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Editorial

Coagulation in Retrospect

THE tempestuous course of the study of blood coagulation is proverbial and even today some of the conflicting concepts have their roots deeply imbedded in the past. Perhaps the trouble started when Petit in 1731 postulated that bleeding was stopped by the formation of a coagulum of blood. The concept that hemostasis centers about a fibrin plug is still widely accepted as a fact rather than regarded as an unproved theory.

An important advance was made when Morawitz and also Fuld and Spiro in 1904 summarized the existing knowledge on blood clotting from the time of Malpighi in 1666 to Alexander Schmidt in the form of the two well known equations:

Thrombokinase + Ca + Prothrombin = Thrombin
Fibrinogen + Thrombin = Fibrin

In accepting calcium as essential for clotting, they overrode Schmidt who vigorously denied that calcium was a primary factor. The work of Hammersten and Pekelharing established that calcium was required for the formation of thrombin but not for the action of thrombin on fibrinogen. Curiously, little more is known today concerning the role of calcium in coagulation.

While the theory of Morawitz is now often called "classic," it received little recognition until 1935. In fact, Pickering in his monograph (1928) wrote, "the hypotheses of Wooldridge and of Morawitz are briefly mentioned, although they are now discarded by almost all research workers." As a result of this discard of the Morawitz hypothesis, the first third of this century became the most chaotic and unproductive period in the study of blood clotting. It is to be remembered that it was during this period that colloidal chemistry and immunologic reactions occupied a dominant place in what is now known as life science research. Some of the leading investigators regarded the conversion of fibrinogen to fibrin as a physiochemical phenomenon. Thus, it was postulated that fibrinogen existed in the blood in a sol-like condition or as hydrated micellae and that coagulation came about by dehydration, thereby converting it to a gel. Thrombin, which earlier investigators regarded as an enzyme, was considered by some as an agglutinin. One prominent investigator explained the formation of fibrin as the result of a combination of fibrinogen with thrombin similar to the union of toxin with antitoxin. Another leading worker did not believe that thrombin was a definite chemical entity but rather an ensemble of unsaturated complexes of fibrinogen. It is not surprising that a few workers denied the very existence of prothrombin.

It is clear in retrospect that one of the basic problems that had to be solved was the nature of the action of thrombin. In this country the prevailing thought had been that it reacted stoichiometrically with fibrinogen. In 1936

it was shown that the concentration of thrombin influenced the speed of the clotting of fibrinogen and that when the clotting time of fibrinogen was plotted against the reciprocal of the thrombin concentration, a straight line was obtained. This clearly suggested that thrombin acted as an enzyme. After the discovery that small peptides are split from fibrinogen through the action of thrombin, thereby activating fibrinogen and causing it to polymerize, this phase of the clotting reaction has become the best understood and least controversial.

The concept that prothrombin was the mother substance of thrombin was firmly established by the leading workers prior to the turn of the century, but how prothrombin is activated physiologically still remains an enigma. Alexander Schmidt postulated that it was converted through the action of a zymoplastic substance which is lipoid in nature. Morawitz regarded the latter as a kinase and designated it thrombokinase. Bordet postulated a direct union of the phosphatides of blood platelets and of tissue juices with prothrombin, which he called serozyme, to form thrombin. One of the great difficulties was lack of knowledge concerning the relationship of platelets to tissue thromboplastin. Until relatively recently many English investigators did not even accept the existence of platelets. A leading English textbook in physiology[3] stated that the platelets were probably artifacts. Until 1947, it was fairly generally accepted that platelets contained preformed thromboplastin.

The view whether tissue extract acts enzymatically or stoichiometrically depended on the experimental method used. Howell postulated that prothrombin was united with an antiprothrombin and that thromboplastin removed this inhibitor, whereupon prothrombin reacted with calcium to form thrombin. With the development of the one-stage prothrombin time, it was shown that as the amount of thromboplastin was increased, the clotting time reached a minimum which could not be further decreased, thus strongly indicating that the reaction is not enzymatic. In 1939, it was demonstrated that the amount of thrombin formed, as measured by the two-stage method, was proportional to the amount of thromboplastin added, which clearly suggested that thromboplastin acts stoichiometrically.

By a different method called the thrombin production test based on Mellanby's observation that when plasma is diluted with cold distilled water and acidified to pH 5, a precipitate is formed which, when dissolved at pH 7 and recalcified, generates thrombin, it could be shown that the speed of thrombin formation is a function of the platelets. This could be interpreted as an enzymatic action. The observation that purified prothrombin dissolved in 25 per cent sodium citrate slowly converts to thrombin makes it questionable whether thromboplastin is required in a system of purified reagents for the conversion of prothrombin to thrombin.

The foregoing illustrates that conflicting concepts can result from observations obtained under different experimental approaches. Because blood coagulation *in vivo* may be quite different from that observed in the test tube, it is understandable why divergent views can readily arise. Often such clashes are superficial, not deep-seated, and actually lead to significant advances. The one- and two-stage prothrombin tests, which were developed almost simultaneously, complemented each other and hastened the introduction of vitamin K into therapy. While there was some difference in opinion concerning the interpretations of results, there has been no dissension concerning the eradication of cholemic bleeding with vitamin K. Yet, the basic concept supported by both methods that vitamin K directly participates in the synthesis of prothrombin is probably erroneous. The academic controversies are usually trivial and harmless and are not to be compared in seriousness to the practice of some clinicians who prescribe vitamin K routinely for patients with all types of bleeding or who give synthetic vitamin K compounds in such large doses that untoward results, such as kernicterus, are induced.

Just as the one- and two-stage methods not only helped to clarify the role of prothrombin in blood clotting but also advanced the clinical approach to hemorrhage and thrombosis, so

the prothrombin consumption time and the thromboplastin generation test furnished effective means to explore the thrombocytopenias, the hemophilias and several related diseases.

As in the past, conflicts will arise because the problems of the future will continue to be attacked by diverse approaches. The clinically orientated will attempt to employ clinical material, the physiologist will use the methods of the physiological laboratory, while the biochemist will exert his major efforts purifying clotting factors and studying their interactions under carefully controlled conditions. In all probability, none of these approaches can furnish the complete answer, but the correlated efforts will, as in the past, penetrate deeper into the mysterious realm of blood clotting.

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