The Circulating Platelet

JOHNSON

THE CIRCULATING PLATELET

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SHIRLEY A. JOHNSON

(April 15, 1922–September 11, 1970)

Our great American, Thomas Jefferson, gave much attention to criteria whereby he estimated the strength of the mind. He listed four qualities in the order of their importance: (1) good humor, (2) integrity, (3) industry, and (4) science. These characteristics can be cultivated. Paying attention to our goals becomes most profitable when they are accurately established. In our Memorial Service, I was interested in utilizing to the utmost the mind qualities exemplified by Shirley Johnson Greenwalt. In each individual these live and work and grow.

Evidently an imprint of basic value was brought from the Canadian prairie provinces of Saskatchewan and Alberta where she was born and lived. Moral soundness, honesty, and uprightness were further nurtured in Victoria College (B. A., 1945) and the Graduate School (Ph.D., 1949) of the University of Toronto. Character builds itself in the stream of life. With Shirley Johnson the commonplace affairs generally proved to be viewed in a special way. Every moment is, of course, new, fresh, and bright, and the one who takes notice of this is soon noticed. On the occasion of her becoming a citizen of the United States of America the oath was administered in just another room by an official. This time, however, the witnesses knew they were especially selected. They appeared in finest dress, and further meaning was added by having a special luncheon after the conferring of citizenship. In her home a dinner or breakfast was prefaced by a short prayer in the finest religious tradition. An ordinary reprint of my Harvey Lecture was presented to me under a special cover before a small group of students, and thus was elevated to extraordinary status. About ten years ago, she and Tibor J. Greenwalt were married. The wedding and the modest reception which followed were attended by only a few, but were very special to all. The marriage was great news for the little world-family of hematologists. Frequently I pass the small church in Grosse Pointe Farms, Michigan, where the cer-

emony took place, and repeatedly it pleases me that this adventure was such a great success.

For the presentation of a scientific paper by Shirley Johnson a manuscript was first perfected and then read. The remarks, which carried clearly, were synchronized precisely with the projection of lantern slides. These were prepared with meticulous care. With color added, the meaning was easy to grasp. The quality of the presentation was a supplement to the excellence of the work itself. Presentations were made at congresses in Paris, Sydney, Moscow, Istanbul, Vienna, Munich, London, and many another city. These left impressions which generated respect for experimental work in the United States, and especially for women in science. This demonstrated that "all virtue and goodness tend to make men powerful in the world; but they who aim at the power have not the virtue" (Newman's law).

Since Jefferson assigned a high rating to good humor or good-naturedness, I feel I must introduce another attitude. When Shirley switched from home economics studies to science technology, it was observed in her characteristic fashion: "There is more to life than cooking cabbage." Consider another incident. Organizing the International Symposium on Platelets at the Henry Ford Hospital was a laborious extracurricular task, but the work was all completed efficiently and, as usual, everything was in good order. The symposium was scheduled to begin on a Friday morning in January. About Thursday noon, or exactly when airplanes from near and far distances were expected to land with participants, a snowstorm started and made safe landing at Detroit impossible for several hours. It was observed good-naturedly that "the timing of the storm was certainly very accurate." On another occasion, there was a heated discussion at a meeting. It was regarded as serious because "even Doctor Jones got very peppery."

The first position after graduation was at Kirksville, Missouri, where the main effort was devoted to instruction. In 1951, research was begun at Wayne State University. She was living with her brother and family in Detroit at the time, and came to the department where I was chairman saying she wanted to work in physiology. It was our first meeting. On such short notice it was not possible to provide for funds. There were only prospects, but this did not stop Doctor Johnson. She began to work on a volunteer basis. This was an unusual beginning for someone destined to rise to the top ranks. Years later, the University presented her with one of its centennial medals. This was in recognition of outstanding scholarship and loyal support of the ideals and programs of the University.

The work at Wayne State University was begun with enthusiasm and continued at a high level of productivity later at the Henry Ford Hospital

(1956–1960), then at Milwaukee County Hospital, the Veterans Administration Hospital, and Marquette University in Milwaukee where Doctor Greenwalt lived. When he became Medical Director, Blood Program, American National Red Cross in Washington, D.C., Doctor Johnson became Chief of the Thrombosis Research Laboratory of the Veterans Administration Hospital and held appointments in physiology at George Washington University and Georgetown University. At each place there was the difficult task of finding funds and help and arranging the laboratory.

The first experiments at Wayne State University were with blood platelets and the blood coagulation mechanisms. Quite soon platelets were obtained in large quantities from bovine plasma, and the substance(s) required to correct the procoagulant power of hemophilia B plasma was found in serum. When oral anticoagulants were being used, this substance could not be found in serum, and its metabolic synthesis was considered to be vitamin K dependent. A theory was advanced to account for the inactivity of the antihemophilic globulin in serum. Activity was recovered by ether extraction. Antifibrinolysin activity was found in platelets. Plasma antithrombin activities were differentiated. Her studies on functional aberration of platelets in clinical states became well known. There was a study of the defects in the blood coagulation mechanisms in polycythemia vera. The morphological location of platelet factor 3 was studied, as well as the suppression of its activity in uremia, in macroglobulinemia, and by dextran. The endothelial supporting function of platelet factor 3 was demonstrated, and this concept was enlarged. This observation was at the level of basic physiology. During the course of histological studies on blood clots, Doctor Johnson prepared herself to do ultrastructural work. The electron microscope was applied to the study of the physiology of hemostasis. The difficult technology was mastered in a short time. A careful analysis was made of a sequence of events in the physiology of hemostasis. A correlation included observations on ultrastructural forms, ADP formation, thrombin formation, fibrin formation, hemostasis, and clot retraction. I quote a summary from a review:

> The main function of platelets, the maintenance of hemostasis, depends on three of their properties, the endothelial supporting function of platelets, the ability to form hemostatic plugs and to release lipoprotein material (platelet factor 3). When the number of circulating platelets is reduced the capillary endothelium becomes weakened as platelets are not available to enter the endothelial cytoplasm to support it and the capillary wall ruptures when exposed to minor trauma. The bleeding through the ruptured vessel wall is arrested by formation of a clump of platelets forming a hemostatic plug. Damage to the vessel wall initiates both the coagulation mechanisms and degradation of adenosine triphosphate. The products of each of these reactions, namely, thrombin and ade-

nosine diphosphate, bring about aggregation of platelets to form the hemostatic plug. These are the platelet functions performed following a transfusion of platelets into a thrombocytopenic recipient. The platelets remaining in the circulation and which are counted in platelet survival studies are those in excess of immediate need of the recipient.

During a time span of less than two decades, Doctor Johnson's name appeared on almost 100 contributions in journals and books. All of these are having their impact. Along with this, three important symposia were programmed. Five books were produced under her leadership and planning. One was coauthored by Tibor J. Greenwalt, while the others contained chapters by various authors. The titles are impressive and sustain the original interest in platelets: (1) "Blood Platelets," (2) "Coagulation and Transfusion in Clinical Medicine," (3) "Physiology of Hemostasis and Thrombosis," (4) "Dynamics of Thrombus Formation and Dissolution," and (5) "The Circulating Platelet."

Such is the nature of an inspiring mind that lives on with good humor, integrity, industry, and science. I quote: "It is not the mere cry of moralists, and flourish of rhetoricians; but it is noble to seek truth, and it is beautiful to find it. It is the ancient feeling of the human heart—that knowledge is better than riches; and it is deeply and sacredly true!" (Sydney Smith).

From my studies in integrative physiology, I learned that I am always gaining. Loss is only apparent. This is a truth which prevails, even if an event is incomprehensible. It is like the problem of evil, which is solved by seeing that it is good not understood. So, under trying conditions, *I remind myself to appreciate all there is*, and proceed in a life-affirming manner.

WALTER H. SEEGERS
Washington, D.C.

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PREFACE

In 1951, the first United States Government funds designated for research into the biochemistry and physiology of platelets became available. The possibility of atomic warfare created a very practical need for the technical knowledge required for successful transfusion of platelets into thrombocytopenic recipients. It is clear from the type of research which resulted that the members of the scientific community responsible for awarding these funds appreciated that practical advances depend on basic knowledge in biochemistry and physiology.

Interdisciplinary backgrounds and tools were focused on this subject, and extraordinary productivity has resulted in the last two decades. Such productivity would only come from a scientific body possessed of health and excitement. As Sir Kenneth Clark has stated "that civilisation ... requires confidence—confidence in the society in which one lives, belief in its philosophy, belief in its laws, and confidence in one's own mental powers" ("Civilisation," Harper and Row, New York, 1969). While scientific achievement is not equated with civilization, it is a part of it.

Although all the contributors submitted comprehensive outlines to the editor before the chapters were written, some repetition of the subject matter has occurred. In each case there is no repetition of point of view so it seemed best to leave the chapter contents unchanged. For the reader interested in these subjects it will be invaluable to read about the same topic from several different vantage points.

The information contained herein has almost all been obtained during the past twenty years, and, I think, represents one of the milestones in expansion of biological knowledge. The success of "The Circulating Platelet" belongs to the contributors, all of whom are authorities in the area they have written about. Their enthusiasm has made organization of this work a pleasure.

SHIRLEY A. JOHNSON

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HISTORICAL PERSPECTIVES AND THE BLOOD PLATELETS

JOHN W. REBUCK

I. THE DISCOVERY OF BLOOD PLATELETS

> That is the Oxford, strong to charm us yet:
> Eternal in her beauty and her past.
>
> *Lionel Johnson**

From this "weathered cloister and worn court" Robb-Smith (1967) has amassed such an array of early Victorian scientific manuscripts as to illumine past perspectives and still leave something more to motivate our

* From *Oxford* written in 1890 and published in *Ireland and Other Poems.*

present-day electronic approach to the twenty-first century. The biologist, unlike the poet, is still confined to the regions circumscribed by his tools and methods. Blood platelets, increasingly more important as determinants of therapeutic life-span, demand, however, exact knowledge of their milieu before they can be studied, indeed, before they can be discovered.

A. Discovery Leading to Function

Before platelets were discovered then Hewson (1773) must necessarily devise the first anticoagulants, which were neutral salts such as sodium sulfate, and discover that hemolysis could be prevented with serum as a diluent. Only then and with the introduction of the achromatic objective to overcome the chromatic aberrations in the compound microscope could Gulliver (cf. Gerber, 1842) describe minute spherules in the blood which were 2.5 mμ in diameter and which then transformed to granulated particles in a few hours. Unfortunately in a later paper as related by Robb-Smith, Gulliver rejected the concept of a relationship between his minute spherules and fibrin formation. The second English discoverer of the platelets, William Addison (1842), observed the beginning of fibrin formation in association with minute bodies in the blood; his drawing (his Fig. 1) illustrates a platelet–fibrin clot. Quite fairly Robb-Smith cites the observations of platelets by the two Germans Simon (1842) and Zimmerman (1846) in the very same period. In 1850, T. W. Jones had induced fibrin thrombi by trauma to the vessel wall in the web of a frog's foot (1851); his Fig. 2 is an excellent drawing of an experimental thrombus but there is little evidence that he envisioned the role of the previously described blood particulate elements as important in its formation. Similarly Virchow (1858), who coined the term fibrinogen which he believed was converted into the fibrin clot, rejected Zimmerman's observation of small colorless bodies, refractile and with well-defined outlines as formed elements of anticoagulated blood. Virchow described the center of the thrombus as composed of fibrin and a faintly granular substance, but having rejected the identity of the platelets, missed the significance of the central fibrin-related granular substance.

In 1865 Schultze devised a warm stage and was able to observe the coalescence of platelets into granular masses, a finding of inestimable importance concerning platelet aggregation. Osler (1874) was able to extend this work by finding that there was no tendency for individual platelets to stick together within the blood vessels of the rat's connective tissue but if blood were removed from the animal, the granular masses formed at once. Furthermore, pseudopodia as "two, three, or even more tail-like processes