Abstract—A review is presented on biorheological studies of platelet activation and platelet-platelet binding events that play key roles in thrombosis and hemostasis. Rheological methods have been used by a number of workers to establish the importance of fluid mechanical shear stress as a determinate of platelet reactions. Fluid mechanical shear stress can be regarded as a platelet agonist that is always present in the circulation and that is synergistic in its actions with other agonists. Early biorheological studies were phenomenological in that they focused on stress effects on measures of platelet function. Subsequent studies have elucidated mechanisms and have shown that the biochemical pathways of platelet activation are very different at elevated shear stresses than in the low shear stress environment used in many platelet activation studies. This finding that biochemical pathways of platelet activations are different at different shear stress levels suggests that it may be possible to develop platelet inhibitors of highly specific action: it may be possible to inhibit pathways important in thrombosis in a partially occluded artery without seriously compromising the normal hemostatic function of platelets. Another aspect of the work suggests that the biorheological approach may make it possible to develop better methods for prediction of thrombotic tendencies in human subjects.

Keywords—Platelets, Aggregation, Blood, Hemostasis, Shear stress.

INTRODUCTION

The purpose of this paper is to review some aspects of the biorheological approach to thrombosis research. That is to say, the subject is the study of the etiology of thromboembolic events as influenced by deformation and flow processes. It is far too large a subject to permit comprehensive coverage in one paper. Here, we will confine our attention to a small subset of the work on early events in thrombosis and hemostasis in which the platelets play the key role. The subset will be limited still more by focusing on events associated with platelet-platelet binding and excluding discussion of the large and important field of platelet adhesion to solid substrates. Thus, a more specific subtitle might be “Introduction to Studies on the Reactions of Human Blood Platelets under Shear Stress.” Even so focused, there has been too much work in this general area to permit any sort of comprehensive discussion in a document of reasonable length. So apologies are offered for the lack of citation of many important contributions.

Some work on the involvement of platelets in thrombosis and hemostasis was performed by a very few pioneering workers in the late nineteenth century. But there was very little research in this area and relatively little understanding of platelet function prior to 1960. In 1962, Born (8) devised a simple method for studying the response of platelets to added chemical agonists. The method involved detection of changes in optical density of a stirred platelet suspension. The methodology (the platelet aggregometer) had a profound effect on the study of platelet reactions, and it rapidly came into routine and widespread use. Since the platelets are stirred to maintain suspension, the reactions in the platelet aggregometer take place under the influence of fluid mechanical shear stress. However, the stress levels are low and vary with position in a complex and uncharacterized way. Several years later, rheological methods were employed to study platelet reactions under known, quantified shear stress, and several workers established the importance of the shear stress field itself as a determinant of platelet reactions (9,10,14, 17,21,27,31-33,36,42,46).

METHODS

Rotational viscometers have been used in many studies to apply a uniform, well-characterized shear field to a fluid sample. Indicators of platelet activity are usually measured after an exposure time of many seconds to a few minutes.

The desire to monitor platelet reactions as they occur led us to modify a cone-and-plate viscometer to allow continuous optical measurement of three indices of platelet response (18): aggregation, release of dense granules, and increase in intracellular calcium ion concentration. Platelet aggregation is monitored using optical density as employed in most commercial “platelet aggregometers.” Release of dense granules from platelets is monitored
by measuring luminescence. Platelet dense granules contain, among other constituents, serotonin, ATP, and ADP. An extremely sensitive luminescence assay for ATP uses the firefly luciferase enzyme. The time course of ATP release is calculated from a continuous measurement of luminescence. The technique for measurement of intracellular calcium ion concentration uses Indo-1, a multicarboxylate calcium ion chelator that changes fluorescence properties upon calcium ion binding.

A schematic drawing of the viscometer showing the optical system for measurement of dual wavelength fluorescence is given in Fig. 1. The optical systems for measurement of optical density (related to platelet aggregation) and luminescence (related to release of granular contents of the platelets) are similar, but simpler, and are not shown.

In a typical experiment, a platelet suspension is subjected to a known, uniform shear stress in the viscometer for 30–300 sec, during which time the three optical indicators of platelet reactions are monitored continuously. Subsequently, specimens are removed from the viscometer for “endpoint” analyses, such as particle size distribution, serotonin release, cell lysis, and platelet morphological changes.

In recent years, flow cytometers have come into use in measuring platelet reactions. The extension of the methodology to employ flow cytometry has a number of significant aspects. By use of fluorescent-conjugated markers, we gain the ability to study platelet reactions in whole blood. Prior platelet aggregation studies were often performed using suspensions of platelets in buffer or using platelet-rich plasma (PRP). Fluorescently labeled antibodies have been developed to detect various epitopes of platelet membrane receptors indicative of function and conformation.

There is widespread appreciation of the utility of the flow cytometric approach to the study of platelet reactions. Thus, a number of monoclonal antibodies that bind to specific platelet membrane receptors are available. Whole blood is subjected to a well-defined, laminar shear stress in the cone-and-plate viscometer. Sheared blood specimens are fixed with formaldehyde and incubated with two monoclonal antibodies. Antibody 6D1, specific for the platelet membrane glycoprotein Ib (GPIb) (13), is used to identify platelets and platelet aggregates. Antibody PAC1, specific for the activated form of the membrane glycoprotein IIb/IIIa complex (GPIIb/IIIa) (45), is used to measure platelet activation. Prior to use in flow cytometry, 6D1 is conjugated with fluorescein isothiocyanate (FITC). PAC1 is biotinylated and subsequently labeled with phycoerythrin–streptavidin. Blood samples are then analyzed in a flow cytometer. Platelets and platelet aggregates are distinguished from erythrocytes and leukocytes on the basis of their characteristic forward-angle light scattering and FITC fluorescence profiles. Use of an antibody that recognizes only the activated form of GPIIb/IIIa yields sensitive determinations of activation.

Details of the various methods for studying mechanisms cannot be given here, we will confine our remarks to a brief note, indicative of the approaches used in certain key studies. Some studies on mechanisms focus on the roles of platelet membrane receptors and adhesive proteins. In this work, we use patients’ platelets that are deficient in a specific membrane receptor. Other patients have disorders associated with deficiencies in specific adhesive proteins. Also, monoclonal antibodies and other agents are used to block specific receptors.

Three disorders associated with platelet function that have been used in mechanistic studies (34,35,39) are listed below.

![FIGURE 1. Schematic of the cone-and-plate rotational viscometer showing the fiber optic measurement system for dual wavelength fluorescence determination of intracellular calcium ion concentration. The labeled platelet suspension (between the two platens) is subjected to a uniform, known shear stress when the upper platen (the cone) rotates at a constant angular velocity.](image-url)