purpura concomitantly with marked increase in peripheral myeloblasts. The platelet count was 262,000 but the adhesiveness curve fell only to 82 per cent of the initial count in eighty minutes. The clotting time and prothrombin time were normal. The patient died of exsanguination three days later.

(f) *Hemophilia: Effect of Antihemophilic Fraction one*⁸*

An 18 year old Negro male of unknown parentage showed the coagulation defects characteristic of hemophilia since early childhood. The bleeding time, prothrombin time, and fibrinogen content of the plasma were normal. The clotting time, when the present study was made, was sixty-nine minutes by the rotating tube method (Normal = four to six and one-half minutes). The platelet adhesive-

* Fraction 1 was prepared from blood collected by the American Red Cross, under a contract between the Office of Scientific Research and Development and Harvard University. It was supplied through the courtesy of Dr. Louis K. Diamond.

ness curve fell to 38 per cent in eighty minutes (upper limit of normal). Anti-hemophiliac fraction 1 was administered intravenously. The coagulation time became reduced to 16 minutes and the adhesiveness of the platelets increased to 23 per cent in eighty minutes (fig. 4).

A 40 year old white male with hemophilia: the diagnosis was established in early childhood. The coagulation time ranged between 37 and 47 minutes by the rotating tube method (normal = four to six and one-half minutes). On numerous occasions, platelet adhesiveness was determined and averaged 32.5 per cent of the initial count in eighty minutes. He was given fraction 1 intravenously, and the clotting time was reduced to between four and ten minutes at various times. Platelet adhesiveness estimated when clotting time was reduced to normal, yielded an average of 24 per cent (two estimations) in eighty minutes.

SUMMARY OF FINDINGS

In the presence of hypercoagulable blood occurring spontaneously in association with thrombosis or artificially induced by large doses of vitamin K, the adhesiveness of the platelets was generally increased. In the spontaneously occurring cases, the change was not constant, instances of normal adhesiveness having been observed even in the presence of marked thrombocytosis. Hypocoagulable blood, whether occurring as part of a blood dyscrasia, or induced by dicumarol, was accompanied more consistently by decreased adhesiveness of the platelets. In hemophilia, the clotting time and the adhesiveness simultaneously were reduced following restoration of coagulability to normal by Fraction 1.

In several cases exhibiting hemorrhagic phenomena, decreased stickiness of the platelets was the only defect found in the coagulation mechanism.

DISCUSSION

Adhesiveness of platelets appears to be dependent upon at least two factors: the intrinsic properties of the thrombocyte surface, and the character of the medium in which the platelets are suspended. It is believed⁹ that fibrinogen is converted into fibrin on the surface of the platelets, and that adhesiveness is the result of this change. It would follow then, that inhibition of the clotting mechanism should impair the adhesive capacity of the thrombocytes and conversely, that augmented coagulability should accelerate adhesiveness. The findings confirm the first hypothesis but support the latter with less constancy in hypercoagulability accompanying thrombosis. Hypocoagulable blood may be induced by deficiency of any component factor of the clotting mechanism. Thrombosis, on the other hand, appears to be the result of a combination of events of which hypercoagulability of the blood may, or may not, be one. In addition, in thrombotic disorders, anticoagulants may be elaborated into the blood stream, presumably as a protective mechanism, so that, when examined in vitro, the behavior of the thrombocytes may vary according to the particular type of response operating at the time the specimen is obtained. Nevertheless it seems logical to assume that in a given case, the detection of increased adhesiveness of the thrombocytes warrants pursuit of the possibility of thrombosis being present or imminent. In earlier studies³ we have found evidence of increased prothrombin activity in the presence of intravascular

thrombosis, and have suggested it as a diagnostic aid, both in the detection of thrombosis (especially in inaccessible locations) as well as in the selection of probable candidates for the complications. The practical application of the detection of hypercoagulability of the blood, particularly in postoperative cases, and following parturition, is obvious. Studies of this kind in inadequately understood disease states accompanied by thrombosis such as thrombocytopenia with platelet thrombus formation¹⁰ might shed some light on the mechanism underlying the disorder.

The correlation noted in some of our cases, between the state of coagulability of the blood and adhesiveness of the platelets, seems to support the fibrin theory of stickiness of thrombocytes. A few observations seem to require additional explanations: microstaining studies have not revealed fibrin on platelet surfaces.¹¹ In the presence of the markedly increased clotting time in hemophilia, the detectable differences in adhesiveness as compared with the normal have been relatively slight, while in dicumarol-induced prothrombinopenia of moderate severity, much greater interference in platelet adhesiveness is the rule. In the cases in which fibrinogen determinations were made, the changes in platelet adhesiveness were found to occur independently of the concentration of this protein in the plasma.

The deficiency in adhesiveness of the platelets noted in hemophilia is of particular interest. Since the blood is hypocoagulable, it is not possible to determine from the present study whether or not the platelets are functionally defective in hemophilia. The stickiness of the thrombocytes is promptly increased when the coagulation defect is corrected by Fraction 1. It is possible that the improved adhesiveness is an expression of the more normal coagulability of the blood.¹² In thrombocytopenic purpura, we have found Fraction 1 to be without effect.¹³ This phase of the study is being extended in this laboratory.

The two cases of bleeding described above (cases c and e) in which deficient adhesiveness of platelets was the only detectable defect in the coagulation mechanism, suggest that a knowledge of the state of the stickiness of thrombocytes may be of assistance in explaining instances of hemorrhagic tendencies which do not fall in categorized syndromes.

SUMMARY AND CONCLUSIONS

The method described by Helen Wright for estimation of the adhesiveness of platelets has been found to be reproducible. Adhesiveness of platelets is reduced in the presence of hypocoagulable blood. It may or may not be enhanced when the blood is hypercoagulable. In hemophilia, the adhesiveness of platelets has been found to become increased beyond the premedication level when the coagulation defect is corrected by Fraction 1 derived from human plasma. Two cases exhibiting bleeding are described in which deficient adhesiveness of the thrombocytes was the only demonstrable defect noted in the coagulation mechanism.

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