A method is described for the preparation of suspensions of washed rabbit-blood platelets, and some of the properties of these platelets are presented. The method has been modified for isolation of human platelets. [The SCImago indicates that this paper has been cited in over 290 publications.]

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After completing my MD thesis at the University of Adelaide, I joined Fraser Mustard's group in 1967 as a fellow of the Ontario Heart Foundation at McMaster University, Hamilton, Canada. Subsequently, I enrolled as a PhD candidate at approximately the same time as two other Australians, Raelene Kinlough-Rathbone and John Lloyd. We still continue our interests in the platelet field. My most vivid recollections from the years at McMaster include the exciting and sometimes exhausting daily activities in the laboratory and the regular evening and weekend discussions that reflected the communicative milieu in which we all worked during those years.

My thesis project involved a study of the mechanism of adenosine diphosphate (ADP)-induced platelet aggregation. In platelet-rich plasma, studies of platelet function are complicated by the presence of plasma proteins, plasma enzymes, and the anticoagulant used to inhibit blood coagulation. Because of this, a variety of methods had been developed for isolating, washing, and resuspending platelets; but there were problems associated with each of these techniques, and we realized that we needed to develop better methods. All of the published methods resulted in suspensions of washed cells that were insensitive to ADP at concentrations effective in plasma. Variability in platelet sensitivity to ADP and spontaneous aggregation upon warming and stirring were additional problems.

We developed methods for preparing suspensions of washed platelets from some animal species and humans by systematically and painstakingly considering various conditions that influence cell functions, including the nature of the anticoagulant, the cations required (particularly calcium and magnesium), the pH, the need for protein, the osmolarity, the need for a source of metabolic energy, and the temperature.

Initially, satisfactory suspensions of isolated platelets could be prepared most consistently from rabbit blood. A preliminary report of this work was presented at the FASEB meeting in April 1968, and the full description of the method for isolating rabbit platelets was published in 1970 [the Classic paper, Ed.]. Washed rabbit platelets stored at 37°C gradually lose their ability to aggregate due to leakage of nucleotides from the cells. We were able to further improve the procedure for isolating rabbit platelets by the addition of apyrase, which degrades nucleotides and consequently maintains platelet sensitivity to ADP. A modification of this method also proved suitable for isolating human platelets. Over the years, the method has been revised in various details, but our original method is still used.

The large number of citations result from the application of the method over the last 15 years to the investigation of a diverse field of platelet functions. The technique that we introduced for isolating platelets from rabbits and other animal species, and subsequently humans, was both very simple and very effective. Other methods for isolating platelets include gel filtration and albumin density centrifugation, but these have a number of obvious drawbacks, and our method is presumably preferred by many other workers. It is gratifying to know that this method has been of some help to others in their efforts to probe platelet function.