

Dr William W. Duke: Pioneer in Platelet Research

SUMMARY OF THE ORIGINAL ARTICLE

The Relation of Blood Platelets to Hemorrhagic Disease: Description of a Method for Determining the Bleeding Time and Coagulation Time and Report of Three Cases of Hemorrhagic Disease Relieved by Transfusion

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JAMA. 1910;55(14):1185-1192

The purpose of this article is to report 3 cases and experiments that furnish additional evidence showing that blood platelets play a part in stopping hemorrhagic disease. In addition, a method for studying hemorrhage called the bleeding time, and a simple method for determining the coagulation time are described.

See www.jama.com for full text of the original *JAMA* article.

Commentary by Thomas Stephen Kickler, MD

IN 1910, WHEN DUKE¹ PUBLISHED AN ARTICLE IN *JAMA* ON the role of platelets in hemostasis, probably few individuals realized that this report would be judged as one of the outstanding contributions to the science of medicine during the first half of the 20th century.² The application of a simple, low-tech approach to answering the elusive role of platelets in hemostasis provided the foundation for many subsequent developments in platelet biology and therapy. This Commentary will focus on Duke's observations and on how his observations have led to improved understanding of the pathophysiology, diagnosis, and treatment of platelet-related disorders.

Humans have always been fascinated by blood. In the late 1800s, poets and philosophers referred to blood as being pure and eloquent—the very essence of life.³ Scientists of the late 19th and early 20th century were motivated with these sentiments and empowered in their study of blood by improvements in microscopy and chemical stains. The easy accessibility of blood by venipuncture clearly puts the investigations of blood diseases ahead of investigation of other less-accessible organ-related diseases. Soon the blood elements erythrocytes, leukocytes, and “platelet dust” were described. With the identification of platelets, a debate arose concerning their physiologic role.

Duke was at the right place at the right time determining the physiologic role of platelets. He received his medical training at Johns Hopkins University in 1908. Two years later, he published his seminal work on platelet physiology while working at the Hunterian Laboratory of Experimental Pathology at Johns Hopkins University. In the early 1900s, Wil-

liam Welch established the Department of Pathology and the Hunterian Experimental Pathology Laboratory with an emphasis on experimental investigation. This was a unique and extraordinary environment in which young Duke could perform his studies. His mentors included the outstanding clinical and laboratory investigators of the time. George Whipple, who was studying hemorrhagic disease of the liver at the time, provided Duke with the opportunity to study mechanisms of coagulation. Studying under the renowned physiologist W. H. Howell allowed Duke to conclude that thrombin generation proceeded despite presence of thrombocytopenia, and as a corollary, the bleeding time is not a test for blood clotting. Another major influence on Duke's studies came from L. Selling, a Hopkins experimental pathologist who wrote the important monograph on benzene as a marrow toxin.⁴ From this observation, Duke took advantage of using benzol to induce thrombocytopenia, which permitted the development of an animal model of thrombocytopenia. All 3 of these great medical investigators are acknowledged in Duke's *JAMA* publication.¹

Although other individuals provided the first description of bleeding time, Duke's report had a greater influence on medical thinking. Duke showed quantitatively that the bleeding time was increased in proportion to the severity of thrombocytopenia. Furthermore, he demonstrated that correction of thrombocytopenia through transfusion of fresh whole blood would correct the bleeding time to normal with the return of the platelet count to a level greater than 100 000/ μ L. This

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was the first report showing that thrombocytopenia was correctable by the transfusion of fresh blood. Instrumental in performing these studies was the ability to perform platelet counts. Counting platelets is now taken for granted but at the time of these studies, it was an accomplishment that could not have taken place without Duke's collaboration with James Homer Wright, who discovered Wright stain at Massachusetts General Hospital.

While little is written about Duke's character or the circumstances that led to his interest in hemostasis, this article illustrates his collaborative nature and ability to ask important questions generated from experimental observations. Moreover, he clearly seemed to appreciate the importance of having good mentors judging by the men with whom he worked.

In Duke's report on the role of platelets in hemostasis, he took 2 approaches: development of an animal model of thrombocytopenia, and clinical and therapeutic observations of thrombocytopenic patients. He was a pioneer in the bedside-to-bench approach of advancing medical science. In his human investigation studies,¹ direct whole blood transfusions were given to 3 thrombocytopenic patients who had severe bleeding. He observed that after the correction of the platelet count, the bleeding time corrected from being markedly elevated for 60 minutes declining to 3 minutes. The tendency to re-bleed returned when the platelets disappeared. However, the degree of bleeding tendency was much less than prior to the transfusions. Duke observed correctly that the correction of the anemia somehow was also responsible for lessening the severity of bleeding. The basis of this finding was much later reexamined. For instance, it is now known that in the bleeding diathesis of uremia, in which the bleeding time can be prolonged due to acquired platelet dysfunction, correction of the hematocrit level to a value of greater than 30% is associated with improvement of the bleeding time and clinical bleeding.⁵ This observation is important in the management of patient cases of chronic renal failure and can now be managed with recombinant erythropoietin to correct the anemia.⁶

Taking his bedside observations to the laboratory, Duke demonstrated that the lack of platelets was the cause of hemorrhage in thrombocytopenic dog studies. Using benzol, he developed an animal model of thrombocytopenia. By using different doses of benzol, he observed in mild-to-moderate thrombocytopenia that no hemorrhage would result. However, with platelet counts of less than 30 000/ μ L spontaneous hemorrhage was likely. The concept of relationship to degree of thrombocytopenia would become an important clinical observation and it provided the basis for what is now known as the platelet transfusion trigger—a platelet value at which spontaneous bleeding occurs and platelet transfusions should be administered.^{7,8} He also demonstrated that this hemorrhagic diathesis was not due to impaired blood clotting factors in plasma, by developing a standardized coagulation time test. Thus, the concept of platelets' primary

role in hemostasis was provided, with plasma clotting proteins playing a related role through the coagulation cascade, known as secondary hemostasis.¹

Two other important concepts introduced by Duke are the short lifespan of platelets and the presence of what he called stimuli in producing platelet regeneration. These observations were documented in his *JAMA* article¹ but were more fully described in a second report on platelet regeneration.⁹ These concepts are now known as platelet kinetics and thrombopoiesis. Duke observed that unlike the red blood cells, after platelets were transfused there was a uniform and rapid disappearance of the platelets. He postulated correctly that the life span of platelets must be much shorter than that of red blood cells. It is now known that the life span of platelets is shorter than 10 days compared with 120 days for red blood cells. Duke also observed in his animal model that the recovery of platelets occurred quickly, and this regeneration response suggested the presence of some stimuli. Thrombopoiesis is indeed under the control of stimuli—the most important of which is thrombopoietin, a growth factor that promotes megakaryocyte maturation and production of platelets. Commercially recombinant agents are now in use to treat thrombocytopenia.¹⁰

While Duke's bleeding time procedure provided important information for his studies, there is no indication that he proposed using the test diagnostically. The bleeding time test was soon modified by other researchers who attempted to improve its standardization. The bleeding time was applied diagnostically to define the cause of bleeding in a variety of clinical settings and it was used in diagnosis of von Willebrand disease, a disorder caused by impaired platelet adhesion due to a quantitative or qualitative deficiency of von Willebrand factor. As with any diagnostic test, the performance of the bleeding time test, in terms of sensitivity and specificity, is dependent on the prior probability of platelet dysfunction.¹¹ The widespread indiscriminate application of bleeding time as a screening method for platelet dysfunction or as a predictor of surgical bleeding proved unreliable. Most systematic reviews of the diagnostic use of the bleeding time test have not led to its being used diagnostically or prognostically.¹²

The usefulness of the bleeding time has been as a specialized test for evaluation of devices or therapies that might affect platelet physiology *in vivo*. The testing has not been applied for several years, and physicians and technologists are no longer trained to perform the test. Multiple advancements in treatment and understanding of bleeding have resulted from the bleeding time being used in clinical investigation. For example, the bleeding time has been useful in the evaluation of the function and viability of new platelet transfusion therapy and platelet storage lesions.¹³ The bleeding time continues to be a key method to evaluating new antiplatelet drugs and devices that may affect platelet function when blood is exposed to a foreign surface. The recognition of platelet dysfunction during cardiac surgery was first documented by performing

bleeding time tests.¹⁴ More recently, by performing bleeding time tests and analyzing the constituents of shed blood, new insights have developed into the role of platelets in microvascular bleeding and the effect of antiplatelet agents.¹⁵

The JAMA Classics article by Duke¹ is historically important for 2 reasons: it defined the role of platelets in hemostasis and it documented the therapeutic efficacy of blood transfusion in treating thrombocytopenia. Duke's key questions have been considered by several generations of platelet investigators, particularly those focusing on platelet life span, control mechanisms in platelet production, and contribution of red blood cells to hemostasis. In terms of the evolution of medical research, the studies by Duke mark a switch from descriptive or morphologic studies to functional studies. Clearly, the model of bedside-to-bench research along with cross-disciplinary investigations continues to lead to better understanding of diseases.

Financial Disclosures: None reported.

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